LOCAL RECIRCULATION OF INJECTED [³H]TESTOSTERONE FROM THE TESTIS TO THE EPIDIDYMAL FAT PAD AND THE CORPUS EPIDIDYIMIDIS IN THE RAT

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Exchange of the inert gases ⁸⁵krypton and ¹³³xenon between the testis and the caput and corpus epididymidis on the ipsilateral but not on the contralateral side has recently been demonstrated in rats. The exchange was probably based on a countercurrent exchange of the gases between the internal spermatic vein and artery (Einer-Jensen, 1974). To investigate if testosterone could be transferred by the same mechanism, the following experiment was performed.

Twenty-four 5- to 6-month-old Charles River male rats were used for the experiment. They were previously used for mating purposes about twice a week.

The animals were anaesthetized with an intraperitoneal injection of 30 mg Mebumal-Na (Nembutal®)/kg. The abdomen was opened through a mid-line incision, the right testis and the epididymis were withdrawn into the abdomen for injection of 0.2 µg (30 µCi) [1,2-³H]testosterone (N) (New England Nuclear, NET 187) dissolved in 0.1 ml ethyl alcohol benzene (10:1). In one group, an intratesticular injection only was given; in a second group, the ductuli efferentes and the whole ligament between the caput epididymidis and the testis was ligated (leaving the testicular artery and vein intact) before the injection. All or most of the lymph vessels from the testes are supposed to be localized in this ligament. To a third group, the injection was given into the caput epididymidis after ligation as described above. The testis and epididymis were replaced in the scrotum and the abdominal incision was closed until removal of the organs 5 or 30 min later.

In four animals from each group, the caput, corpus and cauda epididymidis, as well as the testis and the epididymal fat pad were removed on both sides 5 min after injection. In four other animals, these organs, and the para-aortic and/or the right hypogastric lymph glands were removed after 30 min. The glands were made visible by injecting 0.1 ml of an 8% colloidal suspension of mercurousulphide into the right testis before the ligation. Only stained glands were removed.

Two samples weighing about 10 mg apiece were taken from each organ mentioned. The weight (0.1 mg accuracy) was measured, the organs were dried at 80°C, placed in a coil stand in a standard glass scintillation vial and com-

busted (Gupta, 1966). Instead of a platinum coil, a stainless steel coil was used, and the $^3\text{H}_2\text{O}$ gas was absorbed by 0.5 ml methanol instead of being condensed by freezing (G. N. Gupta, personal communication). After adding 10 ml scintillation fluid (Toluen-Liquifluor R, 925:75), the radioactivity of each sample was counted in a scintillation counter (Packard Tricarb 3375) for 10 min.

The results are given as the mean of four animals $\pm$ S.E. in counts/min/mg wet wt of tissue. The probability for difference between ipsi- and contralateral organs was calculated by Student's *t* test.

The group mean radioactivity of the organs on the injected side was, with few exceptions, higher than in the same organs on the other side (Table 1). No consistent difference was found between the results for the rats from which organs were removed at 5 or 30 min. Ligation of the ductuli efferentes and the lymph vessels did not diminish the uptake. There was significantly less ($P < 0.01$) radioactivity in lymph glands from animals in which the ligament between the testis and the caput was ligated, indicating a successful ligation of the lymph vessels.

The group mean activity in the corpus epididymidis and fat pad on the injected side was about the same when [^3H]testosterone was injected into the testes and into the caput. No uptake in the testes after injection into the caput epididymidis was, however, demonstrated. This lack of uptake cannot be explained at the present time.

The highest uptake of the labelled material was seen in the corpus epididymidis (range 5 to 21 ct/min) and the fat pad (13 to 28 ct/min). The range from the same contralateral organs was 2 to 5 ct/min in both organs. For the rats in the groups receiving intratesticular injections, these values were all significantly different from those of the contralateral organs, the probability varying between $P < 0.05$ and $P < 0.005$.

