would be acceptable. The post vaccination antibody levels measured with either strain by HI after administration of either vaccine were in the range previously found to be protective(6). The results of field trials conducted in 1963 and of observations since on the incidence of influenza in the completely vaccinated military demonstrate that polyvalent vaccines containing either strain have yielded a high degree of protection against Asian influenza(7,8,9). Nevertheless, the superior antigenic potency of the A_2/Japan/170/62 vaccine is apparent and vaccines containing that strain have proven to be protective in the military(9). The observation that vaccine of equal CCA content can differ so markedly in antigenic potency points up the fact that determinants of antigenicity in different strains of influenza virus warrant further investigation.

The findings demonstrate that while there are quantitative differences in the titer values found by HAdI and HI there are no differences in the quality of the information obtained in studies in man directed to the question of the relation of strain variation to vaccine protection.

Summary. A comparison of the antibody response of humans vaccinated with either A_2/Japan/305/57 or A_2/Japan/170/62 monovalent vaccine as measured by HAdI and HI techniques has been presented. Although the HAdI antibody titers were higher the information obtained from comparisons of the frequency of antibody response and of mean fold titer increases was the same for both methods. Thus, it would appear from this experience that the HAdI technique offers no great advantage for the selection of vaccine strains when the criterion is the antibody response in man. In addition, it should be pointed out that the HAdI test is a more laborious, expensive and time consuming procedure than the HI method.

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Incorporation and Fate of Estradiol-17β-6, 7-H^3 in Rabbit Oviducts.* (32605)

YONG JIN KIM,† JOHN B. COATES, III, AND GEORGE L. FLICKINGER
(Introduced by L. Mastroianni, Jr.)
Department of Obstetrics and Gynecology, School of Medicine, University of Pennsylvania, Philadelphia, Pa. 19104

The normal growth and function of certain mammalian tissues are known to be dependent on the continued presence of delicately balanced amounts of steroid hormones. Jensen and Jacobson in their studies with immature rats have shown differences between the incorporation patterns of estradiol-17β in the uterus and vagina, and in those tissues which are not responsive to estrogens (1,2). The affinity of uterine tissue for estradiol, but not estrone, was demonstrated in both *in vivo* and *in vitro* systems. These

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† NIH Postdoctorate Fellow in Reproductive Biology, Training Grant HD130-01. Present address: Department of Obstetrics and Gynecology, Coney Island Hospital, Brooklyn, N. Y.

authors also observed that neither the immature nor the actively growing rat uterus possessed the ability to oxidize estradiol to estrone *in vivo,* whereas estrone was converted to estradiol.

The affinity for estradiol has also been reported for the mouse uterus and vagina(3,4) and for the human uterus(5). Although the Fallopian tube is known to be responsive to sex hormones(6), the affinity for estradiol-17β by the tube has not been reported.

The present experiment was designed to study the incorporation patterns of tritiated estradiol-17β in the uterus, oviduct, skeletal muscle and plasma in the rabbit, and to demonstrate the chemical nature of the radioactive estrogens present in these tissues.

Materials and methods. New Zealand strain female rabbits (2.8-4.7 kg) were castrated and given a subcutaneous injection of 5 μg of estradiol-17β in 0.1 ml peanut oil daily for 5 days. On the 6th day, the rabbits were injected intravenously with 5 μC of estradiol-17β-6,7-H^3 (0.246 μg; S.A. 9.7 C/mM; New England Nuclear) dissolved in 0.1 ml of ethanol and 0.9 ml of saline. Estradiol-17β-6,7-H^3 was purified by paper chromatography prior to its use in the present experiment.

The Fallopian tube, uterus and a portion of the gastrocnemius muscle were obtained by sacrificing the animals at intervals of ½, 1, and 4 hours after injection. 10 ml of whole blood was drawn into heparinized syringes from the right ventricle of the heart at the same intervals.

The above tissues were dissected free of surrounding connective tissues, weighed and homogenized in a glass grinder with distilled water. The proteins of the homogenates were precipitated with 5 volumes of cold acetone, centrifuged for 30 minutes at 3,000 rpm, and the precipitates were washed 2× with acetone. The heparinized blood was centrifuged and 1 ml of plasma was treated the same as the tissues.

