Estrogen Receptor Binding Radiopharmaceuticals: II. Tissue Distribution of 17α-Methylestradiol in Normal and Tumor-Bearing Rats

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Tritiated 17α-methylestradiol was synthesized to investigate the potential of the carbon-11-labeled analog as an estrogen-receptor-binding radiopharmaceutical. In vitro, 17α-methylestradiol is bound with high affinity to the cytoplasmic estrogen receptor from rabbit uterus ($K_d = 1.96 \times 10^{-10} M$), and it sediments as an 8S hormone-receptor complex in sucrose gradients. The compound shows specific uptake in the uterus of the adult rat, within 1 hr after injection. After 30 min the uterine uptake was 1.73% dose/g. In female rats bearing DMBA-induced tumors, specific uterine and tumor uptakes were observed, although at 30 min the tumor uptake was only 23–30% of the uptake in the uterus. Tritiated 17α-methylestradiol with a specific activity of 6 Ci/mmole showed a similar tissue distribution. Our results indicate that 17α-methylestradiol is promising as an estrogen-receptor-binding radiopharmaceutical.

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The estrogen-receptor content of a breast tumor is an important parameter in the choice of appropriate therapy (*1–3*). Patients with receptor-poor tumors are not likely to respond to endocrine therapy, whereas those with receptor-rich tumors have a better prognosis (*4*) and may respond to endocrine therapy (*5*). The availability of estrogen-receptor-binding radiopharmaceuticals for detecting and investigating these tumors in vivo should be of great importance. Because of the potential of carbon-11 (β^+; $T_{1/2}$ = 20.4 min) for the labeling of complex organic compounds, we previously suggested this radionuclide as a label for estrogen-receptor-binding radiopharmaceuticals (*6*). At present the only two radioactive precursors used for labeling steroids with carbon-11 are acetylene and methyl lithium. We developed the synthesis of C-11-labeled 17α-ethynylestradiol and moxestrol (*6,7*), using C-11 acetylene as precursor. We also investigated the potential of these compounds for in vivo location of tumors containing estrogen receptors (*8*). With C-11-labeled methyl lithium, a highly reactive methyl-donating agent, we have prepared labeled 17α-methylestradiol (*9*). To investigate the utility of this C-11 compound as an estrogen-receptor-binding radiopharmaceutical, we prepared tritiated 17α-methylestradiol (sp. act. 57 Ci/mmole). We also studied its interaction with the estrogen receptor in vitro and its tissue distribution in vivo—in mature female rats as well as in rats bearing DMBA-induced tumors. Because of the short half-life of C-11, we were interested mainly in the tissue distribution within 1 hr after injection. We also measured the tissue distribution of tritiated 17α-methylestradiol with a specific activity of 6 Ci/mmole, which is at present the achievable specific activity of the carbon-11-labeled steroids.

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MATERIALS AND METHODS

The following commercially available materials were used, obtained from the indicated sources: [6,7-

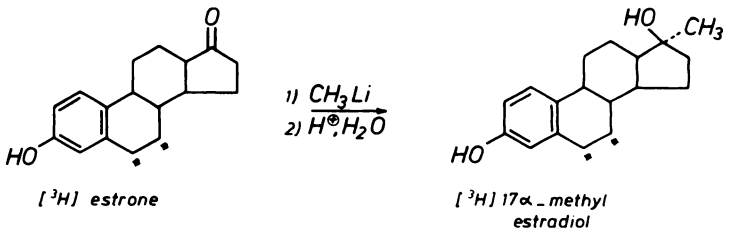

FIG. 1. Synthesis of tritiated 17α-methylestradiol by addition of CH_3Li to tritiated estrone.

^3H(N)]estradiol (51 Ci/mmole), [6,7-^3H(N)]-estrone (57 Ci/mmole), 17β-estradiol, estrone, potassium phosphate, sodium azide, gelatine, 35% aqueous formaldehyde, methyl lithium, charcoal (activated), sucrose (analytical grade), ethylene-diaminetetra-acetic acid, dipotassium salt (EDTA), dextran T-10, solubilizer, Picofluor, Plasmasol, 7,12-dimethylbenz(a)anthracene (DMBA), and hydroxylapatite. (^3H)Estradiol was purified as described earlier (*8*).

