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Uptake of [^{3}H]Testosterone in Several Organs of the Male Viviparous Lizard (*Lacerta vivipara* Jacquin) and Selective Retention by the Epididymis

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[1, 2, 6, 7-^{3}H]Testosterone was injected into castrated male viviparous lizards to establish retention of the hormone by blood, lung, gut, liver, kidney, penis, and epididymis. Radioactivity in these organs was determined in the spring four times during the 12 hr following administration of the isotope. Retention in blood was greater than in mammals, so that significant differences in retention between the epididymis and nontarget tissues appeared only between 6 and 12 hr after treatment. High levels of radioactivity found in the gut indicate that this route is used for clearance of the exogenous steroid. Selective retention of testosterone by the epididymis during its period of activity (spring) was no longer found in regressed or inactive organs (summer and autumn). Autoradiographs of three tissues indicated that testosterone or a metabolite was concentrated in cell nuclei of the epididymis but not in those of liver and gut.

Little is known about the uptake and retention of steroid hormones in reptiles. Administration of [^{3}H]testosterone with a high specific radioactivity permits a demonstration of selective uptake of the labeled hormone in accessory sexual organs of mammals (Tveter and Attramadal, 1968; Stern and Eisenfeld, 1969) such as the prostate and seminal vesicles. Such experiments revealed the presence of steroid receptors for androgens in the so-called target organs (Fang *et al.*, 1969). In an attempt to study steroid receptors in lizard, a nonmammalian vertebrate, we performed experiments to establish which tissues can accumulate androgens. It is well known that in the male lizard three organs are candidates for control by steroid hormones: the epididymis, the sexual segment of the kidney, and the femoral organs (Herlant, 1933; Regamey, 1935). This report deals with the epididymis, a target organ studied in our laboratory, upon which preliminary results on testosterone retention were obtained (Gigon *et al.*, 1973).

Dramatic seasonal variations are an important feature of the physiology of reproduction in reptiles and this paper reports results obtained at different seasonal periods of the sexual cycle. Transport of bound hormone into cell nuclei characterizes the mechanism of steroid hormones action and was established, among other means, by autoradiographic results, first for estrogens (Jensen *et al.*, 1968) and then for androgens (Tveter and Attramadal, 1969; Sar *et al.*, 1970). We have performed such autoradiographic methods in the present study on uptake by the epididymis, gut, and liver.

MATERIALS AND METHODS

Adult male viviparous lizards (*Lacerta vivipara* Jacquin) were collected in Massif Central (France) from March to October and kept for several days or weeks in the laboratory (Gigon-Depeiges and Dufaure, 1977).

Several groups of three to five animals were castrated via an abdominal route 48 hr prior treatment. The lizards' weights ranged between 2, 20 and 4, 60 g. Animals received by subcutaneous injection into the right shoulder 4 to 4, 5 μCi of [1, 2, 6, 7-^{3}H]

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testosterone (85 Ci/mmol, New England Nuclear, purity checked by thin-layer chromatography) given in 10 μl of 10% ethanol:saline. Such a high dose of hormone was chosen because of the plasma level of endogenous testosterone, as determined by radioimmunoassay (40 ng/ml of plasma in the beginning of spring) (Y. Courty, A. Gigon-Depeiges, and J. P. Dufaure, unpublished data).

At 1.5, 3, 6, and 12 hr after [^{3}H]testosterone injection, animals were killed by decapitation. Five to 15-mg samples of blood and organs were placed into preweighed scintillation vials and weighed on a Sartorius balance to within 0.1 mg. The wet weight of organs was obtained after blotting them dry on filter paper. Samples were frozen in liquid nitrogen and stored at −15° before digestion with Soluene 350 (Packard) at 40° for several hours. Scintillation liquid (PPO–POPOP–toluene) was added to the vials, and the radioactivity was determined on an Intertechnique scintillation spectrophotometer with an external standard. Several controls were performed. Possible intrinsic fluorescence of the organs and quenching by the tissues and solubilizer were checked (1) by counting solubilized tissues without added radioactivity, (2) by adding internal standards (25,000 cpm of tritium), and determining the recovery. In another experiment, the influence of coloration was checked in blood and liver by comparing colored and discolored (Soluene–isopropanol and H_2O_2) samples. Because of minimal background, counts reported (dpm/mg wet weight) were not corrected by subtraction of blank values. The precision of the procedure was evaluated by weighing and counting five samples of liver from three different animals injected with the same dose of ^{3}H-labeled hormone; confidence intervals were determined for $p = 0.05$; the coefficient of variation calculated for the five samples in each animal was better than 7.5%.

