Metabolism of sex hormones in the aortic wall

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ABSTRACT The metabolism of labeled sex hormones was examined in human, canine, and rat aortas. Isolated arterial tissue converted estrone to estradiol, estradiol to estrone, and estrone sulfate to estrone and estradiol. The arterial wall also appeared to metabolize testosterone to androstenedione and an unidentified, relatively nonpolar derivative.

Both estrogens and testosterone appeared to enter the arterial wall rapidly. No competition in arterial uptake between the two hormones was apparent. No specific arterial binding of estradiol could be demonstrated.

The concentration of estradiol-^{3}H in the canine aorta exceeded that in the plasma 1–6 hr after estradiol-^{3}H administration. The uptake and disappearance of estradiol-^{3}H in the aorta generally resembled the patterns observed in body tissues other than the adrenal gland and uterus. The uptake of estradiol-^{3}H was greatest in the adrenal gland while its retention was maximum in the uterus.

SUPPLEMENTARY KEY WORDS man · dog · rat · estrogens · testosterone · estradiol binding · serum concentration · adrenal gland · uterus · atherosclerosis

PREVIOUS STUDIES have indicated that sex hormones influence the metabolism of the arterial wall. Malinow and associates have demonstrated a local action of estrogens on arterial oxygen consumption and arterial alkaline phosphatase activity (1, 2) and Werthessen has shown that estrogens can influence lipid synthesis in the perfused calf aorta (3). In addition, recent studies in this laboratory have indicated that estrogens stimulate arterial incorporation of labeled precursors into phospholipid, RNA, and protein, and that testosterone can antagonize the in vitro action of estrogens on phospholipid metabolism (4). Despite these findings and observations in experimental animals and man concerning the possible influence of sex hormones on the atherosclerotic process (5, 6), the fate of sex hormones within the arterial wall is unknown. Entrance of estradiol into arteries has been demonstrated in rabbits (7), but little other information is available concerning the uptake or metabolism of sex hormones in either human or animal blood vessels.

The present investigation was undertaken to examine the entrance, binding, subcellular distribution, and metabolic conversion of tracer doses of labeled estrogens and testosterone in the arterial wall.

MATERIALS AND METHODS

In Vitro Studies

Aortas were removed from human patients within 4 hr of death and from adult mongrel dogs killed under pentobarbital anesthesia. The tissues were prepared as previously described (8) and 0.1–0.3 g portions were incubated for 30–120 min in 10 ml of lactated Ringer's solution containing 17β-estradiol-6,7-^{3}H (0.025–2.5 μc/ml, specific activity 42.4 c/mmole),[1] estrone sulfate-6,7-^{3}H (0.25 μc/ml, 2.85 c/mmole),[1] estrone-6,7-^{3}H (0.25 μc/ml, 42.4 c/mmole),[1] estriol-6-7-^{3}H (0.25 μc/ml, 14.7 c/mmole),[1] or testosterone-1,2-^{3}H (0.25 μc/ml, 42.4 c/mmole).[1] The experiments with estradiol-^{3}H and testosterone-^{3}H were also performed in the presence of unlabeled estradiol or testosterone (10^{-10}–10^{-7} mole/ml). Heat-inactivated aortas, prepared by immersion in boiling water for 1 min, were used as control tissues. The degree of atherosclerosis (9) in the sections of human

The following trivial names and abbreviations have been used. Estrone (E1), 3-hydroxyestra-1,3,5(10)-trien-17-one; estradiol (E2), estra-1,3,5(10)-triene-3,17β-diol; estriol (E3), estra-1,3,5,(10)-triene-3,16α,17β-triol; estrone sulfate (E-SO$_4$), 17-oxoestra-1,3,5(10)-trien-3-yl sulfate; 2-methoxyestrone (2MeO-E1), 2-methoxy-3-hydroxyestra-1,3,5(10)-trien-17-one; testosterone, 17β-hydroxyandrost-4-en-3-one; androsterone, 3α-hydroxy-5α-androstan-17-one; androstenedione, androst-4-ene-3,17-dione; etiocholanolone, 3α-hydroxy-5β-androstan-17-one.

Abbreviation: TLC, thin-layer chromatgraphy.

[1] New England Nuclear Corp., Boston, Mass.

aortas analyzed averaged grade 0 in patient 4, grade 3 in patient 1, and grade 4 in patients 2 and 3.