Synthesis of 17α-methyl [6,7-^3H]estradiol. Tritiated 17α-methylestradiol was prepared by a method already reported for the C-11 analog (*9*); the synthesis is outlined in Fig. 1. One mCi [6,7-$^3H_2$(N)]estrone (sp. act. 57 Ci/mmole) in 1 ml benzene/ethanol was evaporated to dryness. Traces of benzene and ethanol were removed by dissolving the residue in 3 ml of dry tetrahydrofuran (THF) and subsequent evaporation of the THF under reduced pressure. This was repeated twice. The [^3H]-estrone was redissolved in 3 ml of THF and the vessel evacuated. After addition of 0.3 ml of a 5% methyllithium solution in ether, the mixture was stirred for 60 min at 0°C, then quenched with 20 ml of a 20% ammonium chloride solution. The steroid mixture was extracted into chloroform and, after separation from the water layer, the organic layer was dried over $MgSO_4$, filtered, and the solvent removed by vacuum distillation. The residue was dissolved in 1 ml of 10% ethyl acetate in iso-octane and applied to a column of chromosorb/ethanediol (2:1, 10 × 1 cm). The column was eluted with 10% ethylacetate in iso-octane. The radiochemical yield was 54%. The radiochemical purity was checked on TLC (silica gel) with chloroform/hexane/methanol (40:10:1) and benzene/methanol (10:1) as solvent systems, and was found to be better than 99%.

Animals. For in vivo studies we used normal female Wistar rats (12–16 wk, 200 g). To determine the stage of the estrous cycle, a daily vaginal smear was made. Only rats that showed at least two consecutive cycles were used.

Mammary tumors were induced with DMBA in Sprague-Dawley rats as described earlier (*8*). For in vivo studies, only animals bearing tumors with a diameter of 2–3 cm were used, irrespective of the stage of the estrous cycle.

The Wistar and Sprague-Dawley rats were housed in groups of five, maintained in air-conditioned surroundings under controlled lighting conditions, and given standard laboratory food and water ad libitum.

For in vitro experiments, uteri of 6-day-pregnant rabbits were used. The animals were killed and the uteri were immediately removed and stored in liquid nitrogen.

Preparation of cytosol. For in vitro studies, rabbit-uterus cytosol was prepared by pulverizing the uteri, frozen at liquid-nitrogen temperature, to a fine powder in a microdismembrator (*10*). The uterine powder was homogenized in two volumes of buffer A (10 m*M* potassium phosphate, 1.5 m*M* potassium EDTA, 1.5 m*M* sodium azide, pH 7.5). The homogenate was centrifuged at 4100*g* for 30 min at 4°C. The cytosol fraction (supernatant) was pipetted off and, if necessary, was filtered to separate it from lipid contaminants. The cytosol was stored in liquid nitrogen if not used immediately. No differences were noticed between the fresh and frozen cytosols.

Scatchard analysis. A volume of 50 μl cytosol or buffer A was added to 595 μl buffer B (10 m*M* potassium phosphate, 1.5 m*M* potassium EDTA, 1.5 m*M* sodium azide, 250 m*M* sucrose, pH 7.5) and incubated for 18 hr at 0°C in one of six different concentrations of tritiated steroids, ranging from $1.5 \times 10^{-9}M$ to $0.125 \times 10^{-9}M$. The incubations were carried out in triplicate. The steroids were added as 5 μl of the appropriate ethanol stock solution. At each concentration the nonspecific binding was determined by adding $10^{-6}M$ nonradioactive estradiol to the incubation mixture. After incubation, bound steroid was separated from free steroid with hydroxylapatite (*11*). The hydroxylapatite was washed and resuspended in an equal volume of buffer A. After incubation, 0.5 ml of the hydroxylapatite slurry was added and incubated at 0°C for 30 min, with the mixture shaken vigorously several times. Next, 2 ml of buffer A were added and the mixture was centrifuged at 1000*g* for 5 min. After two washings with 2 ml of buffer A, the radioactivity in the pellet was extracted with 2 ml of ethanol at room temperature during 30 min, then counted with an efficiency of 40% in 10 ml of a Picofluor liquid-scintillation mixture. Binding data were analyzed according to Scatchard (*12*). The protein content of the cytosol was determined by the method of Lowry using bovine serum albumin as standard (*13*).

