J. Steroid Biochem. Molec. Biol. Vol. 39, No. 1, pp. 105–113, 1991
Printed in Great Britain.

0960-0760/91 $3.00 + 0.00

A SIMPLE TECHNIQUE FOR COLLECTING BLOOD OF TESTICULAR ORIGIN: APPLICATION TO *IN VIVO* STUDIES ON TESTICULAR STEROIDOGENESIS IN RATS AND *MACACA FASCICULARIS*

CATHERINE BLACKER,* ANTOINE AKAR and ROBERT SCHOLLER
Fondation de Recherche en Hormonologie, 67 boulevard Pasteur, 94260 Fresnes, and 26 boulevard Brune, 75014 Paris, France

(Received 20 December 1990)

Summary—A technique for rapidly collecting blood of testicular origin is described, one which can provide sufficient plasma amounts to investigate some steps of testicular steroid biogenesis *in vivo* in 2 species. In adult male rats, testosterone (T), androstenedione (4A) and 5-androstenediol (5AD) were determined in pampiniform plexus testicular venous blood (PPTV) and peripheral (PV) blood samples before and 2 h after human Chorionic Gonadotropin (hCG). PPTV concentration of 5AD was 0.83 ± 0.1 ng/ml (mean ± SEM) with a PPTV/PV ratio of 7.0 ± 1.0, comparable to a PPTV/PV ratio for 4A of 5.8 ± 1.8. After hCG, PPTV concentration of 5AD significantly increased to 1.28 ± 0.15 ng/ml ($P < 0.05$). Those data are in favor of a participation of 5-ene pathway to testicular biogenesis of T associated to a 4-ene pathway which is predominant. In adult male *Macaca fascicularis*, spermatic vein (SV) concentrations of 5AD and 4A were comparable (3.0 ± 1.2 vs 4.3 ± 1.0 ng/ml) as well as SV/PV ratios under basal conditions (3.5 ± 0.9 vs 5.1 ± 0.1), as well as 48 h after hCG, confirming *in vivo* that both 5-ene and 4-ene pathways are involved in testicular T biogenesis. Testicular production of estradiol (E2), estrone (E1) and their sulfates E2S and E1S showed a SV/PV ratio significantly higher than 1 (3.4 ± 0.6; 2.4 ± 0.1; 1.7 ± 0.2 and 1.6 ± 0.2, respectively).

INTRODUCTION

When the question of the assessment of endocrine function of the testes is addressed, the evaluation of the concentrations of the steroids in peripheral blood (PV) is thought to reflect only partially their production by the gland because they can be affected by many factors including extragonadal steroids production, peripheral metabolism and clearance rate. Steroids concentration in testicular venous blood (TV) provides a more accurate reflection of testicular function and steroid secretion [1]. Therefore, it is worthwhile using a TV sampling technique, but this must be simple and allow the collection of blood volumes sufficient for multiple hormonal assays in a short time period. Moreover, this technique should preserve the integrity of the testis, spermatic cord and vasculature so that it could be applied to *in vivo* studies in different species. In rats, most of the techniques of testicular blood sampling used for testosterone (T) evaluation are derived from the initial works by Suzuki and Eto [2] and Bardin and Peterson [3] where blood is allowed to flow from a testicular superficial vein either after a small incision or through a small catheter; drawbacks of these techniques are either the small volume collected in a short period [4] or the unphysiological conditions produced by TV sampling lasting up to 4 h for the collection of 1–5 ml [2, 5]. Otherwise, if blood is collected from the proximal part of the spermatic vein (SV) [6], T levels are dramatically lower than in testicular veins due both to dilution of TV blood and to the transfer from venous to arterial blood through counter-current exchange in the pampiniform plexus (PP) [1, 7, 8]. In the present study, a technique has been developed in rats for rapid collection of blood from a vein at the start of the PP, in sufficient amounts to allow physiological studies on testicular steroidogenesis. Firstly, it was applied to the evaluation of testicular production of T and its immediate precursors on 4-ene and 5-ene pathways,

*To whom correspondence should be addressed.

Abbreviations: 5-androstenediol, 5-androstene-3β,17β-diol (5-AD); androstenedione, 4-androstene-3,17-dione (4A); estradiol-17β,1,3,5(10)-estratriene-3,17β-diol (E2); estradiol-17β sulfate (E2S); estrone, 3-hydroxy-1,3,5(10)-estratrien-19-one (E1); estrone sulfate (E1S); 17-hydroxy-progesterone (17 OHP); testosterone, 17β-hydroxy-4-androsten-3-one (T).

androstenedione (4A) and 5-androstenediol (5AD), under basal conditions and after stimulation with human Chorionic Gonadotropin (hCG). It was then adapted to *Macaca fascicularis* and used for *in vivo* assessment of the different steps of testicular androgen and estrogen biosynthesis. In particular, it was interesting to find out whether 5AD, the immediate precursor of T in the 5-ene pathway, was secreted by the testis in these 2 different species.