The combined supernatants were evaporated to dryness and the residues were transferred with 2 ml of absolute ethanol to scintillation vials. 15 ml of scintillation media, consisting of 4.6 g of 2,5 diphenyloxazole (PPO) and 116 mg of 1,4 bis-2-(5 phenyloxazolyl)-benzene (POPOP) per liter of toluene was added to each vial. For tissue and plasma backgrounds, a rabbit was injected with non-radioactive estradiol-17β and the tissues and plasma were collected and treated as above. Liquid scintillation was performed on a Packard Automatic Tri-Carb Spectrometer. Sufficient counting time was allotted to yield a maximum relative standard error of $\pm$ 1% for each sample. The count rates (cpm) were corrected for quenching by means of an external standard. All count rates were corrected to 100% efficiency and expressed as disintegrations per minute (dpm). The absolute count rates (dpm) were corrected by subtraction of the background activity for each respective tissue.

For the identification of radioactive estrogens present in tissues and plasma, 2 castrated and estradiol treated rabbits were each injected intravenously with 100 μC (4.8 μg) of estradiol-17β-6,7-H^3. The tissues and blood were obtained by sacrificing the animals ½ hour after injection. The tissues and blood obtained from 2 animals were pooled and homogenized as described above. 50 μg each of carrier estrone, estradiol-17β, estradiol-17α, and estriol was added to each sample before precipitation of the proteins.

The supernatants were evaporated to dryness and the residues, dissolved in 20 ml of distilled water, were extracted 4× with 2 volumes of freshly distilled diethyl ether. Aliquots were taken for assay of radioactivity from the ether and aqueous layers. The ether residues were partitioned between toluene and 1 N NaOH. The NaOH was neutralized with HCl and extracted 3× with 2 volumes of ether. The ether was washed 1× with 1/10 volume of $NaHCO_3$, 2× with 1/10 volume of water, dried over anhydrous sodium sulfate and evaporated.

Chromatography was carried out on Whatman #1 paper strips. Reference estrogens were located on the strips with ferric chloride-potassium ferricyanide reagent(7).

Radioactive zones on paper strips were detected with a radiochromatogram scanner (Baird Atomic; Model RSC-363).

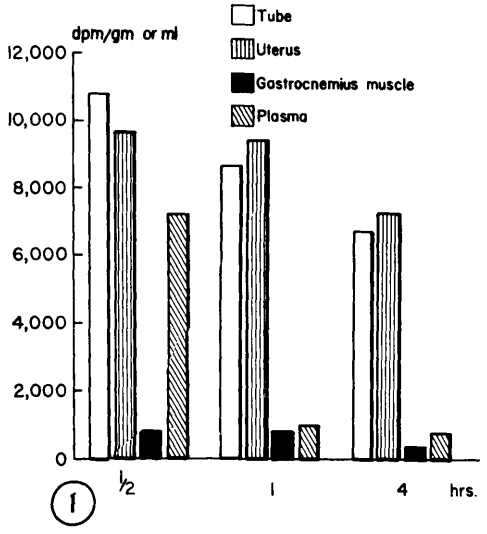
Results. Incorporation of radioactivity. 2 rabbits each were sacrificed at intervals

TABLE I. Concentration of Radioactivity in Tissue and Plasma of Rabbits After Single Intravenous Injections of 5 μC (9.03×10^{-4} μM) of Estradiol-17β-6,7-H^3.

Time (hr)	No. of animals	Concentration of radioactivity (dpm/g of wet wt or ml)			
		Tube	Uterus	Gastrocnemius muscle	Plasma
½	2	10,854 (5.08×10^{-7} μM)	9,766 (4.56×10^{-7} μM)	731 (3.40×10^{-8} μM)	7,265 (3.38×10^{-7} μM)
1	2	8,677 (4.06×10^{-7} μM)	9,454 (4.42×10^{-7} μM)	737 (3.43×10^{-8} μM)	900 (4.19×10^{-8} μM)
4	2	6,797 (3.18×10^{-7} μM)	7,288 (3.41×10^{-7} μM)	232 (1.02×10^{-8} μM)	754 (3.50×10^{-8} μM)

of ½, 1, and 4 hours after injection of estradiol-17β-6,7-H^3. The mean concentrations of radioactivity in the tissues and plasma are shown in Table I. The levels of radioactivity were highest in the tube, uterus and plasma at 30 minutes. There was a sharp decline in the concentration of plasma radioactivity at 1 and 4 hours. In contrast the Fallopian tube and uterus retained high and approximately equal levels of radioactivity at each time interval with the concentration in these tissues being approximately 10× greater than the plasma levels at 1 and 4 hours. The levels of radioactivity in skeletal muscle were relatively low throughout and never exceeded plasma levels. A comparison of the level of radioactivity in the above tissue is depicted in Fig. 1. The average total wet weight of both Fallopian tubes was 1 g; the maximum amount of radioactive steroid present in the Fallopian tube was therefore approximately 0.05% of the injected dose.