Competitive-binding measurements. For competition studies, 50 μl cytosol and 595 μl buffer B were incubated with $5 \times 10^{-9}M$ (^3H)estradiol (51 Ci/mmole) together

with various amounts of nonradioactive steroids, ranging from $10^{-11}M$ to $10^{-6}M$. Steroids were added as 5 μl of the appropriate ethanol stock solution. After incubation, 0.5 ml of a dextran-coated charcoal suspension [0.5% charcoal (w/v), 0.05% dextran T-10, 0.1% gelatin in buffer B] was added and shaken vigorously during 10 min at 0°C. The mixture was centrifuged at 3000g for 15 min at 0°C, and 500 μl of the supernatant was counted in 3 ml of Picofluor. The concentration of competitor that reduced the binding of tritiated estradiol to 50% was measured. The relative binding affinity (RBA) was calculated by taking as unity the estradiol concentration at 50% binding of tritiated estradiol.

Sucrose gradients. Linear 5—20% sucrose gradients (4 ml) were prepared in TD buffer (40 mM Tris, 1 mM DTT, pH 7.4). Cytosol from DMBA-induced mammary tumors was prepared as described for uterine cytosol (*8*) and 300 μl cytosol was incubated with $3 \times 10^{-9}M$ tritiated 17α-methylestradiol or $3 \times 10^{-9}M$ tritiated estradiol for 2 hr at 0°C. Nonspecific binding was determined by incubating an aliquot with $3 \times 10^{-9}M$ tritiated steroid and $3 \times 10^{-6}M$ nonradioactive estradiol. Before the cytosol was layered on the gradients, excess free steroid was removed by dextran-coated charcoal (0.5%, 10 min, 0°C). The gradients were centrifuged at 257,000 g_{av} for 18 hr at 1°C. Alkaline phosphatase (6.2S; EC 3.1.3.1) was used as internal marker in each gradient.

In vivo tissue distribution studies. These were performed as described in the previous paper (*8*). Briefly:

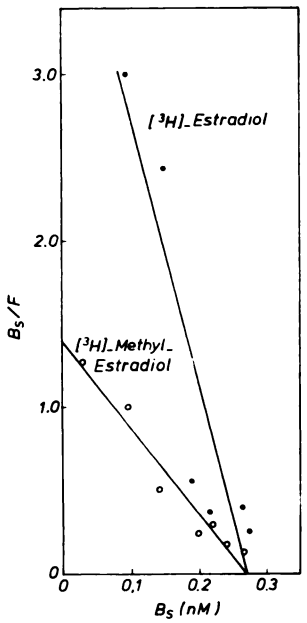

FIG. 2. Scatchard analysis of binding of tritiated estradiol and 17α-methylestradiol to estrogen receptor in rabbit-uterus cytosol. Cytosol was incubated for 18 hr at 0–4°C with various concentrations of tritiated estrogen (1.5 to 0.125 μM) in presence and absence of $10^{-6}M$ estradiol. Receptor-bound estrogen was separated from free estrogen by adsorption to hydroxylapatite.

Wistar rats and Sprague-Dawley rats bearing DMBA-induced mammary tumors were injected by tail vein with tritiated 17α-methylestradiol with a specific activity of 57 Ci/mmole or 6 Ci/mmole. The dose per animal was 5 μCi for the Wistar rats or, because of their greater weight, 7.5 μCi for the Sprague-Dawley rats. In control experiments the rats received a 100-fold excess of nonradioactive estradiol (2.7 and 3.6 μg respectively) just before injection of tritiated steroid. At the indicated times the animals were decapitated, blood was collected in tubes containing a few drops of heparin (5000 IU/ml), and the whole organs and tissues were excised rapidly. Samples of 0.05 to 0.15 g were digested at 50°C overnight in approximately 1 ml of tissue solubilizer. After cooling to room temperature, the colored samples were bleached with 0.1–0.5 ml of commercial bleach and neutralized with an equal volume of 35% aqueous formaldehyde. To 0.5 ml of blood an equal volume of water was added with gentle shaking, followed by 0.5 ml of bleach. After decolorizing, 0.5 ml of 35% formaldehyde was added. All samples were counted in 10–15 ml of Plasmasol, with an efficiency of 20–30%.