EXPERIMENTAL

TV blood collecting device

A special device has been designed in order to allow rapid blood sampling from rat testicular veins into a polyethylene catheter using both systemic blood pressure and capillarity inside the catheter. A 25 gauge needle (5/10 mm dia and 16 mm long) was inserted into a polyethylene catheter (Biotrol No. 2: 0.38 mm i.d., 1.09 mm o.d.). This 5 cm long catheter was in turn forced into a 2.50 m long polyethylene catheter (Biotrol No. 5: 0.86 mm i.d., 1.27 mm o.d.). The distal part of the latter was inserted through a needle into a 5 ml syringe so that a smooth depression could be produced in the system if necessary (Fig. 1). The internal volume of this device was 1.45 ml.

This device was then adapted for TV blood sampling in *Macaca fascicularis*: the vein diameter allowed the use of a 23 gauge needle inserted into a 1.02 mm i.d., 3 m long tygon catheter (Bioblock Ref. B71164) yielding an internal volume of 2.45 ml.

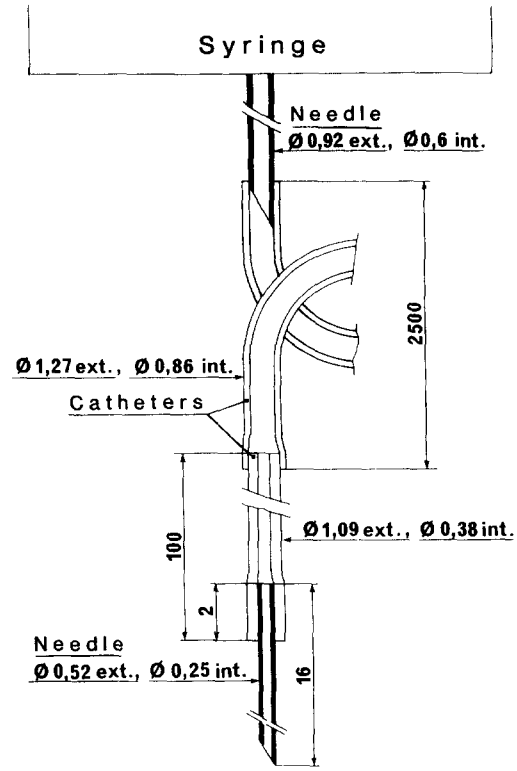

Fig. 1. Scheme of the sampling device (diameters and lengths in mm).

Animals

Rats. Male Wistar rats, 6 months old, weighing 440–660 g, were housed under controlled conditions of temperature (22 ± 1°C) and light (12–24 h); they received standard pelleted diet food and water *ad libitum*.

Monkeys. Adult male *Macaca fascicularis*, 5–11-years-old, weighing 6–11.5 kg, were housed in individual cages under controlled conditions of temperature (26 ± 2°C) and natural photoperiod; they were fed with monkey chow supplemented with fruit and vegetable; water was available *ad libitum* [9].

Experimental protocols

Rats. Animals were divided into 3 groups: group I, control group ($n = 11$); group II, rats were given an i.m. injection of 80 IU/kg hCG (Endo) 1 h before blood samplings ($n = 9$) according to the protocol used by Huhtaniemi *et al.* [10] and group III, rats were given an i.m. injection of 80 IU/kg hCG 2 h before blood samplings ($n = 7$).

PV and TV blood samples were drawn between 11 a.m. and 1 p.m. at the lowest point of T circadian rhythm [11] under ether anaesthesia and after an i.v. injection of 250 IU heparin.

Anaesthetized rats were placed on their backs, and testes were exposed after low midline laparotomy. The fat pad surrounding the PP was carefully dissected. TV blood was drawn with the previously described device from a vein at the start of PP (PPTV) [1]. This technique allowed the withdrawal of a blood volume of 1.2–1.4 ml in approx. 5 min. Immediately after PPTV blood sampling, PV blood was collected from the abdominal aorta.

Blood was centrifuged at 3000 rpm for 10 min and plasma samples were frozen at −20°C until assays. No haemolysis was observed in any sample.

Monkeys. Animals were divided into 2 groups: group I, Control group ($n = 6$); and group II. Animals were given an i.m. injection

of 80 IU/kg hCG 48 h before blood samplings ($n = 6$). This time was chosen because the maximal response occurred at 48 h for most of the steroids in a preliminary study on the kinetics of the testicular responses to a single injection of hCG in 5 animals [12]. Anaesthesia was induced by ketamine (10 mg/kg i.m.) and prolonged with an i.v. infusion of 0.075 g per h of thiopental. The total duration of anaesthesia was 1–2 h.

PV and SV blood samplings were performed in May in all the animals between 9 a.m. and 1 p.m. when the T levels were the lowest and the most steady according to circadian and circannual rhythms [9, 13]. Anaesthetized monkeys were placed on their backs; midline laparotomy was performed under sterile conditions and 1 of the SV was punctured in its retroperitoneal part just above the inguinal ring using the previously heparinized device. Thus, 2.2–2.4 ml of SV blood was collected within 4–5 min. PV blood was simultaneously collected from a saphenous vein; the abdominal wall was then reconstructed in 2 plans. Thereafter, monkeys received a daily i.m. injection of 250 mg/kg ampillicine for 5 days.