After incubation, the aortas were rinsed with ice-cold saline and minced. The steroids in arterial segments incubated with labeled estradiol, estrone, estriol, or testosterone were extracted successively with ethanol, chloroform–methanol 2:1, and diethyl ether. In the tissues incubated with estrone-^{3}H sulfate, the steroids were extracted with 3 × 10 ml of 95% ethanol. The radioactivity in duplicate aliquots of the extracts was measured in a toluene-based scintillation solution by means of a liquid scintillation spectrometer.

Subcellular fractionation was performed on homogenates from aortic segments incubated with estradiol-^{3}H and testosterone-^{3}H as previously described (4). The ^{3}H radioactivity of the particulate fractions was determined as noted above for the total arterial segments. The radioactivity of 1 ml aliquots of the supernatant fraction was assayed in Bray's solution. The radioactivity of representative nuclear fractions was also measured after resuspension of the 800 *g* pellet in 2 ml of cold Ringer's solution and recentrifugation. Portions of the 105,000 *g* supernate were dialyzed for 18 hr at 4°C against 0.9 N saline via Visking dialysis tubing, and the steroid radioactivity of the residue was assayed.

In Vivo Studies

Adult female mongrel dogs (15–20 kg) were injected intravenously with 100 μc (0.6 μg) of estradiol-6,7-^{3}H and killed under pentobarbital anesthesia 1, 3, 6, or 24 hr later. Two animals were used for each time point. Specimens of blood, aorta, uterus, liver, kidney, adrenal gland, subcutaneous fat, and diaphragm were rapidly removed. The tissue steroids were extracted and the radioactivity was determined as described.

Female, immature 21- to 23-day old rats of the Sprague–Dawley strain (Charles River Breeding Laboratories, North Wilmington, Mass.) were injected intraperitoneally with 10 μc (0.06 μg) of 17β-estradiol-6,7-^{3}H (42.4 c/mmole) in 0.5 ml of a 1.0% ethanolic saline solution. The animals were killed by cervical dislocation at 15 min, 1 hr, 3 hr, or 6 hr and the organs were removed at autopsy. The aortic adventitia was stripped away and the arteries were rinsed with saline prior to analysis. The tissues were homogenized and extracted with ethanol, chloroform–methanol, and diethyl ether and the radioactivity was assayed. Combined aortas, uteri, or adrenal glands from groups of six rats and segments of liver from three animals were used in each set of analyses.

Sucrose Gradient Ultracentrifugation

The aortas and uteri from groups of 20 rats killed 1 hr after receiving 10 μc of estradiol-^{3}H (0.06 μg) were homogenized in the cold in Tris–EDTA buffer (0.01 M Tris, 0.0015 M EDTA, pH 7.3). The homogenate was centrifuged at 105,000 *g* for 1 hr to remove particulate material, and 0.2 ml of the supernatant fraction was layered on 4.4 ml linear gradients of 5–20% sucrose in Tris–EDTA buffer. The samples were centrifuged for 16 hr at 37,000 rpm in a Spinco model L ultracentrifuge equipped with a SW-39 rotor. After centrifugation, the tubes were pierced from the bottom and fractions were collected dropwise in 0.2 ml aliquots. The protein content of the samples was estimated by UV absorption at 280 mμ, and the radioactivity was determined in Bray's solution.

Chromatographic Analyses

Estrogens. Representative tissue extracts from the experiments with labeled estrogens were analyzed by TLC on Silica Gel H with benzene–ethyl acetate 1:1 (TLC system 1) and chloroform–acetone 95:5 (TLC system 2) as developing solvents (10). Unlabeled estrone sulfate (courtesy of Dr. Seymour Bernstein, Lederle Laboratories, Pearl River, N.J.), estrone,[2] estradiol,[2] and 2-methoxyestrone[2] (20 μg of each) were added as internal standards prior to development. The plates were stained with iodine vapor. The individual areas were isolated and their radioactivity was determined (8).

Estradiol and estrone fractions from TLC system 1 were also extracted from the silica gel with ethanol, chloroform–methanol, and diethyl ether and the steroids were acetylated with acetic anhydride–pyridine 5:1 (10). The acetylated products were chromatographed on Silica Gel H in petroleum ether–methanol 9:1 (TLC system 3) (10). The steroid acetates were eluted with 3 × 5 ml of acetone and the radioactivity was measured in aliquots of the extract. Carrier estradiol acetate or estrone acetate (100 mg) (both from Endo Laboratories, Inc., Garden City, N.Y.) was added to the remaining material and the acetate derivatives were recrystallized to constant specific activity from hexane–acetone. At least three crystallizations were carried out for each sample. The total radioactivity of the steroid derivatives was calculated from the specific activity and the weight of the carrier material.

