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UPTAKE OF RADIOLABELED ESTRADIOL BY THE CANINE ADRENAL

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Tritiated estradiol injected intravenously into 11 mongrel dogs sacrificed at 3, 7, 10, 17, and 60 min showed maximum uptake in the adrenal gland at 3–7 min. The concentration of radioactivity in the adrenal in percent dose per gram equaled that from ^{131}I-19-iodocholesterol. If a ^{131}I-estradiol could be synthesized that would concentrate similarly in the adrenal, it would offer the advantage of almost instantaneous imaging after the tracer injection and a lower radiation dose.

Early studies with tritiated estradiol in the rat demonstrated two different types of tissue uptake patterns (*1*). In the "nonresponsive" tissue such as liver, kidney, muscle, blood, and adrenal, peak uptake occurred very early, within 10–15 min, with an extremely rapid decline. In the "growth responsive" tissues, uterus and vagina, these organs continued to incorporate and retain radioactivity for much longer, reaching a peak not before 1–2 hr without significant decline for another 1–5 hr.

Further reports confirm these findings in the female rat where highest concentrations of tritiated estrone and estradiol were found in adrenals after 3-hr infusions (*2*). Ullberg, et al (*3*) also had drawn attention to specific localization of radioactive estrone and estradiol in the adrenal cortex and ovaries of the rat. These findings were further confirmed for ^{14}C-labeled mestranol, the cyclopentyl ether derivative of estradiol (*4*).

Because we have been interested and involved in the development of adrenal scanning agents, most recently ^{131}I-19-iodocholesterol (*5–8*), we hoped that if a tritiated estrogen concentrated sufficiently in the adrenal, a gamma-emitting analog might be synthesized for possible diagnostic use.

The studies reported here show that intravenous tritiated estradiol in the dog reaches the highest tissue concentration in the adrenal in 3–7 min and that this level exceeds that of all other tissues.

METHODS

Dogs. Eleven mongrel dogs, four females and seven males, 9–15 kg in weight, were injected intravenously and sacrificed at 3, 7, 10, 14, and 50 min as shown in Table 1. All dogs received 38–89 μCi ^{3}H estradiol.

Radiolabeled steroids. Estradiol-2,4,6,7(n)-^{3}H (85 and 100 Ci/m*M*) was obtained from Amersham/Searle, Chicago, Ill. Radiochemical purity 98% was documented by the suppliers by thin-layer silica gel, paper, and reverse-phase paper chromatography.

The radiosteroid as obtained was dissolved in benzene. The benzene was evaporated and the residue dissolved in absolute ethanol. Polysorbate 80 (1.0%) was added and sufficient normal saline to give a 20% ethanol solution having a specific concentration of 5–10 μCi/ml.

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TABLE 1. RELATIVE TISSUE DISTRIBUTION OF RADIOACTIVITY IN DOGS AFTER INTRAVENOUS INJECTION OF TRITIATED ESTRADIOL 2,4,6,7^{3}H-ESTRADIOL) (% TRACER DOSE/gm OF TISSUE)