RESULTS

In vitro binding studies. An essential requirement for an estrogen-receptor-binding radiopharmaceutical is its binding to the estrogen receptor. Accordingly we investigated the binding of 17α-methylestradiol to the estrogen receptor in rabbit uterine cytosol, which is bound with high affinity. In a competitive binding assay, its binding affinity relative to estradiol (RBA) was essentially 100%. Figure 2 shows representative Scatchard plots for tritiated 17α-methylestradiol and estradiol. 17α-Methylestradiol is bound to the estrogen receptor with only slightly lower affinity ($K_d = 1.96 \times 10^{-10}M$) than estradiol ($K_d = 0.6 \times 10^{-10}M$).

The binding of 17α-methylestradiol to the estrogen receptor in tumor cytosol was analyzed by sucrose-gradient centrifugation. Figure 3 shows the sedimentation patterns of tritiated estradiol and 17α-methylestradiol bound to the estrogen receptor in DMBA-tumor cytosol under low-salt conditions. For both steroids a large 8-9S and a smaller 4S peak are observed. The peaks are displaceable by an excess of nonradioactive estradiol. The sedimentation pattern of tritiated 17α-methylestradiol shows a greater 4S peak than does that of tritiated estradiol. 17α-Methylestradiol also showed more nonspecific 4S binding than estradiol.

In vivo distribution studies in mature female rats. Another requirement for an estrogen-receptor-binding radiopharmaceutical is its localization in tissues containing estrogen receptors. The in vivo distribution of 17α-methylestradiol was investigated in normal female rats at metoestrus, the stage of the estrous cycle in which the estrogen level is low (*14*). Table 1 shows the tissue dis-

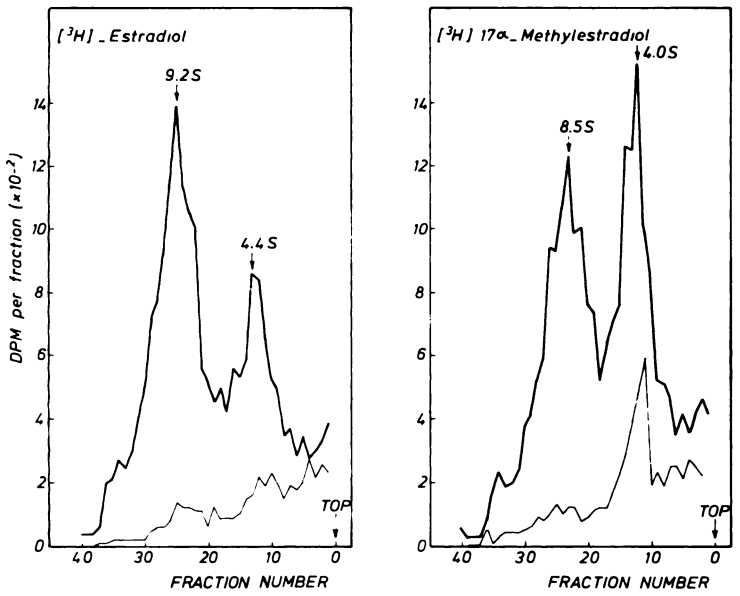

FIG. 3. Low-salt sucrose-gradient analysis of binding of tritiated estradiol and 17α-methyl-estradiol in cytosol from mammary tumors of rat. Gradients with tritium-labeled estrogens are shown as heavy lines, and represent total binding. Light lines show gradients from samples incubated in presence of 100-fold excess of nonradioactive estradiol to determine nonspecific binding.