For autoradiographic studies, samples were quickly frozen in liquid nitrogen or in liquefied propane. Tissues were cut at −25° into 6- to 10-μm thick sections and mounted on desiccated photographic emulsion (Ilford L4) coated slides according to Stumpf's recommendations (Stumpf and Roth, 1966). After 3 to 5 months of exposure at −18°, the slides were developed in D 19 b (Kodak), fixed with sodium thiosulfate, carefully washed in tap water, and stained with methyl green–pyronin or with nuclear fast red.

RESULTS

Levels of Radioactivity in Tissues from 1.30 to 12 hr Following Administration of [^{3}H]Testosterone

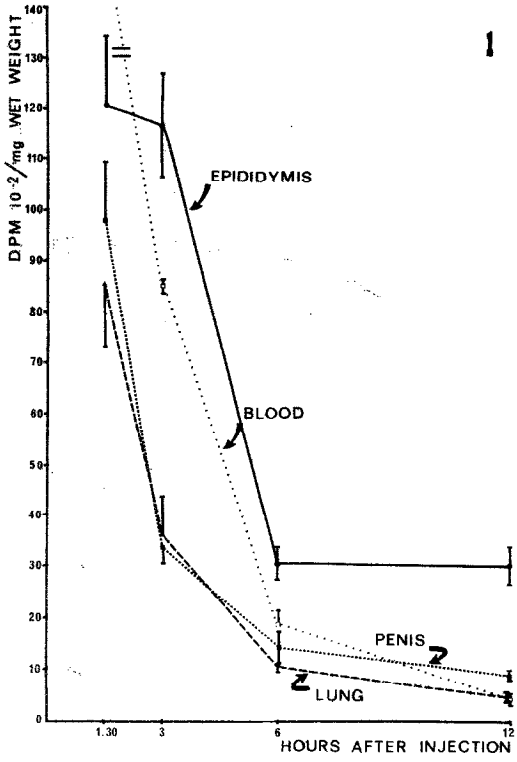
The values of radioactivity remained high and variable in all tissues 1.30 hr after injection (Table 1). Uptake of the radionuclide