Nature of the radioactive material in the tissues and plasma. Most radioactivity in the Fallopian tube, uterus and skeletal muscle was present in the ether layer, whereas in plasma significant amounts of activity were present in the aqueous layer. To determine the chemical nature of the ether-soluble fraction, the ether residues were subjected to paper chromatography as shown in Fig. 2. The radioactive zones were eluted from the final chromatograms and appropriate carrier steroids were added for recrystallization from aqueous ethanol. The steroids were considered radiochemically pure when the specific activ-



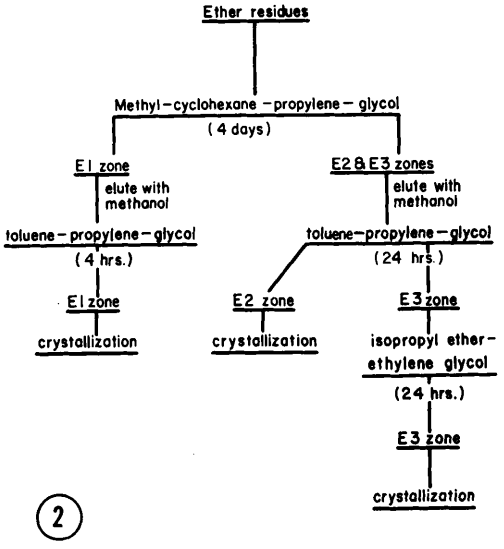


FIG. 1. Radioactivity in tissues and plasma after a single I.V. injection of 5μC of estradiol-17β-6, 7-H^3.

FIG. 2. Purification of radioactive estrogens by paper chromatography. E1 = estrone; E2 = estradiol-17β; E3 = estriol.

TABLE II. Radiochemical Purification of Free Steroids Extracted from Rabbit Tissues and Plasma.

Tissues	Steroids	Steroid carrier (mg)	Expected specific activity (dpm/mg)	Crystallization data Specific activity (dpm/mg) 1st crystals	2nd crystals	3rd crystals
Tube	Estradiol-17β	11.27	13,114	12,675	12,709	12,237
Uterus	Estradiol-17β	23.25	16,740	16,697	17,025	16,820
Gastrocnemius muscle	Estrone	13.55	428	406	417	427
	Estradiol-17β	9.01	4,375	3,518	3,538	3,458
Plasma	Estrone	9.87	1,341	1,260	1,306	1,281
	Estradiol-17β	21.91	32,341	32,178	32,866	31,462
	Estriol	11.08	957	869	862	926

ity of three consecutive crystallizations did not differ by more than $\pm$ 5% from the mean value.

Chromatography and recrystallization of the the ether-soluble fraction demonstrated radioactive estradiol-17β only in the Fallopian tube and uterus. Radioactive estrone and estradiol-17β were identified in the skeletal muscle; and radioactive estrone, estradiol-17β and estriol were found in the plasma (Table II). Radioactivity was not found in the chromatographic zone corresponding to reference estradiol-17α (Toluene-PG) in any of the tissues or plasma.

Discussion. The present study demonstrates that the Fallopian tube of the rabbit incorporates and retains estradiol-17β at the same level as uterine tissue during the time intervals studied. The affinity of the rabbit uterus for estradiol-17β is similar to that demonstrated in the rat(1,2) and mouse(3, 4). Since a limited number of animals and time intervals were used in this experiment, further studies are necessary to define the time relationships of tubal and uterine concentration of estradiol-17β in the rabbit.

Ryan and Engle have demonstrated that a variety of human tissues, including the uterus, have the capacity to interconvert estradiol and estrone *in vitro*(8). A similar conversion to estrone has been found in our laboratory when rabbit tubal tissues were incubated in the presence of estradiol-17β. Jensen and Stone have shown that rat and mouse uteri do not show a specific affinity for estrone as they do for estradiol(1,2,3,4). Absence of significant amounts of radioactive estrone in the tube and uterus in the present study may therefore reflect a similar lack of affinity rather than inability of these tissues to convert estradiol to estrone.

Summary. The incorporation and fate of estradiol-17β-6,7-H^3 was studied in the female rabbit. The Fallopian tube of the rabbit showed an affinity for estradiol-17β at the same level as uterine tissue. The major portion of radioactive steroids present in the Fallopian tube, uterus and skeletal muscle was in the ether-soluble fraction, whereas in plasma significant amounts of activity were present in the aqueous layer. Estradiol-17β was the only radioactive estrogen found in the tubal and uterine tissues. Radioactivity was identified as estrone and estradiol-17β in the skeletal muscle, and as estrone, estradiol-17β and estriol in the plasma.

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