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TESTOSTERONE BINDING CAPACITY IN RELATION TO THE PRODUCTION AND METABOLISM OF TESTOSTERONE IN DOGS, EXPERIENCES OF A NEW METHOD

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SUMMARY

Testosterone dynamics were studied *in vivo* in five male dogs using constant infusion techniques, for the first time comparing conscious dogs with anesthetized ones. Barbiturate narcosis was shown to inhibit testosterone production drastically so that plasma levels were near zero for at least 24 h. This causes a 50% reduction of the specific testosterone binding globulin demonstrated in the male dog using a new two-phase equilibrium partition method[5]. An increased association constant was observed at the same time. This protein was shown not to be identical with transcortin. It bound testosterone but not estradiol-17β. The testosterone–protein complex was efficiently extracted by the liver (85–100%). The hepatic metabolic clearance rate was however only 13–20% of the total MCR. It was unaffected by barbiturate anesthesia but showed a small decrease during halothane + oxygen anesthesia, while the extrahepatic MCR decreased to a greater extent during halothane narcosis or laparotomy.

INTRODUCTION

The purpose of the present investigation is to obtain data on testosterone dynamics in male dogs in relation to anesthesia and surgical trauma. It was previously shown[1] in studies on human male patients that the postoperative decrease in plasma LH concentrations did not last long enough to explain the long-lasting decrease in plasma testosterone concentrations. It is of particular interest, in this connection, to know whether the production or the metabolism of testosterone is affected. Important factors influencing metabolism may be specific binding globulins in plasma, liver blood flow and steroid metabolizing enzymatic activity. Experimental evidence for the existence of a specific testosterone-binding globulin in the dog is contradictory[2–4]. Westphal concluded[3] that available evidence pointed to transcortin as the testosterone-binding globulin in dog plasma. A new method based on a two-phase equilibrium partition system[5] is employed in the present investigation to show the existence of a specific androgen-binding globulin in dog plasma.

Testosterone dynamics are studied using constant infusion of [$7\alpha^3$H]-testosterone and collection of three blood samples at 15 min intervals after reaching steady state in order to determine metabolic clearance rate (MCR) and production rate (PR) for testosterone. Such experiments are for the first time carried out in conscious trained dogs before and after anesthesia and surgery. The immediate effect of atropin ("premedication") is tested. The effect of barbiturate anesthesia is studied over a period of 2 h followed by halothane plus oxygen anesthesia over a period of 1 h before surgery (laparotomy and traction of guts) was carried out for another 1 h while the halothane–oxygen anesthesia was continued. Fifteen minutes after the induction of barbiturate anesthesia the skin and subcutaneous tissues over the external jugular vein on one side of the neck were infiltrated by a local anesthetic and a catheter introduced into a branch of the hepatic vein and the proper site checked by injection of radio-opaque solution under x-ray fluoroscopy. [^{125}I]-Rose Bengal was infused together with [7α-^{3}H]-testosterone in these experiments and blood was simultaneously drawn from a peripheral leg vein and the hepatic vein catheter to enable calculation of liver blood flow and hepatic extraction of testosterone. [^{125}I]-Rose Bengal and [7α-^{3}H]-testosterone are infused in the conscious dogs

assuming that the hepatic extraction of Rose Bengal and of testosterone is the same as initially determined the day before.

The data for liver blood flow and hepatic extraction of testosterone allow the calculation of the hepatic and extra-hepatic plasma MCR for testosterone in the dogs. The specific androgen-binding capacity and the association constant for this activity are determined using [1,2-^{3}H]-5α-dihydrotestosterone together with a saturating amount of cortisol in conjunction with native peripheral or hepatic venous plasma as well as plasma heated at 60° for 20 min.

