The occurrence of C₁₉ steroids in testicular tissue and submaxillary glands of intersex pigs in relation to morphological characteristics

W. D. Booth and C. Polge

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

Summary. Five true hermaphrodite pigs and two male pseudohermaphrodite pigs were studied. A 38XX sex chromosome constitution was found in peripheral leucocytes of three true hermaphrodites and in one male pseudohermaphrodite; XX/XY mixoploidy was present in the leucocytes of the remaining male pseudohermaphrodite. The occurrence of C₁₉ steroids, including 16-androstenes, in the testicular tissue and submaxillary gland of intersex pigs was of a similar pattern to that found previously in mature boars, and masculinization of the genital tract was related to the amount of testicular tissue present. It is postulated that in the absence of germ cells in the testicular tissue of intersex pigs the Sertoli cells may be involved in the metabolism of dehydroepiandrosterone to 5-androstenediol, a possible testosterone precursor in the pig. The high levels of 16-androstenes found in the submaxillary gland of intersex pigs indicates that these steroids are responsible for 'boar taint' in these animals. In contrast to the boar, no consistent relationship was found between the occurrence of C₁₉ steroids and the degree of masculinization of the submaxillary gland; it is postulated that the predominantly female genetic constitution may have affected the response of the salivary gland to androgen.

Introduction

Limited records have shown that the incidence of intersexuality in domestic pigs, in Europe, is 0.5–2.0% (Breeuwsma, 1970). Cytogenetic studies (reviewed by Breeuwsma, 1970) have demonstrated that the majority of intersex pigs are genetic females, and in this regard the pig is similar to the goat (Hamerton *et al.*, 1969), although it has not yet been demonstrated that an autosomal recessive factor is involved in the aetiology of intersexuality in the pig.

Few studies have been made on the production of steroids by gonads of the intersex pig. Navratil *et al.* (1968) found that amounts of 17-oxosteroid and oestrogen in the urine of intersex pigs were lower than those in the urine of boars, but higher than those in sow urine; the levels of steroid were increased following HCG treatment and decreased after castration. Van der Horst & Sybesma (1969) and Breeuwsma (1970) found only small amounts of C₁₉ steroid in the testicular tissue of intersex pigs, whereas testicular tissue in intersex goats produces significant quantities of testosterone (Hamerton *et al.*, 1969; Zlotnik, 1973).

It has been established that 16-unsaturated C₁₉ steroids (16-androstenes), which are produced in large quantities by the boar testis (Gower, 1972; Booth, 1975), are responsible for taint in boar meat (Patterson, 1968; Berry & Sink, 1971). Pig carcasses that are tainted are responsible for economic losses, and Bishop (1969) has suggested that some of these carcasses could be of intersex pigs with a predominantly female phenotype, but possessing intra-abdominal testicular tissue.

The aims of the present study on intersex pigs were (i) to study in greater detail than hitherto the occurrence of C₁₉ steroids in the testicular tissue of the intersex pig; (ii) to find out if 16-androstenes are also present in this testicular tissue and in the submaxillary glands in significant quantities, and therefore likely to be the factors responsible for taint in these animals; (iii) to compare the levels

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

of these two groups of steroid with those previously determined in the boar (Booth, 1975); and (iv) to relate the levels of steroids to the development of male characteristics in the intersex pig.

Materials and Methods

Animals

Six cross-bred intersex pigs between 9 and 15 months of age were studied. They were purchased accidentally with a group of normal gilts because of their predominantly female phenotype. The intersex pigs were eventually distinguished from normal gilts by the fact that each pig had an enlarged clitoris and ventral labial commissure which caused the urine to be voided in upward spurts, and they frequently mounted gilts which were on heat. An intersex pig (belonging to a private owner) with obvious boar-like characteristics was also included in the study. Genital tracts and submaxillary glands were obtained from the pigs within 30 min of slaughter, and a photographic record was made of the tracts. The testicular portion of the ovotestes and the submaxillary glands were weighed, and pieces of tissue from these glands and accessory organs were taken for histology (see Booth *et al.*, 1973; Booth, 1974); pieces of submaxillary gland were also taken for the histochemical demonstration of 3 β -hydroxy-5-ene-steroid dehydrogenase by the method of Booth *et al.* (1973). The remaining tissue was stored over solid CO₂ until required for steroid analysis.

Cytogenetics

The karyotype was determined in five pigs. A 10-ml sample of blood was collected from an ear vein into a heparinized syringe. The blood was allowed to settle at 37°C, and 0.5 ml plasma was added to 10 ml culture medium which was similar to that used by McFee *et al.* (1965). After 3 days in culture, colcemid was added, and the cells were then washed with hypotonic KCl overnight. Cells were fixed with methanol:acetic acid (3:1, v/v), and a cell-fixative suspension was pipetted onto a cold wet slide. The fixative was ignited, the slides air dried, and the cells stained with Giemsa (10 ml) containing 0.2 M-NH₃ (5 ml); after dehydration in acetone and acetone/xylol mixtures, the preparations were mounted in Permount (Fisher Scientific Co., New Jersey, U.S.A.). A number of well-dispersed chromosome spreads were photographed and karyotypes constructed from the cut-out images of the chromosomes according to the scheme of Hsu & Benirschke (1967).

