CHANGES WITH AGE IN THE OCCURRENCE OF C₁₉ STEROIDS IN THE TESTIS AND SUBMAXILLARY GLAND OF THE BOAR

W. D. BOOTH

*A.R.C. Unit of Reproductive Physiology and Biochemistry,
University of Cambridge**

(Received 27th June 1974)

Summary. After extraction from the testes of boars of different ages, C₁₉ steroids including 16-androstenes were determined by gas-liquid chromatography. Similarly, 16-androstenes were determined in the submaxillary glands of these boars. A high concentration of testosterone was found in the testes of 84-day-old fetuses, and this might be significant in the differentiation of male behaviour. The amount of testosterone exceeded that of androstenedione during postnatal development, and dehydroepiandrosterone and 5-androstenediol as free and sulphates were found in high concentrations particularly in postpubertal boars, suggesting that the 5-ene pathway for the synthesis of testosterone might be important. There was a change in the predominance of individual 16-androstenes in the testis during development, which closely paralleled the sequence for the biosynthesis of these compounds proposed from previous studies *in vitro*. Whereas the amount of 5 α -androst-16-en-3 β -ol exceeded that of 5 α -androst-16-en-3 α -ol in post-pubertal testes, 5 α -androst-16-en-3 α -ol was predominant in the submaxillary glands at all ages. The high concentration of 16-androstenes found in the mature boar, are discussed in relation to their release as pheromones and as factors responsible for taint in boar meat.

INTRODUCTION

From his studies with mammalian testis, Lindner (1960) concluded that the relative production of testosterone and androstenedione by the mammalian testis was a significant indicator of the androgenic activity of the gonad. Since that time, numerous investigations have been concerned with the determination of testosterone and androstenedione in the male, including the boar (Baulieu, Fabre-Jung & Huis in't Veld, 1967; Gray, Day, Lasley & Tribble, 1971; Elsaesser, König & Smidt, 1972). Studies on the production of testicular androgens in the dog (Oh & Tamaoki, 1973) have shown that testosterone can be synthesized from 3 β -hydroxy-5-ene-C₁₉ steroids without the intermediate formation of androstenedione. The significant amounts of dehydroepiandrosterone sulphate (Baulieu *et al.*, 1967) and 5-androstenediol sulphate (Raeside &

* Postal address: Animal Research Station, 307 Huntingdon Road, Cambridge CB3 0JQ.

Howells, 1971) which were found in the testis and spermatic vein blood of the boar suggests that the 5-ene pathway to testosterone may be important in this species.

In their search for androgens in boar testis, Prelog & Ruzicka (1944) first isolated large amounts of the musk-smelling steroids 3α -androstenol and 3β -androstenol. It has not yet been shown that these 16-unsaturated C_{19} steroids (16-androstenes) possess androgenic activity in the pig. Patterson (1968a) and Beery & Sink (1971) found 16-androstenes in boar fat and considered that they were responsible for taint in boar meat. The presence of 16-androstenes in the submaxillary glands (Patterson, 1968b), saliva (cited in Gower, 1972) and sweat glands of the boar (Stinson & Patterson, 1972), is of considerable interest since they have been shown to have pheromonal activity in the pig (Reed, Melrose & Patterson, 1974). It has been considered that 16-androstenes might be metabolites of testosterone, but Ahmad & Gower (1968) demonstrated *in vitro* that these steroids are formed from pregnenolone and progesterone before 17α -hydroxylation; when androgens were used as substrates, insignificant quantities of 16-androstene were formed.

Few comparative studies have been made between 16-androstenes and other C_{19} steroids in the pig (Claus, 1970; Claus, Hoffmann & Karg, 1971). The present communication describes some further relationships that have been observed between 16-androstenes and testosterone and related C_{19} steroids in the boar. Preliminary reports of this work have been published (Booth, 1970, 1972; cited in Gower, 1972).

MATERIALS AND METHODS

Animals

Testes and submaxillary glands were obtained within 30 min of slaughter from a number of crossbred boars of different ages (see Tables 2 and 3). The glands were weighed and samples were taken for histology (see Booth, Hay & Dott, 1973; Booth, 1974); the remaining tissue was stored over solid CO_2 until required for steroid analysis.

Chemicals

The following trivial names are used: testosterone (17 β -hydroxy-4-androsten-3-one); androstenedione (4-androstene-3,17-dione); dehydroepiandrosterone (DHA) (3 β -hydroxy-5-androsten-17-one); 5-androstenediol (5-androstene-3 β ,17 β -diol); 3α -androstenol (5 α -andro-16-en-3 α -ol); 3β -androstenol (5 α -andro-16-en-3 β -ol); 3β -androstadienol (5,16-androstadien-3 β -ol); 5α -androstenone (5 α -andro-16-en-3-one); androstadienone (4,16-androstadien-3-one).