Androgens. The extracts from tissue incubated with labeled testosterone were analyzed on Silica Gel H with hexane–ethyl acetate–ethanol 75:20:5 as developer (TLC system 4). Testosterone,[2] androsterone,[2] androstenedione,[2] and etiocholanolone[2] (20 μg of each) were added as internal standards prior to development. The individual areas containing the androgenic compounds were isolated, and the androgenic fractions extracted with 3 × 5 ml of methanol. The extracts were chromato-

[2] Steraloids Inc., Pawling, N.Y.

graphed individually on Silica Gel H in benzene–ethyl acetate–chloroform 60:20:20 (TLC system 5) (11). The steroids were eluted from the silica gel with methanol and the fractions containing testosterone, androstenedione, etiocholanolone, and androsterone were evaporated to dryness. Carrier androstenedione, androsterone, or etiocholanolone (100 mg) was added to the appropriate tube and the material was recrystallized to constant specific activity from hexane–acetone.

The purity of labeled and unlabeled steroids was checked before use by the TLC systems described above. The radiochromatograms were scanned with a Tracerlab 4 π Scanner. The unlabeled steroids on the TLC plates were stained with iodine vapor and their purity was checked by visual inspection. All labeled steroids utilized in the experiments appeared to be more than 99% pure.

Recovery experiments were performed by addition of known quantities of estrone-^{3}H, estradiol-^{3}H, estrone-^{3}H sulfate, and testosterone-^{3}H to untreated segments of tissues. The tissues were treated as described above and the recovery of the labeled hormones was determined. The experimental results were corrected to 100% recovery.

RESULTS

In Vitro Uptake of Estradiol-^{3}H and Testosterone-^{3}H

Arterial radioactivity in tissues incubated with estradiol-^{3}H reached a maximum level within 60 min of incubation in both human and canine vessels. Addition of either unlabeled estradiol or testosterone did not influence estradiol-^{3}H uptake (Table 1). In one canine and two human aortas, 73–77% of arterial radioactivity was recovered in the soluble fraction, 16–23% in the nuclear pellet, and less than 5% in the mitochondrial or microsomal fractions. The percentages of total estradiol-^{3}H radioactivity in the nuclear and supernatant fractions were relatively constant at concentrations of estradiol-^{3}H varying from 0.025 to 2.5 μc (0.00015 to 0.015 μg) per ml. Less than 1% of the total radioactivity in the soluble fraction remained after dialysis, and less than 5% of radioactivity in the nuclear pellet persisted after the pellet was washed with cold Ringer's solution.

Peak testosterone radioactivity was attained within 60 min in a pattern similar to that observed with estradiol-^{3}H. No antagonism in arterial uptake between estradiol and testosterone was apparent (Table 1). On subcellular fractionation of the aorta from patient 1, 71% of the total radioactivity was recovered in the soluble fraction, 19% in the nuclear debris, 4% in the mitochondrial pellet, and 6% in the microsomal fraction.

In Vivo Uptake and Disappearance of Estradiol-^{3}H

Canine Tissues. The concentration of radioactivity in the aorta 1–6 hr after estradiol-^{3}H administration was greater than that in the plasma but considerably less than that in the adrenal gland or uterus (Table 2). The rate of decrease of steroid radioactivity from the arterial wall was generally similar to that observed from plasma, diaphragm, fat, kidney, and liver but greater than that from uterus. The initial uptake of estradiol by the adrenal gland appeared to exceed that by any other tissue, including the uterus. However, the retention of the hormone by the adrenal gland was much less than that by the uterus. After 24 hr, ^{3}H radioactivity was measurable only in the uterus.

