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CHANGES WITH AGE IN THE OCCURRENCE OF C_{19} 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**

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Summary. After extraction from the testes of boars of different ages, C_{19} 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 5α-androst-16-en-3β-ol exceeded that of 5α-androst-16-en-3α-ol in postpubertal testes, 5α-androst-16-en-3α-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 3β-hydroxy-5-ene-C_{19} 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 (17β-hydroxy-4-androsten-3-one); androstenedione (4-androstene-3,17-dione); dehydroepiandrosterone (DHA) (3β-hydroxy-5-androsten-17-one); 5-androstenediol (5-androstene-3β,17β-diol); 3α-androstenol (5α-androst-16-en-3α-ol); 3β-androstenol (5α-androst-16-en-3β-ol); 3β-androstadienol (5,16-androstadien-3β-ol); 5α-androstenone (5α-androst-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 [7α-^{3}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 [7α-^{3}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 5α-[5α-^{3}H]-androstenone (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 ^{3}H.

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. 3α-androstenol and androstadienone, 3β-androstenol and 3β-androstadienol, and 5α-androstenone 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 3α- and 3β-steroid fractions by t.l.c. (i), 3α-androstenol 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 3β-androstenol was separated from the 3β-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 5α-androstenone 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 5α-androstenone 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 5α-androstenone 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 5α-androstenone.

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 5α-androstenone 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 5α-cholestane (Gower & Thomas, 1968). Further information was obtained from g.l.c. for the purpose of identifying steroids. Testosterone and similar C_{19} steroids were submitted to a 1% XE-60 column as free steroids and as chloromethyldimethylsilyl 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); 5α-androstenone and androstadienone were examined as methyl oxime derivatives (Gower, Harrison & Heap, 1970).

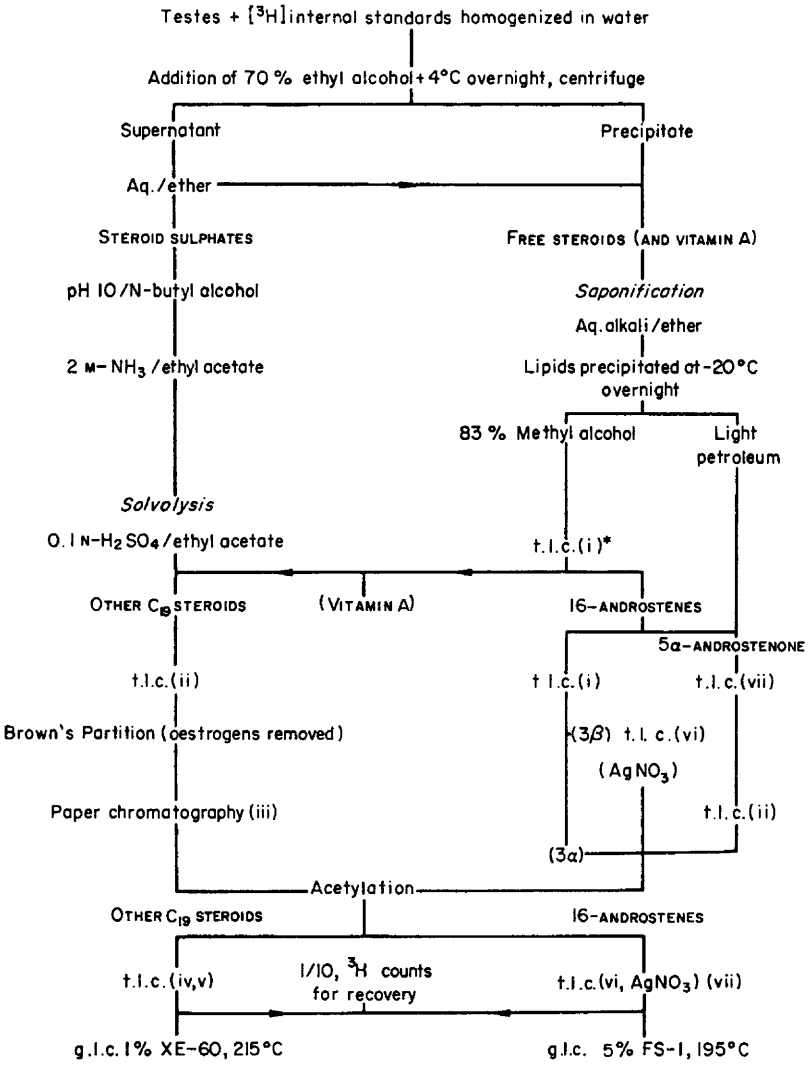
Gas-liquid chromatography–mass spectrometry