Solvents were Analar grade and were purchased from either B.D.H. Ltd (Poole, Dorset, U.K.) or May & Baker Ltd (Dagenham, Essex, U.K.) Most authentic steroids were obtained from either the M.R.C. Steroid Reference Collection by courtesy of Professor W. Klyne or purchased from the Sigma Chemical Co. Ltd (Norbiton Station Yard, Kingston-upon-Thames, Surrey, U.K.). The 5α -androstenone and 3α -androstenol were gifts from Dr C. Hewitt (Organon Laboratories, Lanarkshire, Scotland) and the National Cancer

Institute (NIH, Bethesda, U.S.A.) respectively. The $[7\alpha\text{-}^3\text{H}]C_{19}$ steroids (500 to 1000 mCi/mmol), used as internal recovery standards, were purchased from the Radiochemical Centre (Amersham, Bucks, U.K.). Labelled 5-androstenediol was formed by reducing $[7\alpha\text{-}^3\text{H}]DHA$ with sodium borohydride according to the method of Resko, Feder & Goy (1968), and a yield of 80% was obtained as determined by gas-liquid chromatography (g.l.c.) after purification by thin-layer chromatography (t.l.c.). A gift of $5\alpha\text{-}[5\alpha\text{-}^3\text{H}]\text{-androsthenone}$ (334 mCi/mmol) from Dr R. Claus (Institut für Physiologie, 8050 Friesing, W. Germany) became available for a few recovery determinations.

Measurement of radioactivity

A Packard scintillation spectrometer (Model 3380) was used for the determination of radioactivity. Steroids were dissolved in toluene (10 ml) containing PPO (0.5%, w/v) and POPOP (0.03%, w/v). Counting efficiency was 41% for ^3H .

Extraction of testes

A method for the preparation of lipophilic and hydrophilic fractions of boar testes has been described previously (Booth, 1974). Steroids were determined in these fractions by the following methods (summarized in Flow-sheet 1). After extraction of the testicular homogenate with diethyl ether for free steroids and vitamin A (see Booth, 1974), the aqueous homogenate (20 ml) was then brought to pH 10 with NaOH and extracted for DHA and 5-androstenediol sulphates with *n*-butanol (2×40 ml). The butanol extract was washed to about pH 7 with water and the solvent removed under reduced pressure. Gross impurities were removed by a partition between aqueous 2 M- NH_3 and ethyl acetate according to the method described by Bush & Gale (1961); the steroid sulphates were then hydrolysed to free steroids according to the method of Burstein & Lieberman (1958). The free steroids corresponding to the sulphate fraction, and the free steroids in the initial ether extract of the testis were then subjected to a number of chromatography steps.

Thin-layer chromatography and paper chromatography

Testosterone and similar C_{19} steroids were separated from the 16-androstenes, and were subsequently purified by t.l.c. and paper chromatography according to the method described by Booth (1972); the 16-androstenes were purified by t.l.c. as follows (see legend to Flow-sheet 1 for details of the t.l.c. systems). Three fractions, i.e. $3\alpha\text{-androsthenol}$ and androstadienone , $3\beta\text{-androsthenol}$ and $3\beta\text{-androstadienol}$, and $5\alpha\text{-androsthenone}$ were obtained from the initial thin-layer chromatogram (t.l.c. (i)) according to the method described by Gower (1964). After a repeat purification of the $3\alpha\text{-}$ and $3\beta\text{-steroid}$ fractions by t.l.c. (i), $3\alpha\text{-androsthenol}$ was separated from androstadienone by t.l.c. (vii) according to the method of Gower & Ahmad (1967), following acetylation with acetic anhydride in pyridine (0.1 ml of each overnight). The $3\beta\text{-androsthenol}$ was separated from the $3\beta\text{-androstadienol}$ using argentation t.l.c. (vi) according to the method of Katkov & Gower (1970); these steroids were acetylated and finally purified by t.l.c. (vii). It was found that approxi-

mately 85% of 5 α -androstene was retained in the light petroleum fraction of the testis extract (see Flow-sheet 1), therefore the remaining 15% which was recovered on t.l.c. (i) was added to the petroleum phase. The 5 α -androstene was separated from lipids, first by two t.l.c. steps (vii, ii) and finally after acetylation (to esterify impurities) by argentation t.l.c. (vi). All 16-androstenes were quantified by g.l.c. The recovery of 5 α -androstene from t.l.c. was 36% and the recovery of other 16-androstenes was 24 to 29%; the 16-androstene values have been corrected for a relative recovery in relation to 5 α -androstene.