Estradiol-^{3}H represented an average of 88% of total aortic radioactivity at 1 hr, 83% at 3 hr, and 80% at 6 hr

TABLE 1 IN VITRO UPTAKE OF ESTRADIOL-^{3}H* AND TESTOSTERONE-^{3}H† BY HUMAN AND CANINE AORTA

Tissue Origin	Control	Estradiol (10^{-6} M)	Testosterone (10^{-6} M)
	dpm/g wet weight × 10^{-3}‡		
Estradiol uptake			
Patient 1	2010	2160	2000
Dog 1	2570	2440	2490
Dog 2	1890	1970	1960
Testosterone uptake			
Patient 1	6630	6230	6470
Dog 1	5470	5300	5700

* Concentration of estradiol-^{3}H, 0.0015 μg/ml (specific activity 42.4 c/mmole).
† Concentration of testosterone-^{3}H, 0.0015 μg/ml (42.4 c/mmole).
‡ After 60 min incubation.

TABLE 2 TISSUE ^{3}H RADIOACTIVITY IN THE DOG AFTER ADMINISTRATION OF 100 μc (0.6 μg) OF ESTRADIOL-^{3}H

Time*	Aorta	Uterus	Adrenal	Liver	Kidney	Fat	Diaphragm	Plasma†
hr				*dpm/g dry weight*				
1	6090	30,500	126,000	12,800	7320	10,700	2720	1170
3	4320	18,400	53,400	8780	1320	3990	650	292
6	2890	18,100	17,100	3060	3680	3360	528	296
24	0	17,600	0	0	0	0	0	0

Values represent means for two dogs in each group.
* Interval between estradiol-^{3}H injection and death.
† Specific gravity of plasma assumed to be 1.056.

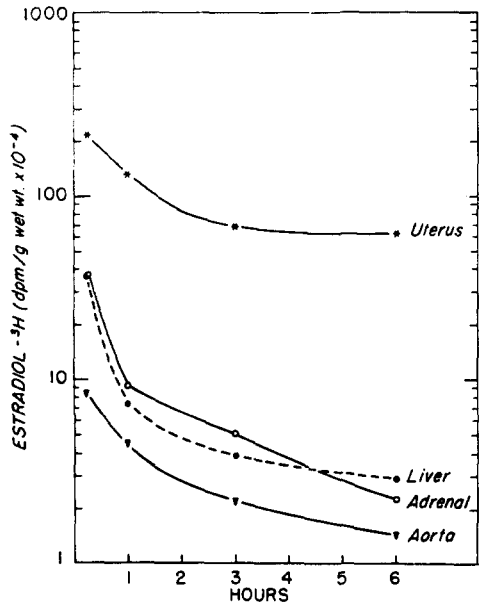

FIG. 1. Tissue estradiol radioactivity in the rat after administration of 0.06 μg of estradiol-³H.

after estradiol-³H administration; most of the remainder was present as estrone-³H (Table 3). Estradiol-³H also accounted for an average of 41–66% of total plasma radioactivity, 38–74% of adrenal radioactivity, and 94–98% of uterine radioactivity.

Rat Tissues. The aortic uptake of labeled estradiol 15 min after its intraperitoneal injection was less than that in the uterus, adrenal gland, or liver (Fig. 1). The disappearance of steroid radioactivity from the aorta as well as from the liver and adrenal gland was more rapid than that from the uterus.

Sucrose Gradient Ultracentrifugation

In uterine homogenates, the radioactivity became separated from the major portion of protein and sedimented further than the protein (Fig. 2). With the aorta, the bulk of radioactivity and protein were also separated from one another. However, in contrast to the uterus, most of the arterial radioactivity remained at the top of the gradient tube in a distribution pattern similar to that observed with free estradiol-³H.

TABLE 3 ESTRONE AND ESTRADIOL RADIOACTIVITY IN THE DOG AFTER ESTRADIOL-³H ADMINISTRATION

	Aorta		Plasma		Uterus		Adrenal	
Time	E1	E2	E1	E2	E1	E2	E1	E2
hr	*% total radioactivity*							
1	9	88	31	66	0	98	24	63
3	13	83	33	47	0	98	23	74
6	16	80	52	41	3	94	35	38

E1, estrone; E2, estradiol. Values represent means for two dogs in each group.