MATERIALS AND METHODS

Animal preparation

Five male mongrel dogs, 22–28 kg of body weight, age 1–4 yr, were trained to participate in the experiments and used for a period of 1–8 months. A recovery period of at least 6 days was provided for between experiments. The plasma testosterone concentration was determined in an absolute first sample of peripheral venous blood (afs) obtained by puncture of a leg vein. Testosterone dynamics were studied in the dogs, while conscious, by connecting a plastic venous cannula (Infart 70 × 1·75 mm, AB Stille, Stockholm) with Teflon tubing to a 50 ml glass syringe attached to a constant infusion pump (manufactured at the Institute of Physiology, Lund). Approximately 34 ml sterile physiological saline (ACO, Sweden), containing the labelled compounds, were infused per hour. The infusion was immediately preceded by a priming dose calculated according to Shipley and Clark[6] in order to reach the approximate constant infusion level almost instantaneously. The time allowed to reach steady state conditions was, as a rule, 75 min. Three blood samples were then taken at 15 min intervals. Experiments were carried out on untreated conscious dogs, then repeated after a week with the injection of 0·5 mg atropin i.v. The effect of atropin was studied for 1·5 h. Experiments on conscious dogs were also carried out on the day after surgery and later, after about 6 months, in three dogs.

A blood sample was taken, at the start of each experiment, for the determination of testosterone in plasma. After fasting overnight, barbiturate narcosis was carried out by the i.v. injection of thiomebumal sodium (Intraval®, Pharma Rhodia, Denmark, or Pentothal sodium®, Abbott), about 0·5 g, and then with doses of 50–150 mg to keep a steady depth of anesthesia. An endotracheal tube was introduced and the dogs were allowed to breath atmospheric air. After two hours the endotracheal tube was connected to a halothane thermostated vaporizer (Fluotec cyprane) with 100% oxygen as carrier gas in a closed system (AGA Anestor Universal). Respiration was spontaneous. The depth of anesthesia was kept at 3:1–2. Sterile Ringer solution was slowly infused i.v. to compensate for loss of blood and body fluid. Blood sampling was started after 45 min. After 1·25–1·5 h a midline skin incision was made, under antiseptic conditions, from below the xiphoid process to halfway between the umbilicus and os pubis and access made to the abdominal cavity. The liver was inspected and the stomach palpated, traction being carried out on the stomach and adjacent parts of the duodenum and jejunum. The opening in the abdominal wall was then closed by sutures. Blood sampling was started after 45 min.

The hepatic vein was catheterized at the beginning of the barbiturate anesthesia. The skin and subcutaneous tissues on one side of the neck were infiltrated with 6 ml of 1% Citanest® (Astra) and a small incision made in order to expose the external jugular vein. After ligation of the vein, an x-ray opaque polyethene catheter (USCI, U.S.A.) was introduced in the central part and passed on until it reached a branch of the hepatic vein. The position was checked at the beginning and at the end of the experiment by hand injection of Urografin (Schering), 15–20 ml of 292 mg I per ml, followed by x-ray photography. The catheterization of the hepatic vein failed in one dog.

Labelled compounds

[7-^{3}H]-testosterone (SA 25 Ci/mmol) was obtained from New England Nuclear (NEN), Boston, Mass. It was specified to contain 85–90% of the tritium at C_7 and approximately 10% at C_4. It was purified before use by paper chromatography in a Bush B3 system and its radiochemical purity checked by rechromatography of a sample with carrier testosterone. It was stored at −20° after being dissolved in absolute ethanol at a concentration of 50 μCi/ml. A suitable sample was diluted approx 1:100 with sterile physiological saline at the day of each experiment and filtered through a Millipore® GSWP 025 00 sterile filter, pore size 0·22 μ. The infusion rate was about 34 ml/h, usually equivalent to about 5 μCi/h. [4-^{14}C]-testosterone (NEN) (SA 50·6 Ci/mol) was purified and checked as described for [7-^{3}H]-testosterone. About 1200 d.p.m. of this compound was added to plasma samples obtained during the infusion experiments to allow a correction for losses. When plasma samples, not containing [^{3}H]-testosterone, were used for analysis of plasma testosterone concentration about 5000 d.p.m. of purified [1,2-^{3}H]-testosterone (NEN) (SA 45–55 Ci/mmol) were added as indicator for recovery determinations. [1,2-^{3}H]-5α-dihydrotestosterone used in the determinations of androgen binding capacity was also purified in the

Bush B3 system. The chromatogram was then scanned in a Frieseke-Hoepfner gas flow-scanner and a peak with $R_f = 0{\cdot}69$ was eluted. [^{125}I]-Rose Bengal sodium was purchased from the Radiochemical Centre, Amersham, as a sterile aqueous solution, SA 150 μCi/mg. About 20 μCi were infused in each dog experiment.