Extraction of submaxillary glands

The initial extraction of this tissue was carried out as previously described (Booth, 1972) and 16-androstenes were determined by the methods described for testicular tissue.

Gas-liquid chromatography

A series 104, Model 64 dual-flame ionization chromatograph (Pye-Unicam, Cambridge, U.K.) was used for the steroid determinations. The detector and the injection port heater were both held at 250°C, and the flow rate of the carrier gas argon was 50 ml/min. Testosterone, DHA and 5-androstenediol were routinely determined by g.l.c. as acetates, or the free steroid in the case of androstenedione (see Booth, 1972). Similarly, 16-androstenes were routinely determined by g.l.c. as acetates, or the free steroid in the case of 5 α -androstene and androstadienone. A 210-cm glass column containing 5% FS-1 on diatomite CQ (100 to 120 mesh) was used for the 16-androstene analysis; the oven temperature was maintained at 195°C and the internal standard was 5 α -cholestane (Gower & Thomas, 1968). Further information was obtained from g.l.c. for the purpose of identifying steroids. Testosterone and similar C₁₉ steroids were submitted to a 1% XE-60 column as free steroids and as chloromethyl-dimethylsilyl ether (CMDS) derivatives (Thomas & Walton, 1968); steroid acetates and androstenedione were also submitted to a 3% SE-30 column (oven temperature 225°C). The 16-androstenes were submitted to the 5% FS-1 column as free steroids and as CMDS derivatives (Gower & Thomas, 1968); 5 α -androstene and androstadienone were examined as methyl oxime derivatives (Gower, Harrison & Heap, 1970).

Gas-liquid chromatography-mass spectrometry

The instrument was fitted with a 2% SE-30 glass column (75 cm) which was maintained at 210°C. The flash heater was held at 240°C and the separator at 200°C; the flow rate of the carrier gas helium was 30 ml/min.

RESULTS

Identification of steroids

Pooled steroid samples were submitted to t.l.c. for qualitative identification; colour reactions and R_F values were found corresponding to authentic compounds. All steroids which were isolated from the testis and submaxillary gland,

Flow-sheet 1. Scheme for the determination of C_{19} steroids (and vitamin A) in boar testis. Solvent systems (v/v): (i) toluene:ethyl acetate (9:1) $\times$ 2; benzene:ether (9:1); (ii) benzene:acetone (4:1); (iii) light petroleum (80 to 100 b.p.):methanol:water (5:4:1); (iv) benzene:ethyl acetate (3:1); (v) benzene:ether (4:1); (vi) cyclohexane:ethyl acetate (1:1); (vii) toluene:ethyl acetate (19:1). The method to the t.l.c. (i) stage (asterisk) has been published in detail (Booth, 1974).

produced relative retention time data corresponding to authentic compounds on g.l.c. (see Table 1). Endogenous 3β -androstadienol and androstadienone were isolated for the first time from both testis and submaxillary gland, and were conclusively identified by g.l.c.-mass spectrometry as shown by the following details of the spectra. The acetate derivative of authentic 3β -androstadienol and the fractions corresponding to this steroid in the tissue extracts produced identical mass spectra showing the distinctive absence of a parent peak (theoretically 314^+), but the presence of diene peaks at 146 and 239.

Table 1. Relative retention times on g.l.c. of C₁₉ steroids isolated from the testis and submaxillary gland of boars

<i>3β-Hydroxy-5-ene-C₁₉ steroid</i>	<i>Column 1% XE-60</i>					
	<i>Free</i>		<i>Acetate</i>		<i>CMDS</i>	
	<i>Testis</i>		<i>Testis</i>		<i>Testis</i>	
DHA	(0.519)		(0.509)		(0.515)	
Free	0.526		0.509		0.515	
Sulphate	0.532		0.511		0.517	
5-Androstenediol	(0.473)		(0.520)		(2.36)	
Free	0.472		0.520		2.44	
Sulphate	0.472		0.520		2.38	
<i>16-Androstene</i>	<i>Column 5% FS-1</i>					
	<i>Free</i>		<i>Acetate</i>		<i>CMDS</i>	
	<i>Testis</i>	<i>Smx. gl.</i>	<i>Testis</i>	<i>Smx. gl.</i>	<i>Testis</i>	<i>Smx. gl.</i>
3α-Androstenol	(0.331) 0.331	(0.336) 0.339	(0.584) 0.585	(0.582) 0.579	(0.602) 0.600	(0.610) 0.613
3β-Androstenol	(0.363) 0.379	(0.384) 0.387	(0.658) 0.657	(0.650) 0.653	(0.806) 0.813	(0.813) 0.816
3β-Androstadienol	(0.345) 0.349	(0.345) 0.350	(0.620) 0.620	(0.623) 0.619	(0.746) 0.741	(0.739) 0.737
5α-Androstenone	(0.780) 0.782	(0.780) 0.782	—	—	—	—
Androstadienone	(1.26) 1.25	(1.25) 1.25	—	—	—	—