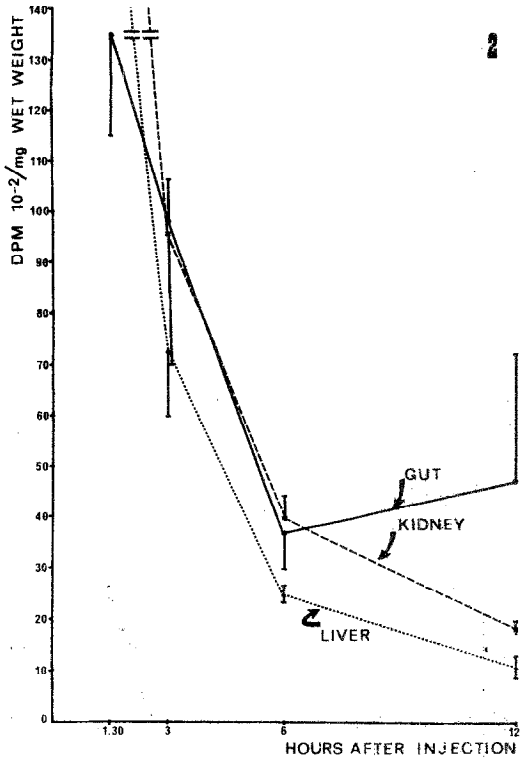
TABLE 1

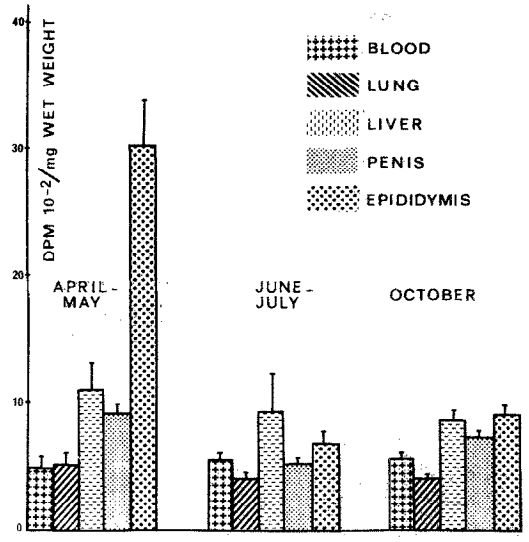
DISTRIBUTION OF RADIOACTIVITY IN SEVERAL TISSUES OF THE LIZARD FOLLOWING A SINGLE INJECTION [^{3}H]TESTOSTERONE[a]

Experiment	Hours after injection	Seasonal period	Number of lizards	Radioactivity in tissues (dpm/mg wet weight)						
				Blood	Lung	Gut	Liver	Kidney	Penis	Epididymis
1	1.30	December	3	15,268 ± 1,838	8,538 ± 1,154	13,444 ± 1,961	16,932 ± 1,413	22,898 ± 2,465	9,800 ± 1,114	12,078 ± 1,339
2	3	April	3	8,518 ± 114	3,633 ± 722	9,812 ± 795	7,250 ± 1,235	9,548 ± 2,540	3,398 ± 276	11,663 ± 1,039
		May								
3	3	December	3	8,342 ± 477	4,943 ± 733	13,968 ± 2,749	11,207 ± 2,055	19,603 ± 2,888	5,948 ± 587	7,208 ± 1,349
4	6	April	6	1,924 ± 234	1,094 ± 89	3,677 ± 689	2,491 ± 155	3,970 ± 399	1,460 ± 270	3,087 ± 281
		May								
5	12	April	5	468 ± 74	510 ± 75 (4)	4,720 ± 2,474	1,103 ± 203 (3)	1,867 ± 96 (3)	917 ± 58	3,052 ± 332
		May								
6	12	June	3	545 ± 51	391 ± 38	3,687 ± 1,263	927 ± 270	1,672 ± 358	508 ± 27	660 ± 85
		July								
7	12	October	4	558 ± 31	406 ± 20	5,760 ± 2,492	861 ± 69	2,551 ± 314	727 ± 43	901 ± 70

[a] The radioactivity values are given as means ± SEM. In Exp. 5 the numbers in parentheses indicate the number of animals utilized in the calculation of the mean values for lung, liver, and kidney.







FIGS. 1–2. Retention of radioactivity by several organs of the male lizard following injection of 4 to 4.5 μ Ci of [1, 2, 6, 7-^{3}H]testosterone. Experiments (see Materials and Methods) were performed during the spring (except for the time lapse of 1.30 hr) and numerical results are given in Table 1. The values are given as means ± SEM.

FIG. 1. Retention of radioactivity by blood, lung, penis, and epididymis.

FIG. 2. Retention of radioactivity by liver, kidney, and gut.