Plasma testosterone

Plasma testosterone concentrations were determined by competitive protein binding as previously described [7]. When [^{14}C]-testosterone was used as indicator it was added to 10–15 ml plasma. Since only 1–2 ml was used for the determination carbon-14 did not disturb

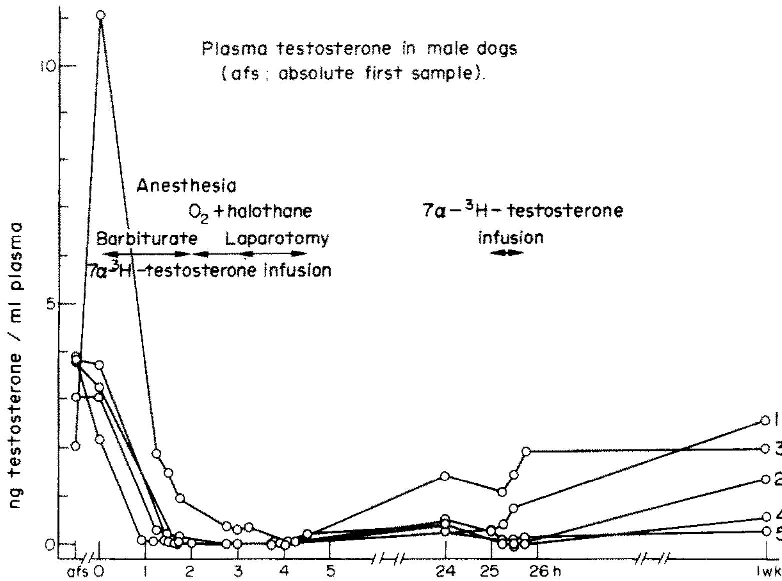

Fig. 1. Plasma testosterone concentration in male dogs. Absolute first sample (afs) and sample in conscious dogs before anesthesia (0). Concentrations were then determined during constant infusion experiments under anesthesia and surgery, then in conscious dogs 25–26 h later. The sample obtained at 24 h was taken at the start of the infusion experiment. A final sample was obtained by venepuncture 1 week later (1 wk) in conscious dogs.

Table 1. Plasma testosterone concentration, ng/100 ml, in dogs before, during and after anesthesia and laparotomy. Constant infusion of [7α-^{3}H]-testosterone and [^{125}I]-Rose Bengal to study testosterone dynamics.

	Experimental conditions	n	Median	Range	Comparison	Significance* (P in parenthesis)
I	Before anesthesia	5	325	217–1107		
II	Thiomebumal anesthesia + vena hepatica catheterization After 75–120 min constant infusion	5	4·3	1·5–142	I	S. (<0·025)
III	O_2 + halothane, 60–90 min	5	1·5	0–35	II	N.S. (>0·05)
IV	Laparotomy, 40–90 min	5	1·5	0–20	III	N.S. (>0·05)
V	1 day later, awake					
a	Before constant infusion	5	41	23–138	I	S. (<0·025)
					IV	S. (<0·05)
b	After 75–105 min constant infusion	5	10·3	0–145	I	S. (<0·025)
					IV	N.S. (>0·05)
					Va	S. (<0·05)
VI	1 week later, awake	5	132	26–253	I	S. (<0·05)
					Vb	S. (<0·05)
VII	6 weeks later, awake	4	132	40–273	I	S. (<0·05)
					Vb	S. (<0·05)

* Lord's t-test based on range, paired, one-tailed[11].

the assay. The fraction used for analysis was adjusted to give a reading in the most sensitive part of the standard curve (beween 0·35 and 1·5 ng). All analyses were performed on duplicate or triplicate plasma samples. Very low or zero concentrations were reanalyzed by radioimmunoassay using a testosterone-3-oxime-BSA antibody purchased from Endocrine Sciences, Tarzana, Cal.[8].

Measurement of radioactivity

Tritium was measured in a liquid scintillation spectrometer (Packard Model 3310) using an automatic external standard for quench correction. ^{125}I was measured in 1 ml plasma samples using an automatic gamma spectrometer (Wallac Decem) with a window setting at 8–16%, a multiplier setting of 0·25 and a high voltage of 840 V. ^{125}I was simultaneously measured in 1 ml samples of diluted infusion solution. No standards were therefore necessary. Enough counts were accumulated to ensure a counting error of less than 2%.