Abbreviations: CMDS, chloromethyldimethylsilyl ether derivative; Smx. gl., submaxillary gland. The column temperatures were 215°C (1% XE-60) and 195°C (5% FS-1). The carrier gas flow rate was 50 ml/min. Values in parentheses correspond to the relative retention times of authentic steroids where the retention time of the internal standard was 1.000. Testosterone, testosterone acetate and testosterone CMDS were used as the internal standards for 3β-hydroxy-5-ene-C₁₉ steroids; 5α-cholestane was the internal standard for 16-androstenes.

Similarly, the mass spectra of authentic androstadienone and the fraction corresponding to this steroid in the tissue extracts, showed a parent peak at 270 and other significant peaks at 146, 147, 228 and 255.

Changes in testosterone and related C₁₉ steroid levels in the testis with age

The concentration of free C₁₉ steroids in relation to age is shown in Text-fig. 1 and the testicular content of free and conjugated C₁₉ steroids is given in Table 2. The amounts of testosterone were generally greater than androstenedione, irrespective of age; the high value for androstenedione in one boar only at 30 weeks may be an unrepresentative result. The 3β-hydroxy-5-ene-C₁₉ steroids, notably 5-androstenediol in both the free and sulphate fractions, were

TEXT-FIG. 1. The concentration of testosterone and related free C_{19} steroids in the testes of the boar from late fetal stage to maturity (results are corrected for extraction losses). Values between 84 days gestation (F) and 2 years are means and are related by a continuous line; values for individual boars older than 2 years are related by a broken line. ●, Testosterone; ○, androstenedione; ■, DHA; Δ, 5-androstenediol.

present in larger quantities than testosterone in mature boars. Although not illustrated, the concentration of DHA sulphate and 5-androstenediol sulphate closely paralleled that of the free steroids. As shown in Table 2, there was a wide variation in steroid levels between boars of the same age even though the testicular weights were similar. Apparently, the azoospermic condition in the 10-year-old boar was not associated with an obvious deficiency in testicular androgens. Histological observations showed that the testes had a normal distribution of Leydig cells.

Changes in 16-androstene levels in the testis with age

The concentration of these steroids in relation to age is shown in Text-fig. 2 and the testicular content in Table 3. A comparison of the levels of 16-androstenes (Table 3) with other C_{19} steroids (Table 2) shows that the testis of the boar preferentially produces the musk-smelling steroids. When the data in Text-fig. 1 are compared with those in Text-fig. 2, it can be seen that the endocrine activity of the testis is characterized by changes in the total production of 16-androstenes and other C_{19} steroids. With increasing age, a developmental sequence occurred in the relative abundance of 16-androstenes. In fetal testes, 3β -androstadienol was the predominant steroid, at birth it was androstadienone, at 6 and 12 weeks it was 3α -androstenol, and in older boars 3β -androstenol. Androstadienone was not measured at 12, 18 and 30 weeks. The high levels of 16-androstene found in the testes at 6 weeks, was associated with the predominance of Leydig tissue seen in histological sections of the testes.

Changes in 16-androstene levels in the submaxillary gland with age

The concentration of 16-androstenes in relation to age is shown in Text-fig. 3.

Table 2. The content of testosterone and related C₁₉ steroids in the testes of boars