FIG. 3. Concentration of radioactivity in blood, lung, liver, penis, and epididymis of the male lizard 12 hr after injection: of 4 to 4.5 μCi of [1, 2, 6, 7-^{3}H]testosterone. Experiments (described under Materials and Methods) were performed at several seasonal periods. Numerical results (mean ± SEM) are given in Table 1.

was greater in kidney, liver, and blood. Then radioactivity declined in all tissues until 12 hr after treatment and the values were more uniform except for the gut and the kidney. The rate of decrease and the final values of the radioactivity at 12 hr were different in different tissues. It appears that the lung and penis retained as little radioactivity as the blood at 12 hr and were considered nontarget tissues. In Fig. 1 the amount of radioactivity is compared between these tissues and the epididymis. The decrease in the concentration of radioactivity was rapid from 1.30 to 3 hr for blood, lung, and penis but not for the epididymis. Although the mean radionuclide content of the epididymis was greater than that of the nontarget tissues at 3 hr after injection, a significant difference was only recorded at 12 hr for experiments performed during spring. The liver and kidney accumulated high levels of radionuclide within 1.30 hr following injection (Table 1), reflecting their role in the metabolism of steroids. The rate of decrease in these organs (Fig. 2) was more rapid than for other tissues between 1.30 and 3 hr after treatment. Important retention of radioactivity was found in the gut until 12 hr after injection and the different values obtained for this organ remained highly variable. In contrast to the gut, the esophagus and stomach did not retain more radioactivity than blood and lung 12 hr after injection: 442 ± 32 dpm/mg in the esophagus and stomach versus 5760 ± 2492 dpm/mg in the gut).

Retention of Radioactivity in Epididymis During the Sexual Cycle

Clear-cut differences in the retention of [^{3}H]testosterone appeared between the epididymis and nontarget tissues 12 hr after injection, and this length of time was chosen to compare the retention of radionuclide at several seasonal periods of the sexual cycle (period of sexual activity in the spring, of atrophy in the summer, of recovery in the autumn) (Fig. 3). Although values of radioactivity were about the same for the different nontarget organs in three periods, retention by the epididymis was significantly greater during the spring than during the summer and autumn. In contrast, differences between summer and autumn values of radioactivity in the epididymis were not significant.

Autoradiographic Study of the Distribution of Radioactivity Following Administration of [^{3}H]Testosterone

Autoradiograms of the epididymis from animals collected in spring were prepared at 3,6 and 12 hr after [^{3}H]testosterone injection. The distribution of radioactivity was rather diffuse at 3 hr, silver grains being visible both in epithelial cells and in extracellular spaces, between tubules. Nevertheless a nuclear concentration was evident (Fig. 4). Maximum nuclear labeling appeared from 6 to 12 hr after injection while silver grains were scarce in the cytoplasm and extra tubular spaces (Figs. 5 and 6). In autoradiograms prepared at 12 hr after in-

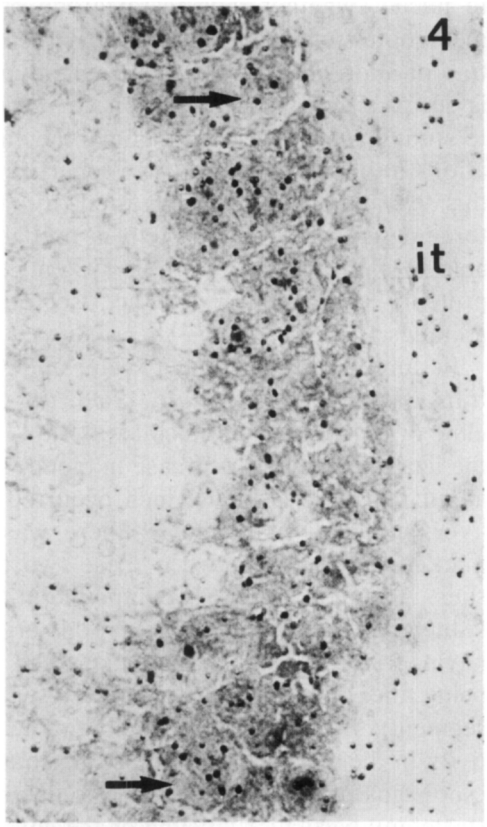
FIGS. 4–7. Autoradiograms of epididymis (4–6) and liver (7) of the male lizard at different intervals (3 to 12 hr) after injection of 4 to 4.5 μ Ci of [1, 2, 6, 7-^{3}H]testosterone.