TABLE 1. TISSUE DISTRIBUTION OF TRITIATED 17α-METHYLESTRADIOL WITH A SPECIFIC ACTIVITY OF 57 CI/MMOLE IN FEMALE RATS*

	% Dose/g†			
Organ	15 min	30 min	45 min	Control‡ 30 min
Blood	0.12 (0.10–0.15)	0.08 (0.06–0.08)	0.05 (0.04–0.06)	0.07 (0.06–0.08)
Uterus	1.57 (1.16–1.71)	1.73 (1.19–2.34)	1.62 (1.30–1.89)	0.29 (0.25–0.32)
Ovary	1.94 (1.52–2.41)	1.22 (0.84–1.62)	0.94 (0.79–1.18)	0.59 (0.51–0.65)
Spleen	0.46 (0.44–0.49)	0.28 (0.21–0.36)	0.19 (0.14–0.22)	0.17 (0.15–0.20)
Kidney	0.80 (0.72–0.88)	0.51 (0.43–0.69)	0.37 0.26–0.56)	0.32 (0.26–0.40)
Adrenals	3.22 (1.87–4.24)	1.36 (1.32–1.41)	1.06 (0.92–1.17)	0.99 (0.83–1.11)
Liver	3.50 (2.76–4.62)	2.11 (1.32–2.66)	1.31 (1.03–1.68)	1.54 (1.21–1.68)
Heart	0.43 (0.35–0.58)	0.36 (0.26–0.47)	0.12 (0.10–0.16)	0.14 (0.12–0.15)
Lung	0.60 (0.58–0.62)	0.42 (0.30–0.56)	0.24 (0.15–0.34)	0.24 (0.18–0.29)
Fat	0.29 (0.20–0.35)	0.46 (0.41–0.71)	0.39 (0.31–0.54)	0.48 (0.39–0.57)
Muscle	0.20 (0.18–0.23)	0.14 (0.09–0.18)	0.16 (0.08–0.35)	0.08 (0.08–0.09)
Small intestine	11.72 (5.60–25.12)	5.81 (2.30–10.63)	2.67 (1.48–4.02)	4.18 (0.90–10.9)

* Wistar rats, age 12–16 wk, weight 200 g. Rats were at the metoestrus phase of their cycle. Mean of 4 rats; range in parentheses.

† Dose: 5 μCi (27 ng).

‡ Rats received a 100-fold dose (2.7 μg) of estradiol before injection of 5 μCi (^{3}H) 17α-methylestradiol with a specific activity of 57 Ci/mmole.

tribution at 15–45 min after i.v. injection of tritiated 17α-methylestradiol. The data are expressed as percentage of the injected dose per gram of wet weight (% dose/g); they show a pronounced uptake of 17α-methylestradiol in such estrogen target tissues as the uterus, vagina, and ovaries. Moreover, organs involved in steroid elimination and metabolism, such as liver, kidneys, and small intestine, showed high uptake percentages. However, only in the uterus and vagina is the 17α-methylestradiol retained at the early level. The decrease of radioactivity in the small intestine should be seen as a reduction in the concentration of metabolized tritiated 17α-methylestradiol in the first part of the intestine due to the mobility of the intestine contents.

It is essential to know whether the observed uptake of tritiated 17α-methylestradiol is due to the interaction with estrogen receptors present in the target organs or to other steroid-protein interactions. Therefore in a control experiment the animals were injected with a 100-fold dose of nonradioactive estradiol, to saturate the estrogen receptors present, just before injection of tritiated 17α-methylestradiol.

TABLE 2. TISSUE DISTRIBUTION OF TRITIATED 17α-METHYLESTRADIOL WITH A SPECIFIC ACTIVITY OF 6 CI/MMOLE IN FEMALE RATS*

Organ	% Dose/g†		
	15 min	30 min	45 min
Blood	0.10	0.07	0.05
	(0.09–0.12)	(0.06–0.09)	(0.04–0.05)
Uterus	1.10	1.19	1.05
	(0.94–0.20)	(0.92–1.62)	(0.92–1.20)
Ovary	1.63	1.01	0.65
	(1.28–2.23)	(0.87–1.18)	(0.57–0.72)
Spleen	0.30	0.18	0.22
	(0.23–0.47)	(0.15–0.20)	(0.12–0.26)
Kidney	0.59	0.40	0.30
	(0.48–0.68)	(0.31–0.45)	(0.25–0.34)
Adrenals	2.31	1.24	0.71
	(1.70–2.55)	(0.90–1.48)	(0.62–0.84)
Liver	2.97	1.74	1.12
	(2.44–3.64)	(1.45–2.04)	(1.01–1.27)
Heart	0.34	0.22	0.20
	(0.28–0.39)	(0.15–0.33)	(0.13–0.29)
Lung	0.44	0.34	0.22
	(0.39–0.49)	(0.25–0.47)	(0.18–0.24)
Fat	0.29	0.40	0.32
	(0.23–0.35)	(0.28–0.56)	(0.25–0.39)
Muscle	0.18	0.11	0.07
	(0.15–0.22)	(0.09–0.12)	(0.06–0.08)
Small intestine	6.20	2.61	3.58
	(0.28–13.43)	(2.13–5.56)	(1.44–8.59)

* Wistar rats, age 12–16 wk, weight 200 g. Rats were at the metoestrus phase of their cycle. Mean of 4 rats; range in parentheses.