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) ×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 C_{19} steroids isolated from the testis and submaxillary gland of boars

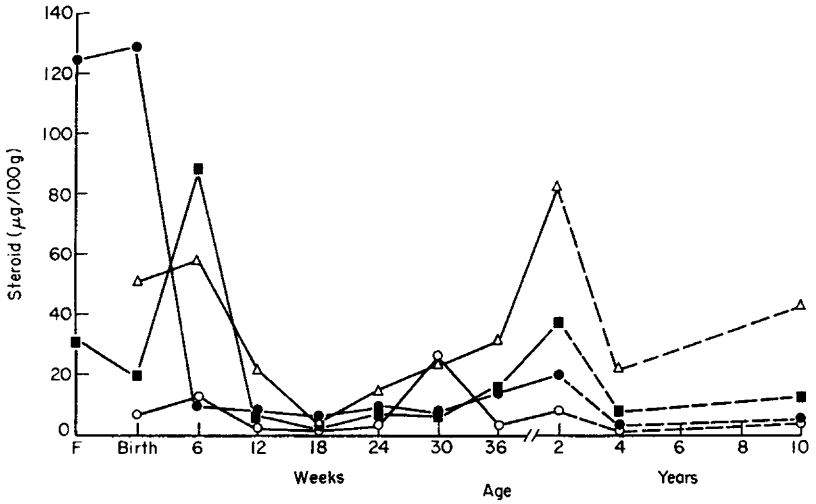
3β-Hydroxy-5-ene-C_{19} steroid	Column 1% XE-60					
	Free		Acetate		CMDS	
	Testis		Testis		Testis	
DHA	(0·519)		(0·509)		(0·515)	
Free	0·526		0·509		0·515	
Sulphate	0·532		0·511		0·517	
5-Androstenediol	(0·473)		(0·520)		(2·36)	
Free	0·472		0·520		2·44	
Sulphate	0·472		0·520		2·38	
	Column 5% FS-1					
16-Androstene	Free		Acetate		CMDS	
	Testis	Smx. gl.	Testis	Smx. gl.	Testis	Smx. gl.
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-C_{19} 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 C_{19} steroid levels in the testis with age

The concentration of free C_{19} steroids in relation to age is shown in Text-fig. 1 and the testicular content of free and conjugated C_{19} 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-C_{19} 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; △, 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 C_{19} steroids in the testes of boars

Age	*Boars (testes assayed)*	*Total wt of testes/animal (g)*	*Stage of spermatogenesis*	*Testosterone*	*Androstenedione*	*DHA*	*DHA sulphate*	*5-Androstenediol*	*5-Androstenediol 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	57·6	Few spermatozoa	4·00	0·89	0·57	N.D.	2·00 (1·00)	0·26
	C13	125		6·22	1·24	2·69	0·33	7·97 (3·88)	1·63
24 weeks	A39L	305	Abundant spermatozoa	20·8	8·81	11·5	2·88	20·4 (8·54)	5·98
	B50C	354		46·5	11·5	40·5	15·3	83·5 (30·4)	40·4
30 weeks	B36M	533		40·5	138	68·5	113	237 (107)	84·2
	B37M	427		12·1	Trace	3·87	3·77	138 (5·55)	8·54
36 weeks	R	465		47·9	14·6	118	60·8	210 (91·1)	142
	G	478		89·0	N.D.	28·2	23·3	79·1 (23·9)	53·5
2 years	A	1004		183	86·0	525	655	1080 (556)	514
	B	935		209	70·9	215	365	591 (250)	269
4 years	'Oscar'	822		22·9	9·68	64·0	41·7	178 (90·4)	74·0
10 years	'Tweedle'	655	Azoospermic	30·6	22·4	77·6	100	279 (98·3)	190

Values are expressed in μg/testes/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 ± 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.