FIG. 4. Three hours; exposure, 3 months; stain, nuclear fast red; ×1000. Silver grains are diffusely distributed between intertubular tissue (it) and the basal part of epithelial cells containing nuclei and ergastoplasm; radioactivity begins to be concentrated in some nuclei (arrows).

FIG. 5. Twelve hours; exposure, 5 months; stain, methyl green–pyronin; ×250. Silver grains are concentrated at the basal part of epithelial tubules (nuclei) and in the lumen of tubules.

FIG. 6. Twelve hours; exposure, 5 months; stain, methyl green–pyronin; ×1000. Radioactivity is concentrated in the nuclei (lightly stained by methylgreen) although silver grains are scarce over the surrounding ergastoplasm (strongly stained by pyronin); a conspicuous concentration of silver grains appears in the upper left (lumen of tubule) over and around the secretion granules.

FIG. 7. Twelve hours; exposure, 5 months; stain, methyl green–pyronin; ×1000.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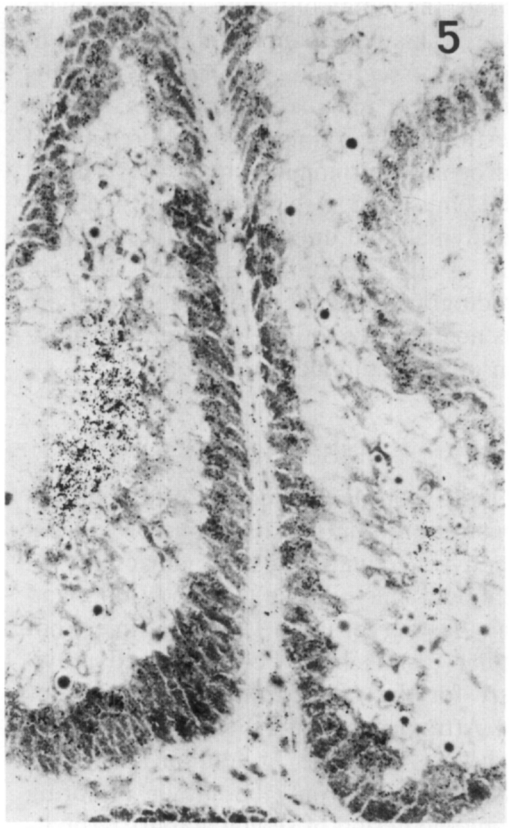
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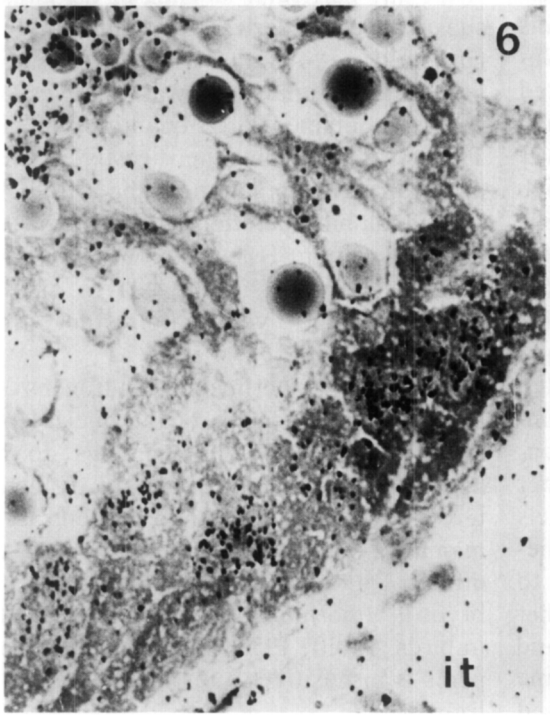
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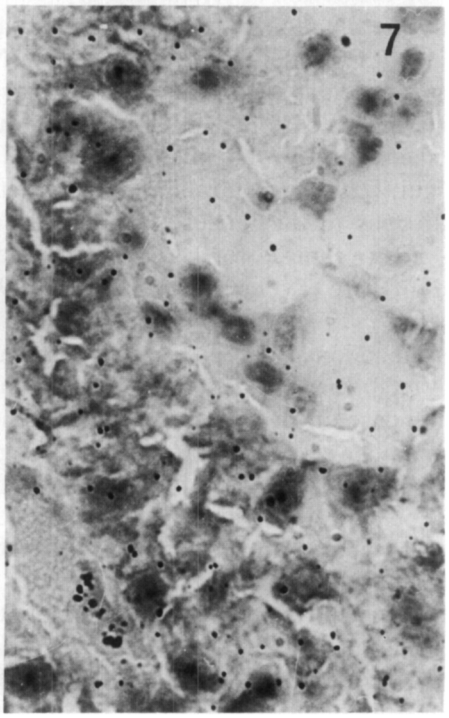
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jection, the lumen of tubules was filled with silver grains which appeared to be concentrated over secretion granules (Fig. 5). This conspicuous picture introduces several questions concerning a possible release of androgen hormones by the lizard epididymis and a binding affinity of the epididymal fluid due or not due to the protein of the secretion granules. In autoradiograms of liver (Fig. 7) and gut there was no evidence for any nuclear concentration of the radionuclide.