Calculations

In vivo steroid dynamics were treated using the principles for the constant infusion method given by Tait and Burstein[9]. Hepatic extraction (HE) was calculated from the difference in plasma concentration of radioactivity (^{3}H or ^{125}I) between peripheral venous blood and hepatic venous blood, assuming that the difference between the blood entering the liver and peripheral venous blood is negligible. Hepatic blood flow (HBF) was calculated from the equation HBF = MCR/HE for [^{125}I]-Rose Bengal sodium[10]. The hepatic metabolic clearance rate for testosterone (MCR_{hep}) was obtained from the equation MCR_{hep} = HE.HBF. Statistical treatment of the data were Lord's paired *t*-test based on range[11] or Student's paired *t*-test according to Pearson[12], using a Hewlett-Packard desk calculator.

RESULTS

Plasma testosterone concentration

The absolute first sample ranged between 254 and 902 ng/100 ml (median 469) and did not differ significantly from the concentration before anesthesia (Table 1). A drastic decrease occurred 75–120 min after thiomebumal anesthesia, continued during halothane

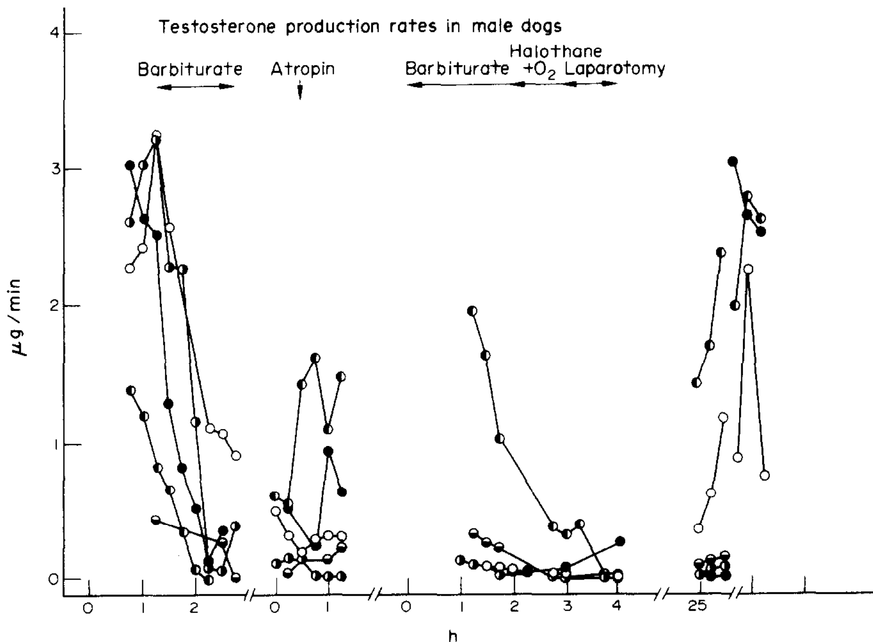

Fig. 2. *In vivo* testosterone production rates in male dogs by the constant infusion method, first in conscious dogs, then during barbiturate (thiomebumal) anesthesia. One week later a new experiment was carried out on the dogs while conscious. After 1 h atropin, 0·5 mg, was injected i.v. After a rest period of at least 1 week an infusion experiment was conducted with the constant infusion of [7-^{3}H]-testosterone + [^{125}I]-Rose Bengal sodium. After induction of barbiturate narcosis a catheter was introduced in one of the external jugular veins under local anesthesia and placed in one branch of the hepatic veins for collection of hepatic venous blood. Halothane + oxygen anesthesia was started after 2 h using an endotracheal tube. Laparotomy was performed after 1h. Production rates were determined in conscious dogs 1 day later using infusion of both [7-^{3}H]-testosterone and [^{125}I]-Rose Bengal. The last infusion experiment was performed in three of the dogs approx. 6 months later while conscious.

Table 2. Testosterone production rates, ng/min, in five dogs during anesthesia, and laparotomy and awake the day thereafter. Constant infusion of [7α-^{3}H]-testosterone and [^{125}I]-Rose Bengal to study testosterone dynamics

	Experimental conditions	Median	Range	Comparison	Significance* (*P* in parenthesis)
I	Thiomebumal anesthesia + vena hepatica catheterization After 75–120 min constant infusion	62	17·5–263		
II	O_2 + halothane After 45–90 min	17·5	0–357	I	N.S. (>0·05)
III	Laparotomy After 40–65 min	14	0–242	I	N.S. (0·05)
				II	N.S. (>0·05)
IV	Awake 1 day later After 75–105 min constant infusion	119	0–1812	I	N.S. (>0·05)
				II	N.S. (>0·05)
				III	N.S. (>0·05)

* Lord's *t*-test based on range, paired, one-tailed[11].