Dog	Sex	Wt (kg)	Sp. act of estradiol (Ci/mM)	Dose (μCi)	Adrenal	Liver	Kidney Cort.	Kidney Med.	Prostate	Uterus/ tube	Ovary	Pancreas	Fat	Nipple	Blood	Pituitary Ant.	Pituitary Post.
									3 Min								
1	M	9.5	100	88	0.64	0.09	0.07	0.04	0.03	—	—	0.03	0.02	—	0.03	0.02	0.03
2	M	—	100	63	0.24	0.08	0.04	0.03	0.02	—	—	0.07	<0.01	—	0.02	—	—
3	F	10.5	100	38	0.23	0.08	0.04	0.04	—	0.01/0.01	0.01	0.01	0.01	0.01	0.03	—	0.02
4	M	11.4	100	62	0.18	0.08	0.08	0.04	0.04	—	—	—	<0.01	—	0.02	—	—
5	F	10.0	100	72	0.24	0.11	0.04	0.04	—	0.02/0.02	0.04	0.06	0.01	<0.01	0.03	—	—
									7 Min								
6	M	11.0	100	89	0.40	0.09	0.03	0.01	0.04	—	—	0.12	<0.01	—	0.01	0.02	0.05
7	M	9.2	85	62	0.37	0.10	0.04	0.04	0.09	—	—	0.03	0.02	—	0.02	—	—
									10 Min								
8	M	—	85	62	0.17	0.08	0.04	0.02	0.04	—	—	—	<0.01	—	0.01	—	—
9	F	10.5	100	53	0.18	0.16	0.07	0.05	—	0.03	0.02	0.03	0.01	0.01	0.03	—	—
									14 Min								
10	F	11.0	100	48	0.13	0.12	0.02	0.02	—	0.01/0.01	—	0.03	<0.01	0.01	—	—	—
									60 Min								
11	M	15.2	85	62	0.05	0.01	<0.01	<0.01	0.03	—	—	0.01	0.01	—	<0.01	—	—

Radioassays. Upon termination of each dog study, the animal was sacrificed with i.v. pentobarbital and 15 tissues were routinely removed (blood, liver, spleen, kidney: medulla and cortex, adrenal, intestines, pancreas, fat, muscle, bladder, heart, lung, and either prostate, vas and testis or ovary, tube and uterus). The tissues were cleaned of fat and extraneous material and weighed. In addition, in three dogs specimens of pituitary, hypothalamus, and cerebral cortex were removed.

An effort was made to keep the weight of samples between 10 and 50 mg. Routinely, duplicate samples of all tissues studied were processed. After weighing, all these samples were placed in counting vials, digested in either 3 ml 10% NaOH or NCS (Amersham/Searle) overnight, and then heated 10–30 min in warm water to complete digestion. Benzoyl peroxide, 0.1 ml, was added to samples requiring decolorization and all samples were neutralized with 0.1 ml glacial acetic acid. Ten milliliters of liquid scintillation fluid were added (PCS-Amersham/Searle) and the radioactivity assayed in a Searle Radiographics (Mark II) liquid scintillation counter. Quench corrections were made using the channels ratio technique. All samples were counted for a sufficient period of time to insure a statistical counting error of less than 5% at the 95% confidence level. Results were expressed in disintegrations per minute per milligram and percent of the administered dose per gram tissue.

RESULTS

In all 11 dogs the highest percent of radioactivity per gram occurred in the adrenal as shown in the table. Furthermore this was true at each time interval studied (3 min–1 hr). The adrenal concentration averaged 0.31% dose/gm at 3 min (0.18–0.64), 0.38% dose/gm at 7 min, and dropped off rapidly to 0.18% and 0.05% at 10 and 60 min, respectively.

Though only four female dogs were studied, (two at 3 min, and one each at 10 and 14 min) the adrenal percent uptake was comparable to that measured in the male dogs at the same time interval.

No significant accumulation of radioactivity was found in any other organ except liver at 3, 7, 10, and 14 min which was still about one-third of the adrenal concentration. The mean percent dose per gram uptake in liver at 3 min was 0.09 (0.08–0.11). Of interest was the lack of significant accumulation in any growth-responsive tissue including uterus, ovary, fallopian tube, and prostate, in fat and muscle, and in neural tissue.

DISCUSSION

De Hertogh, et al (*2*) in studying female rats given long-term intravenous infusions of 6,7-^{3}H 17B-estradiol found that these labeled hormones were taken up by uterus, adrenals, adipose tissue, and muscle mostly in an unchanged state with practically no metabolite uptake. It was his belief that there were target tissue-specific receptors for estrogens in the uterus but that protein binding with large capacity and low affinity might account for the uptake in the liver, adrenals, and muscle. The liver and adrenals, having a higher concentration than that found in plasma, must then display either a higher affinity or a higher concentration of their binding proteins in contrast to plasma binders—mostly albumin.