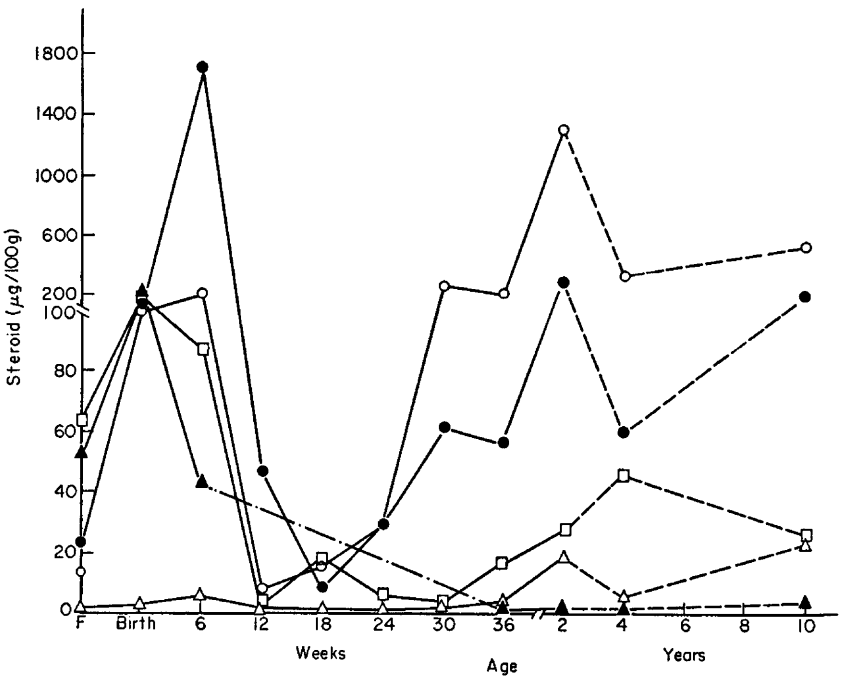
Table 3. The content of 16-androstenes in the testes of boars

Age	*Boars (testes assayed)*	*Total wt of testes/ animal (g)*	*Stage of spermatogenesis*	*3α-Androstenol*	*3β-Androstenol*	*3β-Androstadienol*	*5α-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	57·6	Few spermatozoa	10·8	24·4	N.D.	0·66	N.D.
	C13	125		4·99	6·69	10·4	0·14	N.D.
24 weeks	A39L	305	Abundant spermatozoa	28·9	13·7	22·2	1·10 (4·30)	N.D.
	B50C	354		177	195	19·5	3·19 (14·0)	N.D.
30 weeks	B36M	533		67·7	128	13·2	1·17 (6·22)	N.D.
	B37M	427		470	2151	17·4	14·5 (73·6)	N.D.
36 weeks	R	465		364	1272	103	15·8	2·88
	G	478		162	744	47·3	23·9	3·28
2 years	A	1004		3020	14,080	320	168 (656)	17·7
	B	935		2562	11,136	217	191 (893)	9·76
4 years	'Oscar'	822		492	3275	373	41·1	10·9
10 years	'Tweedle'	655	Azoospermic	1225	3452	167	147 (850)	15·5

Values are expressed in μg/testes/animal and are uncorrected for absolute analytical losses, the exception being those shown in parentheses for 5α-androstenone. % Recovery of 5α-androstenone (mean ± S.D.), 21·6 ± 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

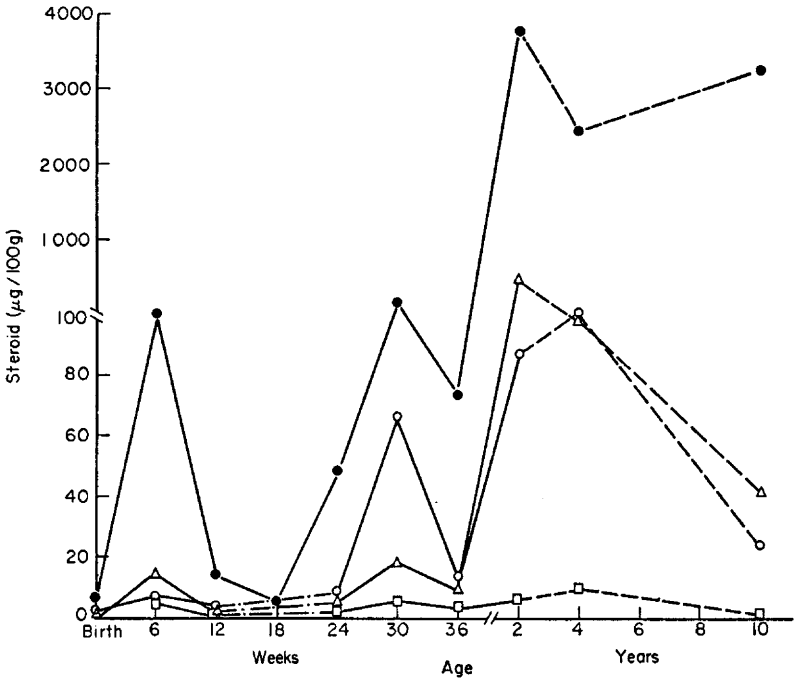


TEXT-FIG. 2. The concentration of 16-androstenes in the testes of the boar from late fetal stage to maturity (results are uncorrected for extraction losses). Values between 84 days of gestation (F) and 2 years are means and are related by a continuous line; values for individual boars older than 2 years 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±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; △, 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 C_{19} 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 3α-androstenol 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 3β-androstenol 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 5α-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).

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