Table 3. Plasma metabolic clearance rates for testosterone in five dogs, ml/min, during anesthesia, laparotomy and awake the day thereafter. Constant infusion of [7α-^{3}H]-testosterone and [^{125}I]-Rose Bengal

	Experimental conditions	Median	Range	Comparison	Significance* (*P* in parenthesis)
I	Thiomebumal anesthesia + vena hepatica catheterization After 75–120 min constant infusion	1251	1083–1409		
II	O_2 + halothane After 45–90 min	1116	870–1213	I	S. (<0·025)
III	Laparotomy After 40–65 min	957	681–1239	I	S. (<0·025)
				II	N.S. (>0·05)
IV	Awake 1 day later After 75–105 min constant infusion	1257	1150–1574	I	N.S. (>0·05)
				II	S. (<0·005)
				III	S. (<0·025)

* Lord's *t*-test based on range, paired, one-tailed[11].

+ oxygen narcosis and the subsequent laparotomy, finally reaching zero values in all dogs (Fig. 1). Concentrations were very low 24 h later and were then decreased further during the infusion experiment that followed. They were higher but not restored to normal 1 and 6 weeks later. Atropin had no effect within 1 h.

Testosterone plasma production rate

Testosterone plasma production rates ranged between 2·3 and 3·3 μg/min in unhandled conscious dogs (Fig. 2). Thiomebumal narcosis caused a pronounced decrease. Zero values were reached in most dogs during halothane + oxygen narcosis or during the subsequent laparotomy (Table 2). Very low production rates were noted on the second day and normal production rates when tested in 3 of the dogs 6 months later. Atropin had no effect within 1 h.

Testosterone plasma metabolic clearance rate

Testosterone plasma MCR was about 1·25 l/min. Barbiturate or atropin had no effect (Fig. 3). A small but significant decrease occurred during halothane + oxygen narcosis and the subsequent laparotomy (Table 3).

Testosterone hepatic plasma clearance rate, extrahepatic plasma clearance rate and hepatic extraction

The MCR_{hep} for testosterone did not change during halothane-oxygen narcosis or laparotomy when compared to values obtained during thiomebumal narcosis or in conscious dogs one day later (Table 4). This was in contrast to the significant decrease observed in the extrahepatic MCR for testosterone during halothane + oxygen anesthesia and laparotomy (Table 5). The

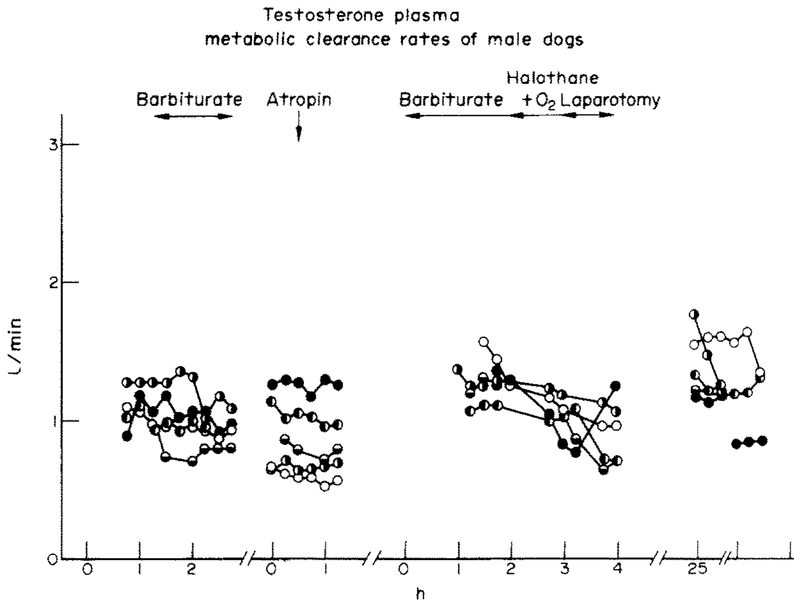

Fig. 3. Testosterone plasma metabolic clearance rates of male dogs at the same experimental conditions as described for Fig. 2.

hepatic extraction of testosterone was 85–100%. A decrease was noted in some, but not all, dogs during halothane narcosis or laparotomy (Table 6) but seemed to be compensated for by an increase in the hepatic blood flow in these dogs (Table 7). The changes in HE and HBF were not statistically significant.