Age	Boars (testes assayed)	Total wt of testes/ animal (g)	Stage of spermatogenesis	Testos- terone	Andro- stenedione	DHA	DHA sulphate	5-Androstene- diol	5-Andro- stenediol sulphate
84 days of gestation*	Pooled (18 boars)	0.12	—	0.15	†	0.04	N.D.	N.D.	N.D.
Birth*	Pooled (12 boars)	0.41	Gonocytes	0.53	0.03	0.08	N.D.	0.20 (0.09)	0.02
6 weeks*	Pooled (12 boars)	7.44	Gonocytes	0.73	1.00	6.60	3.68	4.46 (1.97)	0.97
12 weeks*	Pooled (4 boars)	19.2	Spermatogonia	1.57	0.37	1.26	0.12	4.11 (1.40)	0.25
18 weeks	C11 C13	57.6 125	Few spermatozoa	4.00 6.22	0.89 1.24	0.57 2.69	N.D. 0.33	2.00 (1.00) 7.97 (3.88)	0.26 1.63
24 weeks	A39L B50C	305 354	Abundant spermatozoa	20.8 46.5	8.81 11.5	11.5 40.5	2.88 15.3	20.4 (8.54) 83.5 (30.4)	5.98 40.4
30 weeks	B36M B37M	533 427		40.5 12.1	138 Trace	68.5 3.87	113 3.77	237 (107) 138 (5.55)	84.2 8.54
36 weeks	R G	465 478		47.9 89.0	14.6 N.D.	118 28.2	60.8 23.3	210 (91.1) 79.1 (23.9)	142 53.5
2 years	A B	1004 935	Azoospermic	183 209	86.0 70.9	525 215	655 365	1080 (556) 591 (250)	514 269
4 years	'Oscar'	822		22.9	9.68	64.0	41.7	178 (90.4)	74.0
10 years	'Tweedle'	655		30.6	22.4	77.6	100	279 (98.3)	190

Values are expressed in $\mu\text{g}/\text{testes}/\text{animal}$ and are corrected for analytical losses, except those for 5-androstenediol sulphate which can be compared with uncorrected values for the free steroid (shown in parentheses). % Recoveries (mean $\pm$ S.D.): testosterone, 22.6 ± 3.6 ; androstenedione, 16.1 ± 4.3 ; DHA, 24.7 ± 8.7 ; DHA sulphate, 18.9 ± 6.2 ; 5-androstenediol, 42.8 ± 6.6 . N.D., none detected.

* The testicular weights and steroid values are expressed as the means for these ages.

† Sample lost.

Table 3. The content of 16-androstenes in the testes of boars

Age	Boars (testes assayed)	Total wt of testes/ animal (g)	Stage of spermatogenesis	3 α -Androstenol	3 β -Androstenol	3 β -Androstadienol	5 α -Androstenone	Androsta- dienone
84 days of gestation*	Pooled (18 boars)	0.12	—	0.03	0.02	0.08	0.002	0.06
Birth*	Pooled (12 boars)	0.41	Gonocytes	0.53	0.46	0.64	0.01	0.86
6 weeks*	Pooled (12 boars)	7.44	Gonocytes	136	17.4	7.00	0.44	2.69
12 weeks*	Pooled (4 boars)	19.2	Spermatogonia	8.90	1.49	0.65	0.23	N.D.
18 weeks	C11 C13	57.6 125	Few spermatozoa	10.8 4.99	24.4 6.69	N.D. 10.4	0.66 0.14	N.D. N.D.
24 weeks	A39L B50C	305 354	Abundant spermatozoa	28.9 177	13.7 195	22.2 19.5	1.10 (4.30) 3.19 (14.0)	N.D. N.D.
30 weeks	B36M B37M	533 427		67.7 470	128 2151	13.2 17.4	1.17 (6.22) 14.5 (73.6)	N.D. N.D.
36 weeks	R G	465 478		364 162	1272 744	103 47.3	15.8 23.9	N.D. 2.88
2 years	A B	1004 935	Azoospermic	3020 2562	14,080 11,136	320 217	168 (656) 191 (893)	3.28 17.7
4 years	'Oscar'	822		492	3275	373	41.1	9.76
10 years	'Tweedle'	655		1225	3452	167	147 (850)	10.9 15.5

Values are expressed in $\mu\text{g}/\text{testes}/\text{animal}$ and are uncorrected for absolute analytical losses, the exception being those shown in parentheses for 5 α -androstenone. % Recovery of 5 α -androstenone (mean $\pm$ S.D.), 21.6 $\pm$ 3.3. N.D., not determined.

* The testicular weights and steroid values are the means for these ages.