Hormone assays

All steroids were determined by RIA after column chromatography on either celite for T, 4A and 5AD [14, 15], or Sephadex LH20 for estrone (E1), estradiol (E2) [16] and 17-hydroxyprogesterone (17 OHP) [17]. Estrogen sulfates, E1S and E2S, were solvolyzed after the preliminary extraction of unconjugated steroids [18]. All the methods were validated for assay in rat and *Macaca fascicularis* plasma, according to the statistical procedures described by Scholler [19].

Statistical analysis

Normality of distributions was assessed using both Lilliefors (Kolmogorov–Smirnov modified) and Shapiro–Wilk tests. Whenever normality could be assumed, comparisons of unpaired or paired samples were made with 1-tailed Student's t-test; Mann–Whitney or Wilcoxon non-parametric tests were otherwise addressed [20, 21].

For 3 samples comparisons (rats) weighted 1-way analysis of variance (weighted ANOVA) was performed using RS1 procedures (BBN Software Products Cor., Cambridge, MA) as implemented on a DEC M3500 Microvax. Then, when underlying assumptions were tenable, the Duncan new multiple range test and Newman–Keuls test were performed.

RESULTS

Rats

Mean concentrations of T, 4A and 5AD in PPTV and PV under basal conditions and after hCG administration are given in Fig. 2.

Under basal conditions, mean concentrations of T, 4A and 5AD were significantly higher in PPTV than PV blood; the PPTV/PV ratio was 164.4 ± 60.7 (mean $\pm$ SEM) for T, 5.8 ± 1.8 for 4A and 7.0 ± 1.0 for 5AD, indicating a testicular production of the 3 steroids. After hCG administration, mean PPTV blood concentrations of T, 4A and 5AD were also significantly higher than PV ones, 1 h after hCG (group II) and 2 h after hCG (group III); the PPTV/PV ratio was 92.6 ± 26.5 for T, 16.5 ± 4.3 for 4A and 9.6 ± 1.3 for 5AD in group II and 54.8 ± 7.6 for T, 12.0 ± 2.2 for 4A and 14.6 ± 2.7 for 5AD in group III. The response of the steroids to hCG stimulation was tested with weighted ANOVA and results are shown in Table 1. The most significant increase for T and 4A in PV and PPTV blood was observed 2 h after hCG. The increase of 5AD after stimulation was not significant in PV but was significant in PPTV blood 2 h after hCG.

Monkeys

Mean concentrations of T, 4A, 5AD and 17 OHP in PV and SV blood before and 48 h after hCG are represented in Fig. 3, those of estrogens and estrogen sulfates under the same conditions in Fig. 4.

Before hCG, the mean concentrations in SV were significantly higher than in PV blood for all the steroids but E1. Mean SV/PV ratios are represented in Table 2. The highest SV/PV ratios were observed for 17 OHP and T.

Forty-eight hours after hCG, the mean concentrations in SV were significantly higher than in PV blood for all the steroids. As under basal conditions, the highest mean SV/PV ratios were observed for T and 17 OHP. A significant increase 48 h after hCG stimulation was observed in the SV as well as in PV blood for all the steroids except 5AD (Table 3).

In 5 out of the 6 monkeys investigated after hCG administration, blood could be drawn from right and left testicular veins at 20 min

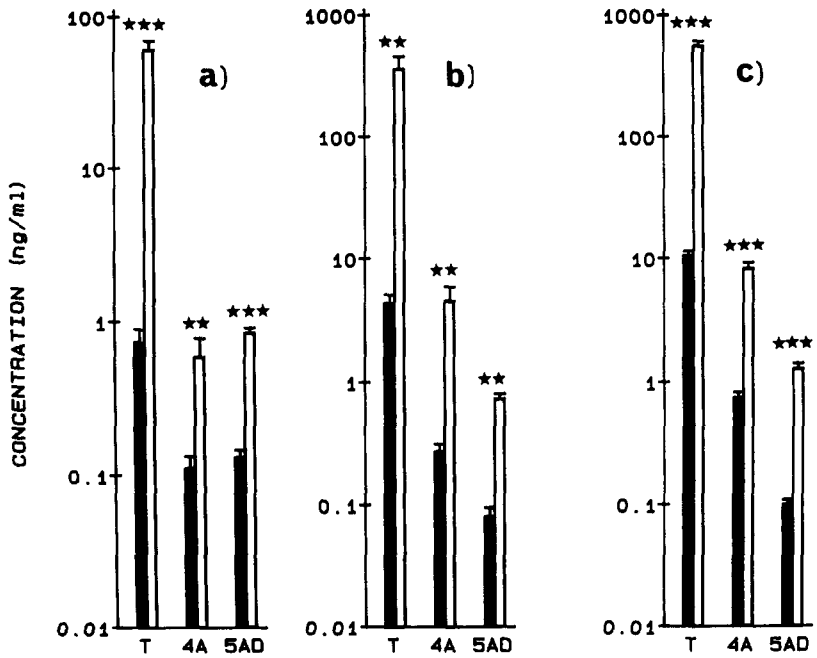

Fig. 2. Concentrations (logarithmic scale) of T, 4A and 5AD in PV (■) and PPTV (□) blood in rats before (a), 1 h (b) and 2 h (c) after 80 IU hCG/kg i.m. $^{**}P < 0.01$, $^{**}P < 0.001$.

intervals (Table 4). These data show that, under hCG stimulation, the production by the testes of T and its immediate precursors 4A and 5AD is steady over a short period of time.