DISCUSSION

Our experiments demonstrate that administration of [^{3}H]testosterone provides a selective accumulation of radioactivity in the epididymis relative to blood, lung, and penis, lasting for up to 12 hr after injection. Similar results have been obtained previously for the rat ventral prostate (Tveter and Attramadal, 1968; Fang *et al.*, 1969). Such a selective retention of [^{3}H] testosterone can only be shown in spring during the period of sexual activity. Apparently the functional state of the epididymis influences the uptake of testosterone. Nuclear concentration of radioactivity existed in epithelial cells of the epididymis although no such selective labeling occurred in liver or gut. The concentration of radioactive androgens in the nuclei of epithelial cells has been reported for rat seminal vesicles and prostate (Tveter and Attramadal, 1969; Sar *et al.*, 1970). A two-step mechanism for steroid action as first proposed by Jensen *et al.* (1968) for estrogen could be postulated in the case of the lizard epididymis, a nonmammalian target organ. The androgen-retaining process described in this paper may be pertinent to androgen action models in mammals (Mainwaring, 1977). Nevertheless, several controls are necessary to confirm the model, such as in the use of other steroids and of anti-androgens (Stern and Eisenfeld, 1969). We have also to determine the nature of the ^{3}H-labeled steroid retained in nuclei, whether it is testosterone or a metabolite such as DHT. If selective retention of androgens by the lizard epididymis is due to a receptor protein, it is of interest to study in such a seasonal animal the variations in occurrence and (or) properties of this protein. But, as suggested by autoradiograms, another protein is probably able to bind androgens in our system, the protein component of the secretion granules.

In contrast to mammals (Tveter and Attramadal, 1968; Fang *et al.*, 1969), the decline of radioactivity in blood was slow. In the lizard the rate of decrease in blood was about the same as that which occurred in liver, although in mammals there was a marked difference between these two tissues. Accumulation of ^{3}H compound in the kidney has probably two causes, this organ having an excretory function as well as being the target for testosterone (sexual segment). We found a high level of radioactivity in the gut but not in the stomach and esophagus, and we were unable to demonstrate any particular labeling of the gut tissues; consequently we do not think that the gut is a target organ. During the dissection of ^{3}H-injected animals, it was observed that the gut contained large quantities of bile. Thus, it is clear that the digestive tract is used for the elimination of the ^{3}H-labeled steroid via bile secretion; a high radioactivity content after [^{3}H]testosterone administration has occurred in the bile of another nonmammalian vertebrate, the trout (Schreck, 1973).

In similar experiments, androgens accumulated in the penis of the duck (Horst and Paulke, 1977). Our findings do not agree with a selective retention of testosterone by the lizard penis. In contrast to mammals, one of us has shown that the differentiation of this organ in lizard embryos is anhormonal (Dufaure, 1966) as it is in birds (Wolff, 1950). We confirm that the penis is insensitive to testosterone in adult lizards.

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