In contrast to the testis, the relative concentrations of these steroids in the submaxillary gland did not vary significantly in relation to age; 3α -androstenol was the predominant steroid. The trace amounts of androstadienone isolated in each extraction were below the sensitivity of the g.l.c. method. Pooled samples provided enough steroid, however, for a definitive identification of this compound by g.l.c.-mass spectrometry. Comparing the data in Text-fig. 2 with those in Text-fig. 3, it can be seen that fluctuations in the level of 3α -androstenol were similar in both the testis and submaxillary gland. In individual

TEXT-FIG. 2. The concentration of 16-androstenes in the testes of the boar from late fetal stage to maturity (results are uncorrected for extraction losses). Values between 84 days of gestation (F) and 2 years are means and are related by a continuous line; values for individual boars older than 2 years are related by a broken line (---). The lack of values for androstadienone between 6 weeks and 36 weeks are shown as (-.-.-.-). ●, 3α -Androstenol; ○, 3β -androstenol; □, 3β -androstadienol; △, 5α -androstenone; ▲, androstadienone.

boars of the same age, the animals with the highest levels of 3α -androstenol and 5α -androstenone in the testis also had the highest levels of these steroids in the submaxillary gland. The % recovery of 5α -androstenone gave a mean $\pm$ S.D. of 21.3 ± 7.3 .

DISCUSSION

The results show that the endocrine activity of the boar testis fluctuates considerably with age. The high activity from late fetal stage to 6 weeks of age, and in the mature animal, can be related to the abundance of Leydig tissue (Bascom & Osterud, 1927; W. D. Booth, unpublished observations). Steroids in

TEXT-FIG. 3. The concentration of 16-androstenes in the submaxillary glands of the boar from birth to maturity (results are uncorrected for extraction losses). Values between birth and 2 years are means and are related by a continuous line; values for individual boars older than 2 years are related by a broken line (---). The lack of values between 12 and 24 weeks are shown as (-.-.-.-). ●, 3α -Androstenol; ○, 3β -Androstenol; □, 3β -Androstadienol; Δ, 5α -Androstenone.

both the testes and submaxillary glands reached their highest levels in the 2 year-old boars; this suggests that in the boar, as in man (Bishop, 1970), the endocrine activity of the testis reaches a peak early in adult life. It appears that testosterone is preferentially synthesized in the testis of the 84-day-old fetal boar. In keeping with these findings, it is noteworthy that Moon & Raeside (1972) observed 3β -hydroxy-5-ene-steroid dehydrogenase activity in the testes of fetal boars during late gestation, and Attal (1969) found more testosterone than androstenedione in the testes of ram fetuses towards the end of pregnancy. This increased production of testosterone in late gestation is probably necessary for the differentiation of neural centres concerned with the control of male behaviour (Brown-Grant, 1973). The finding that amounts of testosterone were greater than androstenedione in the postnatal boar, is in agreement with the findings of Elsaesser *et al.* (1972); Skinner, Booth, Rowson & Karg (1968) also found a similar relationship in the ram lamb. The differences in steroid levels between boars of the same age are probably related to differences in the endocrine activity of the testis since the testicular weights were similar; Elsaesser *et al.* (1972) also found considerable variations in steroid levels between boars of the same age. The significant amounts of DHA and 5-androstenediol that were isolated in both the free and sulphate fractions is in agree-

ment with the observations of Baulieu *et al.* (1967) and Raeside & Howells (1971) respectively. The finding of higher levels of 5-androstenediol than DHA, particularly in older boars, could be due to the conversion of DHA to 5-androstenediol in the seminiferous tubules as Richards & Neville (1973) described for the rat. In addition to their rôle as potential precursors for testosterone synthesis in the testis (Oh & Tamaoki, 1973), these steroids may also function as testosterone precursors in other tissues of the pig (Booth *et al.*, 1973).

The high concentration of 16-androstenes found in relation to other C₁₉ steroids in the testes of boars is in agreement with the findings of Claus (1970) and Claus *et al.* (1971); these results support the conclusions from the in-vitro studies of Ahmad & Gower (1968) that 16-androstenes are preferentially synthesized in boar testis. Furthermore, the change in the predominance of individual 16-androstenes that was found in the testis during development reflects the proposed biosynthetic pathway for these compounds (Brophy & Gower, 1972). The similar pattern in the occurrence of 3 α -androstanol in both testis and submaxillary glands strongly suggests that the concentration of this steroid in the salivary gland is directly dependent upon the testicular production of this steroid or its precursors (Katkov, Booth & Gower, 1972); failure of the submaxillary gland to accumulate higher levels of 3 β -androstanol may be due to a limited presence of specific receptors for this steroid in the salivary gland, or to the inactivation and excretion of this steroid as the glucosiduronate conjugate in urine (Saat, Gower, Harrison & Heap, 1972).

The simultaneous occurrence in the submaxillary gland of the mature boar of high levels of 16-androstene, testosterone and 5 α -dihydrotestosterone (Booth, 1972), and hypertrophy of the serous cells (Booth *et al.*, 1973) has been demonstrated. This shows that the full capacity of the salivary gland to secrete 16-androstenes as pheromones is reached only when other secondary sexual characteristics are well developed.