DISCUSSION

The measurement of T and other steroids in TV blood is considered to be a better way of assessing testicular function as peripheral levels can be affected by various parameters including peripheral metabolism, clearance rate or modifications of peripheral blood flow [22] as well as extragonadal steroid production mainly by adrenals.

In rats, different techniques of TV blood sampling have been described. Most of them consist of a blood collection from a testicular superficial vein near the head of the epididymis, either through a small catheter [2, 5, 23] or directly into a large tube after incision of the vein wall [3, 4, 24, 25]. The limitation of this technique is the volume of blood collected: it is very small, from 25–400 μl [4, 24, 25] except if the collection period is sufficiently long, up to 4 h, to collect 5 ml (2); this causes unphysiological conditions due to prolonged anaesthesia including modifications of the testis vasculature and temperature. Another procedure is the cannulation of a spermatic vein near the point of entry into the caval vein [6]; but it has been demonstrated that a dramatic reduction of T concentration occurs from the superficial testicular veins to the spermatic veins at the proximal part of the spermatic cord due both to the dilution of the TV blood cord and to the transfer of T from venous to arterial blood in the PP through counter-current exchange [1, 7, 8]. In the present study, TV blood sampling was performed at the distal (testicular) end of the PP; in this region T concentration is not different from the one observed in the testicular superficial vein [1]. Our collecting device allows, under the effect of capillarity in the long catheter, a rapid sampling of a volume of approx. 1.5 ml, which is sufficient

Table 1. Weighted ANOVA for concentrations of T, 4A and 5AD in PV and PPTV blood in control rats, 1 h and 2 h after 80 IU hCG i.m.

	T		4A		5AD	
Comparisons	PV	PPTV	PV	PPTV	PV	PPTV
1 h vs control	$P < 0.001$	$P < 0.01$	$P < 0.05$	$P < 0.01$	NS*	NS
2 h vs control	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS	$P < 0.05$
2 vs 1 h	$P < 0.001$	NS	$P < 0.001$	$P < 0.01$	NS	$P < 0.05$

*NS = no significant difference.

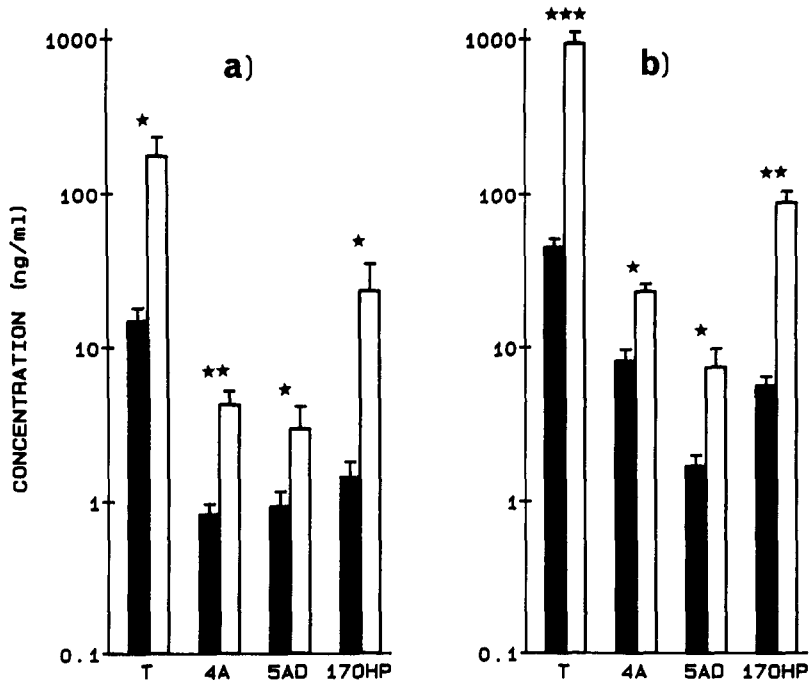

Fig. 3. Concentrations (logarithmic scale) of T, 4A, 5D and 17 OHP in PV (■) and SV (□) blood in *Macaca fascicularis* before (a) and 48 h after (b) 80 IU hCG/kg i.m. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

for biogenetic and metabolic studies. The short duration of sampling is an important factor for physiological studies because anaesthesia using ether, ketamine, pentobarbital or halogenated anaesthetics is responsible for a decrease in T production by the testes which is time dependent [26].

T concentration observed in PPTV blood under basal conditions is concordant with most of the previous studies [1, 4, 6, 23, 24, 26]