Testosterone binding globulin capacity and association constant

TeBG capacity and K_A were evaluated from complete Scatchard plots (Fig. 4) and show a significant decrease in binding capacity the day after anesthesia + laparotomy and a concomitant significant increase of K_A (Table 8) when measured in peripheral plasma. There was a small, however not statistically significant, increase in capacity during thiomebumal anesthesia. No difference was observed between hepatic vein and peripheral vein plasma. The small increase of K_A observed in hepatic vein plasma was not very significant. In one dog when testosterone was used as ligand a specific binding capacity of 1·12 μg/100 ml was found. Estradiol-17β did not bind.

Table 4. Hepatic plasma clearance rates for testosterone in four dogs, ml/min, during anesthesia and laparotomy and awake during the day thereafter, assuming the same hepatic extraction as on the previous day under thiomebumal anesthesia. Constant infusion of [7α-^{3}H]-testosterone and [^{125}I]-Rose Bengal

	Experimental conditions	Median	Range	Comparison	Significance* (P in parenthesis)
I	Thiomebumal anesthesia + vena cava catheterization After 75–120 min constant infusion	189	121–243		
II	O_2 + halothane After 45–90 min	162	116–293	I	N.S. (>0·05)
III	Laparotomy† After 40–65 min	190	131–196	I	N.S. (>0·05)
				II	N.S. (>0·05)
IV	Awake 1 day later After 75–105 min constant infusion	173	117–255	I	N.S. (>0·05)
				II	N.S. (>0·05)
				III	N.S. (>0·05)

* Lord's t-test based on range, paired, one-tailed[11].
† n = 3.

Table 5. Extrahepatic plasma clearance rates for testosterone in four dogs, ml/min, during anesthesia and laparotomy and awake during the day thereafter, assuming the same hepatic extraction as on the previous day under thiomebumal anesthesia. Constant infusion of [7α-^{3}H]-testosterone and [^{125}I]-Rose Bengal

	Experimental conditions	Median	Range	Comparison	Significance* (*P* in parenthesis)
I	Thiomebumal anesthesia + vena cava catheterization After 75–120 min constant infusion	1095	840–1288		
II	O_2 + halothane After 45–90 min	887	738–1035	I	S. (<0·05)
III	Laparotomy† After 40–65 min	828	526–919	I	S. (<0·05)
				II	S. (<0·025)
IV	Awake 1 day later After 75–105 min constant infusion	1349	1033–1557	I	N.S. (>0·05)
				II	S. (<0·025)
				III	S. (<0·025)

* Lord's *t*-test based on range, paired, one-tailed[11].
† $n = 3$.

Table 6. Hepatic extraction of testosterone from plasma by simultaneous determination in hepatic vein and peripheral plasma during constant infusion of [7α-^{3}H]-testosterone in dogs during anesthesia and laparotomy

	Experimental conditions	Extraction (%) Dog 1	Dog 2	Dog 3	Dog 4	Comparison	Significance* (*P* in parenthesis)
I	Thiomebumal anesthesia						
	75 min	84·7	93·3	100			
	90 min	92·6	97·2	100	99·4		
	105 min	96·8	100	100	99·4		
	mean	*91·4*	*96·8*	*100*	*99·4*		
II	O_2 + halothane						
	45 min	94·0	97·8	94·6	77·2		
	60 min	94·5	98·4	96·1	44·6		
	90 min			93·1			
	mean	*94·3*	*98·1*	*94·6*	*60·9*	I	N.S. (>0·05)
III	Laparotomy						
	40–60 min	50·3	39·0	96·7	—	I	N.S. (>0·05)
						II	N.S. (>0·05)

* Lord's *t*-test based on range, paired, one-tailed[11].