The relatively low levels of 16-androstene found before 24 weeks of age in the present study are in keeping with the observation that taint is negligible in meat from boars less than 200 days old (Rhodes, 1972).

ACKNOWLEDGMENTS

The author would like to thank Dr R. L. S. Patterson of the A.R.C. Meat Research Institute, Langford, Bristol, for identifying steroids on combined gas-liquid chromatography-mass spectrometry. Special thanks are due to Dr C. Polge and Dr A. G. C. Renwick for their advice during the work, and to Dr Polge and Professor T. R. R. Mann for their advice in the preparation of the manuscript.

REFERENCES

- AHMAD, N. & GOWER, D. B. (1968) The biosynthesis of some androst-16-enes from C₂₁ and C₁₉ steroids in boar testicular and adrenal tissue. *Biochem. J.* **108**, 233-241.
ATTAL, J. (1969) Levels of testosterone, androstenedione, oestrone and oestradiol-17 β in the testes of fetal sheep. *Endocrinology*, **85**, 280-289.
BASCOM, K. F. & OSTERUD, H. L. (1927) Quantitative studies of the testis. III. A numerical treatment of the development of the pig testis. *Anat. Rec.* **37**, 63-82.

- BAULIEU, E. E., FABRE-JUNG, I. & HUIS IN'T VELD, L. G. (1967) Dehydroepiandrosterone sulfate: a secretory product of the boar testis. *Endocrinology*, **81**, 34–38.
- BEERY, K. E. & SINK, J. D. (1971) Isolation and identification of 3 α -hydroxy-5 α -androst-16-ene and 5 α -androst-16-ene-3-one from porcine adipose tissue. *J. Endocr.* **51**, 223–224.
- BISHOP, M. W. H. (1970) Ageing and reproduction in the male. *J. Reprod. Fert.*, Suppl. **12**, 65–87.
- BOOTH, W. D. (1970) The occurrence of some C₁₉ steroids and vitamin A in boar testis. *J. Reprod. Fert.* **23**, 533–534.
- BOOTH, W. D. (1972) The occurrence of testosterone and 5 α -dihydrotestosterone in the submaxillary salivary gland of the boar. *J. Endocr.* **55**, 119–125.
- BOOTH, W. D. (1974) Vitamin A in testicular tissue of the boar and intersex pig. *J. Reprod. Fert.* **40**, 219–222.
- BOOTH, W. D., HAY, M. F. & DOTT, H. M. (1973) Sexual dimorphism in the submaxillary gland of the pig. *J. Reprod. Fert.* **33**, 163–166.
- BROPHY, P. J. & GOWER, D. B. (1972) 16-Unsaturated C₁₉ 3-oxo-steroids as metabolic intermediates in boar testis. *Biochem. J.* **128**, 945–952.
- BROWN-GRANT, K. (1973) Recent studies on the sexual differentiation of the brain. In *Foetal and Neonatal Physiology*, pp. 527–545. Eds. K. S. Comline, K. W. Cross, G. S. Dawes and P. W. Nathanielsz. Cambridge University Press.
- BURSTEIN, S. & LIEBERMAN, S. (1958) Hydrolysis of ketosteroid hydrogen sulfates by solvolysis procedures. *J. biol. Chem.* **233**, 331–335.
- BUSH, I. E. & GALE, M. (1961) Techniques and apparatus. In *The Chromatography of Steroids*, chap. 3, pp. 197–200. Pergamon Press, London.
- CLAUS, R. (1970) *Bestimmung von Testosteron und 5 α -androst-en-3-on, einen Ebergeruchsstoff, bei Schweinen*. Dr. agr. dissertation, Technical High School, Munich.
- CLAUS, R., HOFFMANN, B. & KARG, H. (1971) Determination of 5 α -androst-16-en-3-one, a boar taint steroid in pigs with reference to relationships to testosterone. *J. Anim. Sci.* **33**, 1293–1297.
- ELSAESSER, F., KÖNIG, A. & SMIDT, D. (1972) Der Testosteron-und Androstenediongehalt im Eberhoden in Abhängigkeit vom Alter. *Acta endocr., Copenh.* **69**, 553–566.
- GOWER, D. B. (1964) Chromatographic separation of C₁₉-16-dehydrosteroids. *J. Chromat.* **14**, 424–431.
- GOWER, D. B. (1972) 16-Unsaturated C₁₉ steroids, a review of their chemistry, biochemistry and possible physiological role. *J. Steroid Biochem.* **3**, 45–103.