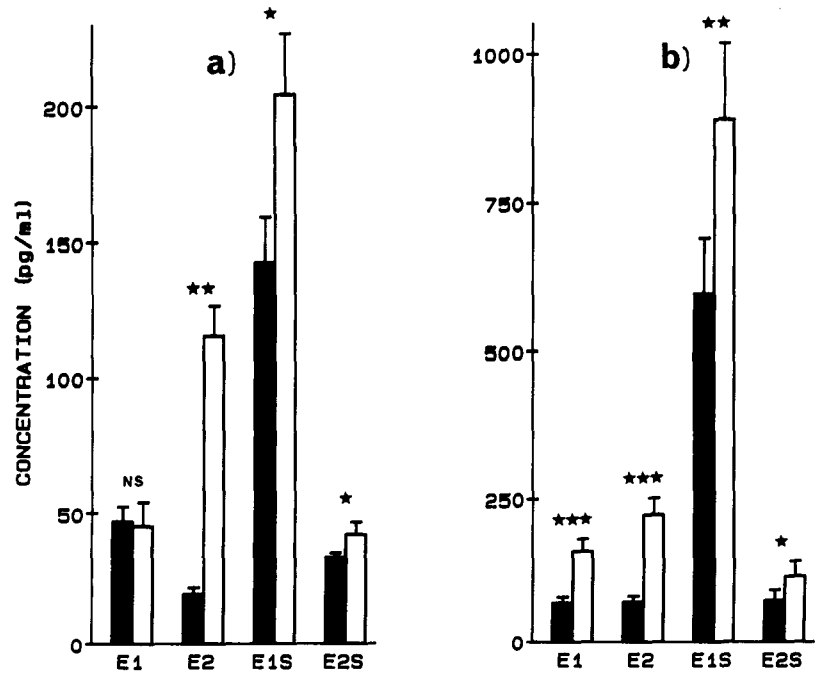

Fig. 4. Concentrations of E1, E2 and their sulfates (E1S and E2S) in PV (■) and SV (□) blood in *Macaca fascicularis* before (a) and 48 h after (b) 80 IU hCG/kg i.m. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 2. SV/PV blood concentration ratio for T, 4A, 5AD, 17 OHP, E1, E2, E1S and E2S in *Macaca fascicularis* before and 48 h after 80 IU hCG i.m.

	SV/PV before hCG	SV/PV after hCG
T	11.4 ± 3.0	21.6 ± 3.5
4A	5.1 ± 0.1	3.1 ± 0.3
5AD	3.5 ± 0.9	5.3 ± 1.5
17 OHP	20.8 ± 11.3	15.5 ± 1.0
E1	1.1 ± 0.4	2.4 ± 0.1
E2	6.5 ± 0.9	3.4 ± 0.6
E1S	1.5 ± 0.1	1.6 ± 0.2
E2S	1.3 ± 0.1	1.7 ± 0.2

Results are expressed as mean ± SEM.

including those where blood was taken from testicular superficial veins. This confirms that, at the point where the vein is punctured, there is no effect of dilution or counter-current exchange. PPTV blood concentration of 4A as well as PV one has not been previously determined with success because of the poor sensitivity of assays and high blanks [2, 3, 5]. The present data show 4A concentrations in PPTV blood approx. 10 times higher than in PV blood. 5AD has been previously measured in peripheral blood [27, 28] and our data are in agreement with the results of these authors. For the first time, the present study presents evidence of higher concentrations of 5AD in TV blood, and with a PPTV/PV ratio similar to that of 4A. This finding is all the more interesting because 5AD has been found in testicular tissue [27, 28], whereas Chubb and Ewing [30] were unable to detect it in perfusion medium of rat testis.

The early testicular response to hCG was studied 1 and 2 h after i.m. administration of 80 IU/kg hCG. Concerning peripheral T after hCG, our results confirm the data of Tremblay and Belanger [28] on a maximal response at 2 h whereas for Huhtaniemi *et al.* [10] higher levels were observed at 1 h. Moreover, the response was more homogeneous in group III, at 2 h, than in group I at 1 h in peripheral as well as testicular veins. Similarly, a peripheral 4A peak

Table 4. Comparison of concentrations (ng/ml) of T, 4A and 5AD in right (RSV) and left (LSV) spermatic venous blood at a 20 min interval, 48 h after 80 IU hCG/kg i.m. in *Macaca fascicularis*

	RSV 48 h			LSV 48 h + 20 min		
Animals	T	4A	5AD	T	4A	5AD
1	559.8	18.5	5.9	598.1	10.6	7.6
2	1581.1	29.2	15.5	1503.4	36.1	16.8
3	723.5	20.1	4.2	584.2	19.2	3.2
4	823.9	18.7	1.8	919.2	21.6	5.7
5	1236.1	33.4	13.5	892.3	25.7	12.1

was observed at 2 h as previously described by Tremblay and Belanger [28] and at this time, PPTV/PV ratio was higher than in basal conditions. Concerning peripheral 5AD, we did not observe any significant rise after hCG, whereas Tremblay and Belanger [28] found a small increase. But interestingly, in PPTV blood, 5AD levels were significantly higher 2 h after hCG.

Those data show a testicular production of 5AD which is in favor of an involvement of the 5-ene pathway in the testicular biogenesis of T, associated to the 4-ene pathway which is predominant in rats [31]. The minor concentration of 5AD among the steroids in testicular veins and the weak rise after hCG could be explained less by the rapid conversion of 5AD into T [32] than by the switch from the 5-ene to 4-ene pathway which mainly occurs at the 17-hydroxypregnenolone level [33].