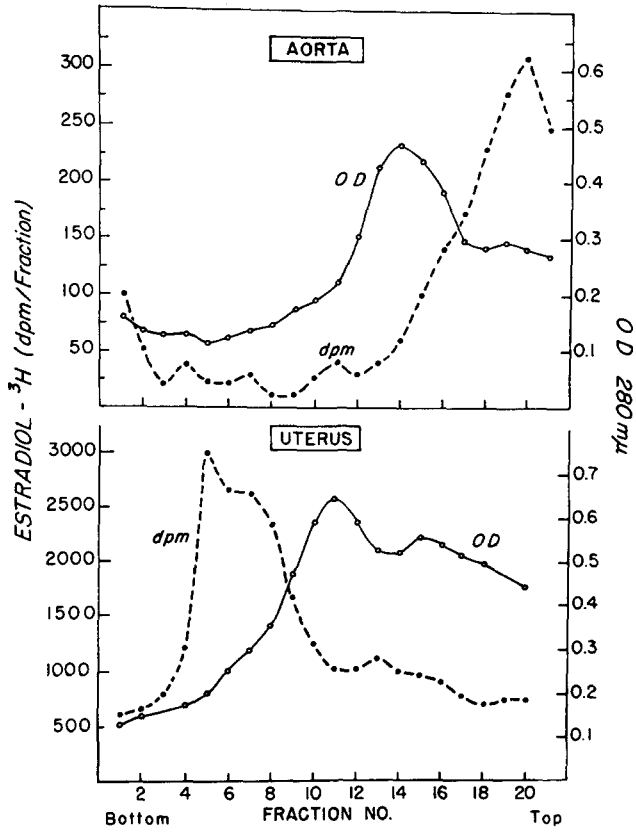

FIG. 2. Density gradient patterns of soluble fractions from aorta and uterus of 20 rats treated with 0.06 μg of estradiol-³H. Sedimentation is from right to left.

Metabolic Conversion of Estrogens and Testosterone by the Aortic Wall

Estrone-³H. After incubations with estrone³H (Table 4), 2.1–3.7% of the total radioactitivity at 1 hr and 3.4–6.0% at 2 hr was isolated as estradiol. Less than 1% of total radioactivity was recovered in either the estriol or 2-methoxyestrone fractions, and because of the paucity of counts, no attempt was made to purify these fractions by recrystallization procedures. In the heat-inactivated tissues, more than 99% of total radioactivity was recovered unchanged as estrone-³H.

Estradiol-³H. In the arteries incubated with estradiol-³H, after 1 hr, 1.9–3.6% of total tissue radioactivity was present as estrone-³H. At 2 hr, 4.3–5.9% of total radioactivity was recovered with estrone. Less than 1% of total radioactivity was isolated with estriol or 2-methoxyestrone fractions. In the heat-inactivated tissues, more than 99% of total radioactivity was present as estradiol-³H.

Estriol-³H. Less than 1% of total ³H radioactivity was present in any estrogenic fraction other than estriol at both 1 and 2 hr.

Estrone-³H Sulfate. After 1 hr of incubation with estrone-³H sulfate, 22–63% of total extractable ³H

TABLE 4 In Vitro Conversion of Estrone-^{3}H, Estradiol-^{3}H, Estriol-^{3}H, and Estrone-^{3}H Sulfate by Aortic Intima

Tissue Origin	Estrone		Estradiol		Estriol			Estrone Sulfate		
	E1	E2	E1	E2	E1	E2	E3	E1	E2	E-SO_4
					*% total radioactivity**					
Patient 2			1.9	97.0				63.0	3.2	32.2
Patient 3	95.0	2.1	2.6	95.2	0	0.5	96.6	50.5	1.9	43.9
Patient 4	93.9	2.1	3.2	94.6						
Dog 3	93.6	3.7	3.6	93.3	0.6	0.9	97.0	56.2	5.3	38.4
Dog 4			3.1	95.0				22.2	1.8	72.3

E1, estrone; E2, estradiol; E3, estriol; E-SO_4, estrone sulfate.
* After 60 min incubation.

radioactivity was isolated with estrone and 2–3% with estradiol. At 2 hr, 41–80% of the radioactivity was recovered with estrone and 3–5% with estradiol. In the heat-inactivated tissues, no conversion of estrone-^{3}H sulfate was apparent.

Testosterone-^{3}H. 1.0–3.9% of arterial radioactivity, as determined by carrier dilution experiments, was present as androstenedione-^{3}H (Table 5). Up to 1.2% of arterial radioactivity was recovered as androsterone. Labeled etiocholanolone was isolated from three of the five aortas but it represented less than 1% of total radioactivity. 2.3–4.0% of total radioactivity was recovered in an unidentified fraction which had an R_f value in both TLC systems 4 and 5 higher than that of the other derivatives. In the heat-inactivated tissues, more than 99% of total arterial radioactivity was isolated as testosterone-^{3}H.