Ullberg, et al (*3*) studied the distribution of ^{14}C-estrone and ^{3}H-estradiol autoradiographically in mice. They found a selective accumulation in the adrenal cortex, the granulosa layer of large ovarian follicles, and in the endometrium. They believed that

these sites were target areas of the estrogens. The greatest concentration was found in the adrenal cortex at 1 hr. The adrenal medulla showed no specific uptake. After 4 hr, a relatively increased activity was found at the sites of excretion, particularly the liver and intestine. By 24 hr the concentration in all the tissues except the excretory pathways was greatly reduced. After 4 days, no activity could be found in any tissues but the liver.

The peak uptake of radiolabeled estradiol observed by us in the adrenal of dogs at 3–7 min is remarkable in that the uptake in percent dose per gram equals the uptake we have previously observed with ^{131}I-19-iodocholesterol in the dog (*9*) and in the human (*5,7,8*). If a ^{131}I- or ^{11}C-labeled estradiol could be synthesized that would concentrate in the adrenal cortex of man in a concentration similar to the ^{3}H-estradiol used here, it would allow almost instantaneous imaging of the adrenal after a tracer rather than the 3–14 days necessary with ^{131}I-19-iodocholesterol (*10*). It would also offer the advantage of a shorter biologic half-life and lower radiation dose to the adrenal.

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REFERENCES

1. JENSEN EV, JACOBSON HI: Basic guides to the mechanism of estrogen action. *Recent Prog Horm Res* 18: 387, 1962

2. DE HERTOGH R, EKKA E, VANDERHEYDEN I: Equilibrium concentration of [6,7-^{3}H] oestrone and [6,7-^{3}H] 17β-oestradiol metabolites in female rat tissues during long-term intravenous infusions. *Acta Endocrinol* 66: 401–416, 1971

3. ULLBERG S, BENGTSSON G: Autoradiographic distribution studies with natural oestrogens. *Acta Endocrinol* 43: 75–86, 1963

4. APPELGREN LE, KARLSSON R: The distribution of labelled mestranol in mice. *Acta Endocrinol* [*Suppl*] 138: 84, 1969

5. BEIERWALTES WH, LIEBERMAN LM, ANSARI AN, et al: Visualization of human adrenal glands in vivo by scintillation scanning. *JAMA* 216: 275–277, 1971

6. COUNSELL RE, RANADE VV, BLAIR RJ, et al: Tumor localizing agents IX. Radioiodinated cholesterol. *Steroids*, 16: 317–328, 1970

7. LIEBERMAN LM, BEIERWALTES WH, CONN JW: Diagnosis of adrenal disease by visualization of human adrenal glands with ^{131}I-19-iodocholesterol. *N Engl J Med* 285: 1387–1393, 1971

8. MORITA R, LIEBERMAN LM, BEIERWALTES WH, et al: Percent uptake of ^{131}I radioactivity in the adrenal from radioiodinated cholesterol. *J Clin Endocrinol Metab* 34: 36–43, 1972

9. BLAIR RJ, BEIERWALTES WH, LIEBERMAN LM, et al: Radiolabeled cholesterol as an adrenal scanning agent. *J Nucl Med* 12: 176–182, 1971

10. BEIERWALTES WH, ANDERSON BG, MORITA R, et al: Some new isotopic procedures and techniques for endocrine diagnosis. In *Clinical Uses of Radionuclides: Critical Comparison with Other Techniques*, Symposium Series 27, Oak Ridge, Tenn, USAEC, 1972, pp 393–411

11. ANDERSON BH, BEIERWALTES WH: Adrenal imaging with radioiodocholesterol in the diagnosis of adrenal disorders. *Adv Intern Med* 19: 327–343, 1974