In monkeys, only a few studies have been dedicated to the evaluation of T in TV or SV blood [34, 35, 36]; no one, to our knowledge, addressed *Macaca fascicularis*, and in only one has another steroid, E2, also been assayed [36]. Blood of testicular origin has been previously collected in *Macaca mulatta* either from a testicular superficial vein [35] or from a branch of the SV [36]. In the present protocol, SV blood was drawn from a retroperitoneal SV; the diminution of T concentration from testicular superficial vein to retroperitoneal SV seems, in monkeys, of minor importance [37]. So, at the

Table 3. Concentrations (ng/ml) of T, 4A, 5AD, 17 OHP and concentrations (pg/ml) of E1, E2, E1S and E2S in PV and SV blood in control *Macaca fascicularis* and 48 h after 80 IU hCG i.m.

	PV		SV	
	Control	48 h	Control	48 h
T	14.9 ± 3.3	45.4 ± 6.3[a]	177.9 ± 56.6	954.4 ± 161.4[a]
4A	0.8 ± 0.1	8.2 ± 1.5[a]	4.3 ± 1.0	23.4 ± 2.6[b]
5AD	0.9 ± 0.2	1.7 ± 0.3[a]	3.0 ± 1.2	7.5 ± 2.3
17 OHP	1.4 ± 0.4	5.7 ± 0.8[a]	23.7 ± 11.6	88.6 ± 16.2[a]
E1	47 ± 6	68 ± 10[c]	45 ± 9	160 ± 21[a]
E2	19 ± 2	70 ± 10[b]	116 ± 11	224 ± 27[a]
E1S	143 ± 17	596 ± 95[a]	205 ± 22	890 ± 128[a]
E2S	33 ± 2	72 ± 19[a]	42 ± 5	116 ± 27[a]

Results are expressed as mean ± SEM.
Significant increase after 48 h.
[a] $P < 0.01$; [b] $P < 0.001$; [c] $P < 0.05$.

point where SV blood was drawn, steroids concentration can be considered as reflecting the testicular endocrine function.

In the present study, peripheral T and 4A levels, under basal conditions, were in agreement with the previous data of Dang and Meusy-Dessolle [9], Steiner *et al.* [13] and Meusy-Dessolle and Dang [38] in non-anaesthetized *Macaca fascicularis*. Peripheral E2 levels were lower than those reported by Meusy-Dessolle and Dang [38]; there is no clear technical explanation for this discrepancy but it should be noted that our data are closer to those found in *Macaca mulatta* [36, 39, 40] and in men [10, 18]. Peripheral concentrations of the other steroids have not been previously evaluated in *Macaca fascicularis*: 17 OHP and 4A levels were comparable to those observed in *Macaca mulatta* [39, 40] and our data showed equal peripheral concentrations for 4A and 5AD. Concerning estrogens, E1 levels were higher than those of E2 as it has been reported in *Macaca mulatta* [39, 40] and E1S was the major circulating estrogen. In SV blood, T was more than 10 times higher than in PV, as reported in *Macaca mulatta* by Einer-Jensen and Waites [35] whereas Resko *et al.* [36] found T concentrations only 3 times higher in blood drawn from a distal SV than in PV. The second major component in SV blood was 17 OHP for which the SV/PV ratio was the highest observed under basal conditions. The concentrations of the immediate precursors of T in the 4-ene pathway, 4A, and in the 5-ene pathway, 5AD, were similar in SV, as it was observed in PV blood; their SV/PV ratios showed testicular production of both of them. Concerning estrogens, levels were higher in SV than in PV blood for all but E1. The SV blood concentration of E2 was identical to the one observed in *Macaca mulatta* [36].

After hCG stimulation, peripheral T response is biphasic with a first rise between 2 and 4 h and a major increase 48 h after i.m. injection in *Macaca fascicularis* [12] and in *Macaca mulatta* [41]. 48 h after hCG, all the steroids were significantly increased in PV blood except 5AD which showed no significant variation at any time of the stimulation [12]. The increase of 17 OHP is concordant with the data of Davies *et al.* [42] in *Macaca mulatta*. The stimulation of testicular production of 4A and E2 has been previously reported only after chronic hCG administration in *Macaca mulatta* [43]. In SV blood, all the steroids were increased 48 h after hCG, but the rise did not reach a significant level for 5AD. As under basal conditions, the highest SV/PV ratios were observed for T and 17 OHP and the SV/PV ratios for 4A and 5AD were comparable. The SV blood concentration of all the estrogens, including E1, was significantly higher than in PV blood after hCG.

The data collected in the present study show firstly testicular production *in vivo* of 5AD in *Macaca fascicularis*. This is in agreement with the *in vitro* results of Preslock and Steinberger [44] and Higashi *et al.* [45] in *Macaca mulatta* suggesting that T is formed through both the 4-ene and 5-ene pathways; moreover, the latter group found 5AD tissue concentrations greater than those of 4A and demonstrated the formation of 5AD from its precursors of the 5-ene pathway. Secondly, 17 OHP appears as the second major steroid released by the testis, a likely effect of the low activity of C17–20 lyase for 17 OHP [45]. Finally, testicular production of estrogens as well as estrogen sulfates is also demonstrated in *Macaca fascicularis* as it has also been shown in man [18].