- GOWER, D. B. & AHMAD, N. (1967) Studies on the biosynthesis of 16-dehydrosteroids. The metabolism of [4-¹⁴C]pregnenolone by boar adrenal and testis tissue *in vitro*. *Biochem. J.* **104**, 550–556.
- GOWER, D. B., HARRISON, F. A. & HEAP, R. B. (1970) The identification of C₁₉-16-unsaturated steroids and estimation of 17-oxosteroids in boar spermatic vein plasma and urine. *J. Endocr.* **47**, 357–368.
- GOWER, D. B. & THOMAS, B. S. (1968) Gas-liquid chromatography of androst-16-enes as trimethylsilyl and chloromethyldimethylsilyl ethers. *J. Chromat.* **36**, 338–340.
- GRAY, R. C., DAY, B. N., LASLEY, J. F. & TRIBBLE, L. F. (1971) Testosterone levels of boars at various ages. *J. Anim. Sci.* **33**, 124–126.
- KATKOV, T., BOOTH, W. D. & GOWER, D. B. (1972) The metabolism of 16-androstenes in boar salivary glands. *Biochim. biophys. Acta*, **270**, 546–556.
- KATKOV, T. & GOWER, D. B. (1970) The biosynthesis of androst-16-enes in boar testis tissue. *Biochem. J.* **117**, 533–538.
- LINDNER, H. R. (1960) *Testicular endocrine function in domestic animals*. Ph.D. thesis, Cambridge University.
- MOON, Y. S. & RAESIDE, J. I. (1972) Histochemical studies on hydroxysteroid dehydrogenase activity of fetal pig testes. *Biol. Reprod.* **7**, 278–287.
- OH, R. & TAMAOKI, B. (1973) *In vitro* biosynthesis of androgens in canine testes. *Acta endocr., Copenh.* **74**, 615–624.
- PATTERSON, R. L. S. (1968a) 5 α -Androst-16-ene-3-one:— compound responsible for taint in boar fat. *J. Sci. Fd Agric.* **19**, 31–38.
- PATTERSON, R. L. S. (1968b) Identification of 3 α -hydroxy-5 α -androst-16-ene as the musk odour component of boar submaxillary salivary gland and its relationship to the sex odour taint in pork meat. *J. Sci. Fd Agric.* **19**, 434–438.
- PRELOG, V. & RUZICKA, L. (1944) 7. Untersuchungen über Organextrakte. Über zwei moschusartig riechende Steroide aus Schweinen testes-Extrakten. *Helv. chim. Acta*, **27**, 61–66.
- RAESIDE, J. I. & HOWELLS, G. A. (1971) The isolation and identification of androstenediol sulfate from spermatic vein blood and testes of the boar. *Can. J. Biochem.* **49**, 80–84.
- REED, H. C. B., MELROSE, D. R. & PATTERSON, R. L. S. (1974) Androgen steroids as an acid to the detection of oestrus in pig artificial insemination. *Br. vet. J.* **130**, 61–67.
- RESKO, J. A., FEDER, H. H. & GOY, R. W. (1968) Androgen concentrations in plasma and testis of developing rats. *J. Endocr.* **40**, 485–491.
- RHODES, D. N. (1972) Consumer testing of pork from boar and gilt pigs. *J. Sci. Fd Agric.* **23**, 1483–1491.

- RICHARDS, G. & NEVILLE, A. (1973) Androgen metabolism in rat interstitial tissue and seminiferous tubules. *Nature, Lond.* **244**, 359–361.
- SAAT, Y. A., GOWER, D. B., HARRISON, F. A. & HEAP, R. B. (1972) Studies on the biosynthesis *in vivo* and secretion of 16-unsaturated C₁₉ steroids in the boar. *Biochem. J.* **129**, 657–663.
- SKINNER, J. D., BOOTH, W. D., ROWSON, L. E. A. & KARG, H. (1968) The post-natal development of the reproductive tract of the Suffolk ram, and changes in the gonadotrophin content of the pituitary. *J. Reprod. Fert.* **16**, 463–477.
- STINSON, C. G. & PATTERSON, R. L. S. (1972) C₁₉-Δ¹⁶ steroids in boar sweat glands. *Br. vet. J.* **128**, 41–43.
- THOMAS, B. S. & WALTON, D. R. M. (1968) Chloromethyldimethylsilyl ethers in the routine assay of urinary 11-deoxy-17-oxosteroids by gas-liquid chromatography. *J. Endocr.* **41**, 203–211.