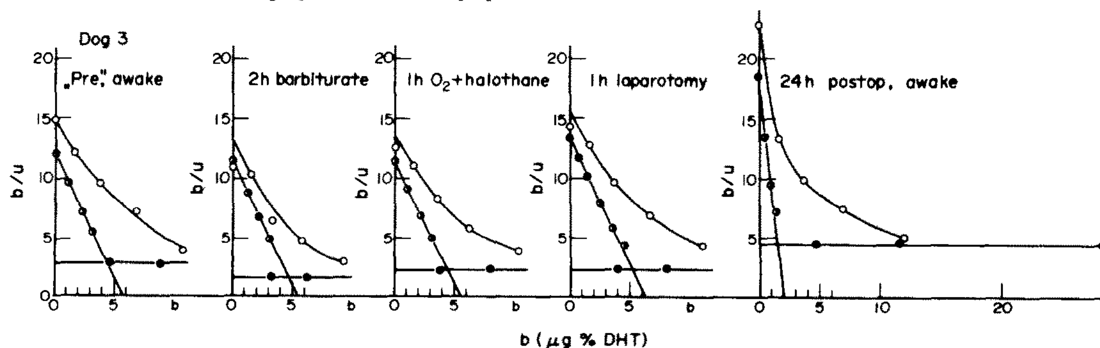

Fig. 4. Typical Scatchard plots in one dog used to determine the specific binding capacity in μg of 5α-dihydrotestosterone (DHT) per 100 ml of plasma. Equilibrium concentrations were determined for DHT in the lower phase of the two-phase system and the ratio of bound/unbound (b/u) plotted against bound (b). The curves were obtained from samples before the infusion experiment in the conscious animal, 2 h after barbiturate narcosis, 1 h after O_2 + halothane narcosis, 1 h after surgery and 24 h postoperatively in the conscious state.

Table 7. Hepatic plasma flow estimated in dogs during anesthesia and laparotomy and awake during the day thereafter from the metabolic clearance rate of constantly infused [^{125}I]-Rose Bengal. The hepatic extraction of Rose Bengal was determined during the first experimental day and assumed to be the same during the second day as on the first day during thiomebumal anesthesia

	Experimental conditions	ml/min Dog 1	Dog 2	Dog 3	Dog 4	Comparison	Significance* (*P* in parenthesis)
I	Thiomebumal anesthesia						
	75 min	119	175	208			
	90 min	124	204	223	170		
	105 min	147	174	297	230		
	mean	*130*	*184*	*243*	*200*		
II	O_2 + halothane						
	45 min	113	173	270	185		
	60 min	132	189	352	337		
	90 min			308			
	mean	*123*	*181*	*310*	*261*	I	N.S. (>0·05)
III	Laparotomy						
	40–60 min	261	502	196	—		
	90 min			151	—		
	mean	*261*	*502*	*174*	—	I	N.S. (>0·05)
						II	N.S. (>0·05)
IV	Awake 1 day later						
	75 min	142	240	269	131		
	90 min	142	219	251	127		
	105 min	146	203	245	125		
	mean	143	221	255	128	I	N.S. (>0·05)

* Lord's *t*-test based on range, paired, one-tailed[11].

DISCUSSION

Many studies of hormone *in vivo* dynamics in animals are complicated by the fact that the animals have to be anesthetized. Testosterone production and metabolism was studied for the first time in trained conscious dogs. Contrary to the belief of Tremblay *et al.*[13] it was shown that barbiturate anesthesia caused a pronounced decrease of plasma testosterone concentration due to an inhibition of the production while the metabolic clearance rate was unchanged. Barbiturate anesthesia

Table 8. Testosterone binding globulin capacity and association constant (K_A) in male dogs before and during the anesthesia and laparotomy experiment in which testosterone dynamics were studied. Mean and standard error for peripheral vein plasma (PV) and for hepatic vein plasma (VH).

		Before	Thiomebumal anesthesia	O_2 + Halothane anesthesia	Laparotomy	The day after
$\mu g DHT/100$ ml						
	PV	3·88[1]	4·22[2]	3·82	3·25[2, 3]	2·01[1, 3]
	SE	0·67	0·48	0·58	0·53	0·18
	n	5	5	5	5	4
	VH		4·10[4]	4·13	3·29[4]	
	SE		0·66	0·62	0·78	
	n		4	4	4	
$K_A \times 10^{-8}$						
	PV	8·70[5, 6, 8]	5·81[6, 7, 10]	6·32[8, 9, 11]	6·65[12]	15·8[5, 10, 11, 12]
	SE	1·45	0·79	0·58	0·85	2·68
	n	4	4	4	4	4
	VH		7·31[7]	7·21[9]	6·71	
	SE		0·40	0·36	0·53	
	n		4	4	4	