TABLE 5 In Vitro Conversion of Testosterone-^{3}H by Aortic Intima

Tissue	Testosterone	Androstenedione	Androsterone	Etiocholanolone	"Nonpolar"
			*% total radioactivity**		
Patient 1	87.2	2.2	1.0	0.9	4.0
Patient 2	93.3	1.0	0.1	0.4	2.3
Patient 3	93.1	2.1	0.6	0.8	2.9
Dog 3	88.6	3.6	1.2	0	2.7
Dog 5	89.5	3.9	1.1	0	3.6

* After 60 min incubation.

DISCUSSION

These studies demonstrate for the first time the chemical conversion of sex hormones by the arterial wall. The interconversion of estradiol and estrone by the blood vessel is similar to that reported for other tissues (12, 13) and suggests the presence of a 17β-dehydrogenase enzyme in the artery. The metabolism of estrone sulfate to estrone and estradiol also indicates activity of an arterial sulfatase. The importance of the arterial metabolism of estrogens in determining the arterial content of the hormones is unclear. Since plasma steroids can rapidly enter the arterial wall, the steroid content of plasma presumably has an important influence on arterial steroid composition. However, an important role of arterial steroid metabolism in determining this composition is not excluded, and it is of interest that the relative arterial concentrations of estradiol-^{3}H and its metabolites after estradiol-^{3}H administration differed from those in the plasma. The nature of the steroid in the intima may be critical in determining the vascular effects of a given hormone, since differences may exist between individual estrogens with respect to their influence on arterial wall metabolism (4) and on the atherosclerotic process (14, 15).

The rapid rate of entrance of estradiol-^{3}H into the intima is similar to that previously observed with aldosterone (16). In contrast to this rapid uptake of steroids, cholesterol and other sterols seem to equilibrate rather slowly between plasma and artery (9, 17, 18).

The arterial uptake of estradiol in the dog was generally of the same order of magnitude as that observed in the human tissues other than uterus and adrenal gland. The unusual capacity of the uterus to concentrate estradiol is apparently due to specific nuclear and cytoplasmic "receptors" with marked affinity for the hormone (19, 20). Similar specific binding substances for estradiol could not be demonstrated in the aortic wall. In contrast to the results with uterus, most of the arterial estradiol radioactivity was recovered from the 105,000 *g* supernate rather than the nuclear fraction; unlabeled estradiol did not compete with the labeled hormone for arterial uptake in vitro; and the labeled estradiol was easily liberated from both the nuclear material and the soluble fraction. In addition, on sucrose density gradient ultracentrifugation of the 105,000 *g* supernatant material, the migration of aortic estradiol resembled that of the free hormone rather than that of uterine estradiol, which binds to a macromolecule with a sedimentation coefficient of about 9.5 S (19). A nonspecific form of binding is not excluded since in both the in vitro and in vivo experiments, the aortic concentration of estradiol-^{3}H was

greater than that of the perfusing solution. However, such binding forces, if present, seem to be relatively weak. The present results also suggest that, at least in the aorta, specific binding of estradiol may not be critical for its metabolic action.

The major arterial metabolites of testosterone appeared to be androstenedione and an unidentified product which by TLC analyses appeared to be less polar than testosterone. Oxidation of testosterone has previously been demonstrated in other tissues, and androstenedione has also been identified as the major product of testosterone metabolism in various human tissues (21–23).

The unusual ability of the dog adrenal gland to concentrate estradiol was unexpected. Similar ability was not observed in the adrenal glands of sexually immature rats in either the present or previous investigations (24). The metabolic significance of this observation is unknown, although recent studies in man suggest that estrogens may be involved in the regulation of adrenal function (25). The rapid rate of disappearance of estradiol from the adrenal gland and the high concentration of estradiol metabolites recovered there may indicate an active adrenal metabolism of estrogens, but further studies are needed to confirm this hypothesis.

The complete pathway of arterial estrogen metabolism could not be ascertained from these experiments since the study was designed to examine only the major products of the hormones. However, the absence of labeled 2-methoxyestrone or estriol after incubations with estradiol-^{3}H or estrone-^{3}H suggests that, under these conditions, the 2-oxygenation or 16-hydroxylation pathways that have previously been described (26) are not operative in the isolated arterial intima.

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