When [^3H]testosterone was injected into the testes, the mean uptake in the caput was smaller than that in the corpus. The differences between the ipsilateral and contralateral capita were, however, significant ($P < 0.05$ to $P < 0.005$) in three out of the four groups.

The difference between the uptake of labelled material from the caudae on the injected and uninjected sides was small or non-existent. In three out of four cases, the difference was not significant. In the remaining case, $P < 0.05$.

As an increase in radioactivity was found even when the distribution by way of the ductuli efferentes and the lymph vessels was curbed, distribution through the blood seems to be the only possibility. Because the concentration was higher in the organs on the injected side, the arterial blood leading to these organs must have had a higher concentration of labelled material. There is no reason to believe that the blood flow to or the extraction from the blood was more effective on the injected side. The only possible explanation for the increase in blood levels on the one side seems to be a local exchange from the internal spermatic vein to the internal spermatic artery in the region of the pampiniform plexus. The cauda epididymidis, in contrast to the caput, corpus and fat pad, receives blood from the vasal artery (Greene, 1968; Harrison & Kidd, 1970), and shows no uptake of radioactivity.

The experiment suggests not only that some of the testosterone is exchanged

Table 1. Distribution of radioactivity in the male genital organs in the rat 5 or 30 min after injection of [³H] testosterone, into the testis or the caput epididymidis

	Right side (injection site)					Left side (no injection)					Abdominal lymph glands
	Testis	Caput	Corpus	Cauda	Fat pad	Testis	Caput	Corpus	Cauda	Fat pad	
5 min											
Intact, injection in testis	—	5 ± 1.5 N.S.	19 ± 4.8 **	4 ± 0.5 N.S.	14 ± 1.6 ***	2 ± 0.4	3 ± 0.8	4 ± 0.4	4 ± 1.0	2 ± 0.2	
Ligated, injection in testis	—	5 ± 0.9 **	13 ± 2.0 ***	5 ± 1.9 N.S.	13 ± 3.1 ***	2 ± 0.2	2 ± 0.2	5 ± 1.2	2 ± 0.3	2 ± 0.2	
Ligated, injection in caput	2 ± 0.3 N.S.	—	21 ± 5.4 *	6 ± 1.0 N.S.	14 ± 5.1 N.S.	3 ± 0.8	3 ± 0.7	5 ± 1.6	4 ± 0.8	5 ± 0.9	
30 min											
Intact, injection in testis	—	4 ± 0.5 ***	5 ± 0.5 ***	2 ± 0.4 N.S.	16 ± 4.1 **	1 ± 0.0	2 ± 0.2	2 ± 0.2	2 ± 0.2	2 ± 0.3	17 ± 3.8
Ligated, injection in testis	—	8 ± 2.3 *	13 ± 4.0 *	5 ± 1.3 *	23 ± 4.0 ***	2 ± 0.8	2 ± 0.5	3 ± 0.5	2 ± 0.3	2 ± 0.3	3 ± 0.3 ***†
Ligated, injection in caput	2 ± 0.3 N.S.	—	8 ± 1.6 *	6 ± 1.5 *	28 ± 10.1 *	2 ± 0.2	3 ± 0.5	3 ± 0.4	2 ± 0.5	2 ± 0.3	4 ± 0.7 ***†

Each group contained four rats. The values are expressed as Means ± S.E. (counts/min/mg wet wt of tissue).
P values: N.S. > 0.05; * < 0.05; ** < 0.01; *** < 0.005.
 † Against non-ligated group.

and returned to the testis in the arterial blood (Free, Jaffe, Jain & Gomes, 1973; Jacks & Setchell, 1973) but, at least in the rat, that the epididymal fat pad and parts of the epididymis also benefit from the exchange.

The physiological importance of this special way of distribution of testosterone is obvious for the epididymis, but remains unknown at this point for the fat pad. The latter may possibly act as a depôt, diminishing the local blood level fluctuations.

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