† Dose: 5 μCi (260 ng).

TABLE 3. UTERUS-TO-BLOOD RATIOS OF TRITIATED 17α-METHYLESTRADIOL IN FEMALE RATS*

Time	Uterus-to-blood†	
	6 Ci/mmole	57 Ci/mmole
15 min	10.8	13.6
	(9.2–12.1)	(9.5–16.8)
30 min	17.6	23.0
	(10.4–22.3)	(18.8–30.7)
45 min	22.7	34.5
	(18.9–24.9)	(29.1–42.1)

* Rats were at the metoestrus phase of their cycle. Mean of 4 rats; range in parentheses. Ratios are calculated from data shown in Tables 1 and 2.

† Dose: 5 μCi.

As shown in Table 1, preinjection with estradiol markedly reduced the uptake of 17α-methylestradiol in the uterus, in contrast to the uptake in nontarget tissues. The ovarian uptake is also decreased; the uptake in the adrenals is not influenced by preinjection of estradiol (1.06 and 0.99% dose/g at 45 min respectively). These experiments showed that 17α-methylestradiol localizes in vivo in an estrogen-specific way, i.e., by interaction with estrogen receptors in estrogen-target organs.

The specific activity of labeled 17α-methylestradiol obtainable with C-11 is dependent on the carbon-11 production facility and on the radiochemical synthesis, e.g., on the total synthesis time, on the CO_2 content of the $LiAlH_4$ solution, or on the maximum amount of $^{11}CO_2$ that can be produced. We achieved about 6 Ci/mmole for the final product (*15*). In order to investigate the utility of carbon-11-labeled 17α-methylestradiol, the distribution study was repeated with tritiated 17α-methylestradiol of the 6 Ci/mmole specific activity. Mature cycling rats at the metoestrus phase of their cycle were used, injected with a dose of 5 μCi. In Table 2 the results of these experiments are shown. Again a pronounced uptake and retention is seen in the uterus and vagina. Compared with the uptake of 17α-methylestradiol with a specific activity of 57 Ci/mmole, the uptake of radioactivity is decreased (Table 1). However, this decrease is less than the tenfold reduction expected from the tenfold decrease in specific activity. This indicates the nonsaturation of the receptors under these conditions. The uterus-to-blood ratios of tritiated 17α-methylestradiol are shown in Table 3.

In vivo studies in rats bearing DMBA-induced mammary tumors. Most of these tumors are hormone-dependent and contain estrogen receptors, although at a lower concentration than in the uterus (*16*). We mea-

TABLE 4. UPTAKE OF TRITIATED 17α-METHYLESTRADIOL IN RATS BEARING DMBA-INDUCED TUMORS*

	% Dose/g† at 30 min		
	6 Ci/mmole†	57 Ci/mmole†	Control‡
Blood	0.06	0.06	0.07
	(0.05–0.07)	(0.05–0.07)	(0.06–0.08)
Uterus	1.01	1.71	0.20
	(0.89–1.40)	(0.74–2.34)	(0.17–0.24)
Liver	1.64	1.93	1.78
	(1.33–1.93)	(1.84–2.15)	(1.68–1.93)
Tumors	0.34	0.39	0.19
	(0.20–0.42)	(0.18–0.52)	(0.16–0.23)
Fat	0.26	0.23	0.24
	(0.18–0.35)	(0.13–0.34)	(0.10–0.23)
Muscle	0.11	0.09	0.10
	(0.08–0.14)	(0.07–0.11)	(0.08–0.13)

* Sprague-Dawley rats, age 12–16 wk, weight 250 g, were used at random stages of their cycle.

† Dose = 7.5 μCi; mean (and range) for at least 28 tumors in 4 animals.

‡ Rats received a 100-fold dose (3.6 μg) of estradiol before injection of 7.5 μCi (^{5}H)17α-methylestradiol with a specific activity of 57 Ci/nmole. Each point represents the mean (and range) of 11 tumors in 3 animals.

sured the tissue distribution of tritiated 17α-methylestradiol at two specific activities. The results are summarized in Table 4. In general the distribution of 17α-methylestradiol is similar to that obtained in rats without tumors. Again we observe a high uptake in the uterus, the estrogen target. The tumor uptake of 17α-methylestradiol is considerably less than the uptake in the uterus (23–30%) over a wide range of specific activities.