Significant differences using Student's paired *t*-test, one-tailed, are indicated by numbers:
$P < 0·001$ 11
$P < 0·025$ 3, 10, 12
$P < 0·05$ 4, 5, 7
$P < 0·1$ 1, 2, 6, 8, 9

was previously shown by Rowe *et al.*[14] to cause a decrease of plasma testosterone levels in male rabbits. Halothane anesthesia caused a small decrease of testosterone plasma MCR in the dogs. The combined procedure of barbiturate + halothane + laparotomy caused a pronounced decrease of plasma testosterone over the subsequent 24 h approaching zero levels. Testosterone metabolism may depend upon specific binding proteins in blood plasma, organ metabolizing enzymes and organ blood flow. One metabolizing organ of particular interest is the liver. It was therefore of interest that we could demonstrate that the hepatic metabolic clearance rate in the dog was only 13–20% of the total, while the hepatic extraction of testosterone approached 100%. We could also show the existence in dog plasma of a specific androgen binding protein that bound testosterone but not estradiol-17β. The assumption that the testosterone binding protein in dog plasma was transcortin has thus been disproved [15]. The capacity of this protein was higher than that of TeBG in the human male but its affinity was slightly lower. After near exhaustion of the body with regard to testosterone for 24 h the capacity of the androgen binding protein was reduced to half its normal value. Testosterone may thus stimulate the production of this protein in the dog which may be different from the situation in the human in whom estrogen is a well-known stimulator. In this connection it is notable that testosterone metabolism was shown to be equal in male and female dogs[16]. It must be suspected that this is due to compensating differences in organ metabolism in male and female dogs. The high extrahepatic metabolism of testosterone in the male dog is in accord with the extrahepatic metabolism of cortisol recently shown in male and female dogs[17]. The increased affinity found for testosterone binding globulin one day after anesthesia + laparotomy is difficult to interpret. The most likely explanation is a conformational change of the protein in the absence of testosterone giving rise to an increased affinity. Another explanation may be the existence of two different proteins of which one disappeared, disclosing the other one with a higher affinity. Work is in progress to elucidate this question.

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DISCUSSION

Schrader:

I'm a little curious about your concept of the equilibrium constant for the binding of the steroid changing from one day to the next. That constant ought to be varied only by changing essentially the amino acid composition of the protein. It ought to be a function of the conformation of the protein. It would seem to indicate to me that perhaps there is some other error in your determination which is artificially changing what you think is the constant.

Carstensen:

I don't think there is any error in the determination. We have thought of the possibility that the absence or almost complete absence of testosterone for 24 h may induce the conformational change in the protein. Of course, other factors have to be investigated, for example, post-operative increases of fatty acids and maybe other types of fats in the plasma. We don't know about that.

Schrader:

Well, in cases of receptors within the cells, clearly as the receptor titers go up and down within the cells in a number of systems the equilibrium constants stay very much the same and it's merely the number of binding sites that change.

Tait, J. F.:

Dr. Carstensen, with such a high hepatic extraction and the extra hepatic clearance being five times the hepatic clearance do not your total metabolic clearance rates equal or even exceed the cardiac outputs?

Carstensen:

We haven't really measured the cardiac output in these dogs, but the figures for the metabolic clearance rates for testosterone are close to the cardiac output as measured by Bradbury.

Hansson:

We have studied only five dogs for the presence of a TeBG-like protein in serum. Corvol reported recently that only 13 out of 27 species examined did contain any TeBG, using polyacrylamide gel electrophoresis at 0°C. We are also unable to identify any TeBG-like protein in the dog serum with properties similar to TeBG in human, monkey and rabbit sera. I therefore wonder if you know more about the size or molecular properties or any other properties of this binding component which we did not demonstrate by gel electrophoresis.

Carstensen:

We have done polyacrylamide electrophoresis and found that the steroid stripped off the protein very easily. Of course, your finding is exactly the same as Corvol has reported some years ago in the dog so I think the difference in technique will explain the difference in the results.

Hansson:

What was the equilibrium constant at 0°C?

Carstensen:

We have the equilibrium constant at 4°C and that is about 8×10^8 l/mol in the dog.