In summary, assessment of steroids in TV blood appears as an adapted way to evaluate, *in vivo*, testicular endocrine function. This requires a blood sampling procedure which is rapid and simple and yields a sufficient blood volume for metabolic studies. The technique described first allowed us to demonstrate 5AD testicular production in rats *in vivo*. In *Macaca fascicularis*, it allowed us to describe *in vivo* the participation of the 4-ene and 5-ene pathways in T biogenesis, as proved by *in vitro* studies in *Macaca mulatta*, and to demonstrate testicular production of E1 and E2 as well as their sulfates.

Acknowledgements—The authors are particularly grateful to Dr C. Dang for his help and useful advise in managing the *Macaca fascicularis* in the laboratory of anatomy of the Saints Pères UER, Paris.

REFERENCES

1. Maddocks S. and Sharpe R. M.: Dynamics of testosterone secretion by the rat testis: implications for measurement of the intratesticular levels of testosterone. *J. Endocr.* **122** (1989) 323–329.
2. Suzuki Y. and Eto T.: Androgens in testicular venous blood in the adult rat. *Endocr. Jap.* **9** (1962) 277–283.
3. Bardin C. W. and Peterson R. E.: Studies of androgen production by the rat: testosterone and androstenedione content of blood. *Endocrinology* **80** (1967) 38–44.
4. Knorr D. W., Vanha-Perttula T. and Lipsett M. B.: Structure and function of rat testis through pubescence. *Endocrinology* **86** (1970) 1298–1304.