Reduction of the specific activity of tritiated methylestradiol did not result in a decrease of the uptake of radioactivity in the uterus. As already mentioned, this implies that the dose used in the high-specific-activity preparation did not saturate the estrogen receptors in the uterus.

The specificity of the tissue distribution of 17α-methylestradiol was investigated by injection of a 100-fold excess of nonradioactive estradiol before the administration of tritiated steroids as in the normal rats. Preinjection with estradiol markedly reduced the uptake of tritiated 17α-methyl-estradiol in the uterus.

DISCUSSION

The highly reactive methyl lithium offers unique possibilities for the incorporation of C-11 methyl groups into organic or bio-organic molecules such as steroid hormones. With this carbon-11-labeled precursor, estrone can be converted to carbon-11-labeled 17α-methylestradiol. The reaction is almost stereospecific (*9*). With the tritiated analogs, we investigated the potential of 17α-methylestradiol as an estrogen-receptor-binding radiopharmaceutical. 17α-methylestradiol is bound with high affinity to the estrogen receptor, as shown by direct binding analysis and by sedimentation on sucrose gradients. Compared with estradiol, it is bound to the estrogen receptor with somewhat lower affinity, which is in agreement with the results of Ojasoo (*17*). After centrifugation on sucrose gradients, both estradiol and 17α-methylestradiol showed specific 8–9S and 4S binding in cytosol from rat mammary tumor. The higher proportional 4S binding observed with 17α-methyl-estradiol might be explained partly by a dissociation of the 8S estrogen-receptor complex during centrifugation, with subsequent binding of the steroid to nonspecific binding components (*18*).

In vivo 17α-methylestradiol (57 Ci/mmole) showed an estrogen-receptor-specific tissue distribution in mature female rats and in rats bearing DMBA-induced mammary tumors, within 45 min after injection. High uptake percentages were obtained in the uterus, and the radioactivity was retained at the same level during the investigated period of time. The uptake in tumor tissue was only 23–30% of the uterus uptake, which can be explained by its lower receptor concentrations (*16*).

When tritiated 17α-methylestradiol is used for in vivo studies, with approximately the same specific activity as the carbon-11-labeled analog, high uterus uptake and uterus-to-blood ratio were again obtained. The tumor-to-blood ratios were approximately 5 to 6.

The low tumor-to-fat ratio can be the result of the nonspecific binding of 17α-methylestradiol as measured in vitro.

When 17α-methylestradiol, 17α-ethynylestradiol moxestrol and 11β-methoxy-17α-ethynylestradiol are compared for their potential as estrogen-receptor-binding radiopharmaceuticals, the following parameters should be considered. First its selectivity of distribution and extent of uptake by target tissue; second, the availability of the radioactive precursor and the specific activity of the product with methods available. All steroids mentioned show a selective tissue distribution. Moxestrol showed the highest tumor-to-blood and tumor-to-fat ratios (*8*). For the preparation of carbon-11-labeled 17α-ethynylestradiol and moxestrol, C-11acetylene is used as precursor. When this precursor is produced by irradiation of a calcium carbide target, the specific activity of the steroids is far too low for in vivo studies (*6,7*). When the method described by Crouzel (*19*) is used for the production of carbon-11-labeled acetylene with our maximum beam current, a specific activity of 5–10 Ci/mmole seems to be achievable for 17α-ethynylestradiol and moxestrol. Carbon-11-labeled methyllithium, the radioactive precursor of 17α-methylestradiol, is

synthesized from $^{11}CO_2$ via the intermediary $^{11}CH_3I$ (*15*). The specific activity of the steroid obtained with this precursor is about 6 Ci/mmole (*9*) and might be upgraded to approximately 250 Ci/mmole by preparing the intermediary $^{11}CH_3I$ directly by a hot-atom reaction in a target containing HI and N_2 (*20*). Given the availability of a compact medical cyclotron, specific activities of more than 1000 Ci/mmole are anticipated (*21*).

In conclusion, though 17α-methylestradiol may not be the most selective steroid studied, it has certain promise as receptor-binding radiopharmaceutical because it can be prepared with a specific activity suitable for estrogen-receptor studies in vivo.

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