5. Hashimoto I. and Suzuki Y.: Androgens in testicular venous blood in the rat, with special reference to pubertal change in the secretory pattern. *Endocr. Jap.* **13** (1966) 326–337.
6. Damber J.-E. and Janson P. O.: Testicular blood flow and testosterone concentrations in spermatic venous blood of anaesthetized rats. *J. Reprod. Fert.* **52** (1978) 265–269.
7. Free M. J. and Jaffe R. A.: Dynamics of venous–arterial testosterone transfer in the pampiniform plexus of the rat. *Endocrinology* **97** (1975) 169–177.
8. Punjabi U., van Hoecke J., Verdonck L. and Vermeulen A.: Testicular blood flow in young and old rats and influence of hCG. *J. Androl.* **5** (1984) 223–226.
9. Dang D. C. and Meusy-Dessolle N.: Annual plasma testosterone cycle and ejaculatory ability in the laboratory-housed crab-eating macaque (*Macaca fascicularis*). *Reprod. Nutr. Dev.* **21** (1981) 59–68.
10. Huhtaniemi I., Bolton N. J., Martikainen H. and Vihko R.: Comparison of serum steroid responses to a single injection of hCG in man and rat. *J. Steroid Biochem.* **19** (1983) 1147–1151.
11. Kinson G. A. and Liu C-C.: Diurnal variation in plasma testosterone of the male laboratory rat. *Horm. Metab. Res.* **5** (1973) 233–234.
12. Akar A., Blacker C., Dang D. C., Nahoul K., Castanier M. and Scholler R.: Etude de la sécrétion testiculaire de testostérone, d'androstènedione et de 5-androstène-3-beta, 17-beta-diol chez *Macaca fascicularis*, avant et après hCG, par prélèvements simultanés dans les veines périphérique et spermatique. *Path. Biol., Paris* **34** (1986) 924–925.
13. Steiner R. A., Peterson A. P., Yu J. Y. L., Conner H., Gilbert M., terPenning B. and Bremner W. J.: Ultradian luteinizing hormone and testosterone rhythms in the adult male monkey, *Macaca fascicularis*. *Endocrinology* **107** (1980) 1489–1493.
14. Roger M., Nahoul K., Toublanc J. E., Castanier M., Canlorbe P. and Job J. C.: Les androgènes plasmatiques chez le garçon de la naissance à l'adolescence. *Anns Pédiat.* **26** (1979) 239–245.
15. Nahoul K., Bournique B., Adeline J. and Scholler R.: Radioimmunoassay of 5-androstene-3-beta, 17-beta-diol in plasma and in breast cyst fluid. *J. Steroid Biochem.* **24** (1986) 835–842.
16. Castanier M. and Scholler R.: Dosage radioimmunologique de l'estrone et l'estradiol-17 bêta plasmatiques. *C. R. Acad. Sci., Paris* **271** (1970) 1787–1789.
17. Tea N. T., Castanier M., Roger M. and Scholler R.: Simultaneous radioimmunoassay of plasma progesterone and 17-hydroxyprogesterone, normal values in children, in men and in women throughout the menstrual cycle and in early pregnancy. *J. Steroid Biochem.* **6** (1975) 1509–1516.
18. Scholler R., Nahoul K., Castanier M., Rotman J. and Salat-Baroux J.: Testicular secretion of conjugated and unconjugated steroids in normal adults and in patients with varicocele. Baseline levels and time-course response to hCG administration. *J. Steroid Biochem.* **20** (1984) 203–215.
19. Scholler R.: 1. Exactitude. Fidélité. Limite de détection. In *Contrôle de Qualité en hormonologie. I. Steroides urinaires, Paris-Fresnes* (Edited by R. Scholler) S.E.P.E., Paris (1977) pp. 31–77.
20. Conover W. J.: *Practical Nonparametric Statistics.* John Wiley, New York (1980) p. 493.
21. Zar J. H.: *Biostatistical Analysis.* Prentice-Hall, Englewood Cliffs, NJ (1984) p. 718.
22. Maddocks S. and Setchell B. P.: The physiology of the endocrine testis. *Oxf. Rev. Reprod.* **10** (1988) 53–123.
23. Cooper T. G. and Waites G. M. H.: Testosterone in rete testis fluid and blood of rams and rats. *J. Endocr.* **62** (1974) 619–629.
24. Mock E. J. and Frankel A. I.: Response of testosterone to hemicastration in the testicular vein of the mature rat. *J. Endocr.* **92** (1982) 231–236.
25. Handelsman D. J., Spaliviero J. A. and Turtle J. R.: Testicular function in experimental uremia. *Endocrinology* **117** (1985) 1974–1983.
26. Free M. J., Jaffe R. A. and Cheng H-C.: Effect of anesthetics on testosterone production in rats. *J. Androl.* **1** (1980) 182–196.
27. Punjabi U., Deslypere J. P., Verdonck L. and Vermeulen A.: Androgen and precursor levels in serum and testes of adult rats under basal conditions and after hCG stimulation. *J. Steroid Biochem.* **19** (1983) 1481–1490.
28. Tremblay T. and Belanger A.: Changes in plasma steroid levels after single administration of hCG or LHRH agonist analogue in dog and rat. *J. Steroid Biochem.* **22** (1985) 315–320.
29. Bélanger A., Caron S. and Picard V.: Simultaneous radioimmunoassay of progestins, androgens and estrogens in rat testis. *J. Steroid Biochem.* **13** (1980) 185–190.
30. Chubb C. and Ewing L. L.: Steroid secretion by *in vitro* perfused testes: secretions of rabbit and rat testes. *Am. J. Physiol.* **237** (1979) E231–238.
31. Preslock J. P.: A review of *in vitro* testicular steroidogenesis in rodents, monkeys and human. *J. Steroid Biochem.* **13** (1980) 965–975.
32. Slaunwhite W. R. Jr and Burgett M. J.: *In vitro* testosterone synthesis by rat testicular tissue. *Steroids* **6** (1965) 721–735.
33. Chubb C. and Ewing L. L.: Steroid secretion by *in vitro* perfused testes: testosterone biosynthetic pathways. *Am. J. Physiol.* **237** (1979) E247–E254.
34. Scardino P. T., Bergman S., Terrill R. and Javadpour N.: Chapter XVI. Urologic surgery. Countercurrent exchange of testosterone in the primate spermatic cord. *Surg. Forum* **27** (1976) 579–581.
35. Einer-Jensen N. and Waites G. M. H.: Testicular blood flow and a study of the testicular venous to arterial transfer of radioactive krypton and testosterone in the rhesus monkey. *J. Physiol.* **267** (1977) 1–15.
36. Resko J. A., Quadri S. K. and Spies H. G.: Negative feedback control of gonadotropins in male rhesus monkeys: effect of time after castration and interactions of testosterone and estradiol-17-beta. *Endocrinology* **101** (1977) 215–224.
37. Dierschke D. J., Walsh S. W., Mapletoft R. J., Robinson J. A. and Ginther O. J.: Functional anatomy of the testicular vascular pedicle in the rhesus monkey: evidence for a local testosterone concentrating mechanism. *Proc. Soc. Exp. Biol. Med.* **148** (1975) 236–242.
38. Meusy-Dessolle N. and Dang D. C.: Plasma concentrations of testosterone, dihydrotestosterone, delta-4-androstenedione, dehydroepiandrosterone and oestradiol-17-beta in the crab-eating monkey (*Macaca fascicularis*) from birth to adulthood. *J. Reprod. Fert.* **74** (1985) 347–359.
39. Aso T., Goncharov N., Cekan Z. and Diczfalusy E.: Plasma levels of unconjugated steroids in male baboons (*Papio hamadryas*) and rhesus monkeys (*Macaca mulatta*). *Acta Endocr., Copenh.* **82** (1976) 644–651.
40. Franz C. and Longcope C.: Androgen and estrogen metabolism in male rhesus monkeys. *Endocrinology* **105** (1979) 869–874.
41. Sundaram K., Thau R. B., Goldstein M., Phillips D. M., Rivier J., Vale W. and Bardin C. W.: Effect of an LHRH agonist on pituitary and testicular function in rhesus monkeys. *J. Reprod. Fert.* **72** (1984) 365–371.

42. Davies T. F., Hodgen G. D., Dufau M. L. and Catt K. J.: Regulation of primate testicular luteinizing hormone receptors and steroidogenesis. *J. Clin. Invest.* **64** (1979) 1070–1073.
43. Wickings E. J. and Nieschlag E.: Serum levels of testicular and adrenal steroids after dexamethasone and HCG-administration in the laboratory-maintained rhesus monkey. *Acta Endocr., Copenh.* **87** (1978) 650–658.
44. Preslock J. and Steinberger E.: Testicular steroidogenesis in the rhesus monkey *Macaca mulatta*. *Steroids* **34** (1979) 527–537.
45. Higashi Y., Takahashi J., Yoshida K.-I., Winters S. J., Oshima H. and Troen P.: Seasonal changes in steroidogenesis in the testis of the rhesus monkey (*Macaca mulatta*). *J. Androl.* **5** (1984) 70–79.