Steroid determinations

The methods used for the extraction, identification, and measurement of C₁₉ steroids including 16-androstenes in testicular tissue and submaxillary glands, were identical to those previously described (Booth, 1975).

Steroid nomenclature

The following trivial names are used: testosterone (17 β -hydroxy-4-androsten-3-one); 5 α -dihydro-testosterone (17 β -hydroxy-5 α -androstan-3-one); androstenedione (4-androstene-3,17-dione); de-

EXPLANATION OF PLATE I

Fig. 1. The genital tract of a true hermaphrodite, B16M, with a 38XX chromosome complement. Note the well-developed uterus with blind oviducts (o), ovary with cystic follicles (f), corpora lutea or luteinized follicles (l) on the ovarian portion of the ovotestis, epididymis (e) and ductus deferens (d) on both sides, and an enlarged clitoris (c).

Fig. 2. The genital tract of a male pseudohermaphrodite, D17B, with a 38XX chromosome complement. Note the immature uterus with blind oviducts (o), well-developed testes (t), epididymides (e), ductus deferentes (d) overlying the base of the uterus and merging with a pair of seminal vesicles (s), prostate (p), thick muscle surrounding the urethra (m), and an enlarged clitoris (c).

PLATE 1

(Facing p. 116)

hydroepiandrosterone (DHA) (3 β -hydroxy-5-androsten-17-one); 5-androstenediol (5-androstene-3 β ,17 β -diol); 3 α -androstanol (5 α -androst-16-en-3 α -ol); 3 β -androstanol (5 α -androst-16-en-3 β -ol); 3 β -androstadienol (5,16-androstadien-3 β -ol); 5 α -androstenone (5 α -androst-16-en-3-one); androstadienone (4,16-androstadien-3-one).

Results

Anatomy and morphology

A number of the more distinctive anatomical and morphological characteristics that were observed are listed in Table 1. Five pigs were true hermaphrodites (Pl. 1, Fig. 1), and two were male pseudohermaphrodites (Pl. 1, Fig. 2). A well-developed uterus was found in the true hermaphrodites, and in some animals it was distended with a putrid fluid apparently caused by the back-flow of urine through a relaxed cervix. The oviducts in all animals ended blindly at the gonad. Cystic follicles were prevalent in the ovarian tissue, but follicles of normal appearance were also present in the ovaries and the ovarian portion of ovotestes. Only pig B16M had regular oestrous cycles and corpora lutea or luteinized follicles were present in the ovary and ovarian portion of the ovotestis (Pl. 1, Fig. 1).

In all pigs, an epididymis and ductus deferens were present on the side ipsilateral to testicular tissue. In pigs B16M and D16B, however, an epididymis was also present on the side with only ovarian tissue; in all cases the ductus deferens ended blindly in the region of the cervix. The most abundant testicular tissue occurred in the male pseudohermaphrodites and was associated with small uteri. A prostate and seminal vesicles were present in these animals, and the histological appearance of the glands showed that they were actively secretory. The testicular tissue in all pigs contained an abundance of Leydig tissue. Germinal cells were absent and only Sertoli-like cells were seen in the seminiferous tubules. The seminiferous tubules in the scrotal testis of pig A17M were well developed and each tubule contained a large lumen.

The largest submaxillary glands were found in the two male pseudohermaphrodites and in pig A20B which had other well-developed male secondary sexual characteristics. The amount of serous cells was greatest in two of the masculinized pigs, A20B and A17M (Pl. 2, Fig. 5). However, some of the heaviest submaxillary glands were not associated with serous cell hypertrophy, e.g. pigs D17B and B16M (Pl. 2, Fig. 3). In the four pigs investigated, the histochemically demonstrable activity of 3 β -hydroxy-5-ene-steroid dehydrogenase in the serous cells (Pl. 2, Figs 4 and 6) was related to the abundance of testicular tissue; the activity in the ducts was strong to intense in all samples. Pig A17M had boar-like libido, and was used to detect heat in gilts.

Cytogenetics

Four of the five pigs investigated cytogenetically had the normal female karyotype of 38XX (see Table 1), while the pig A17M had a 38XX/XY karyotype.

EXPLANATION OF PLATE 2

Figures 3 and 5 are paraffin wax-embedded sections of the submaxillary gland of intersex pigs showing the relative abundance of serous cells (S) and mucous cells (M). Haematoxylin, Alcian blue and chlorantine fast red. $\times 160$. Figures 4 and 6 are frozen sections showing 3 β -hydroxy-5-ene-steroid dehydrogenase activity in the submaxillary gland of intersex pigs. $\times 160$.

Fig. 3. True hermaphrodite, B16M, with a 38XX chromosome complement. Note the preponderance of mucous cells (M).

Fig. 4. True hermaphrodite, D16B, with a 38XX chromosome complement. Note the strong activity in ducts (d), and the slight activity in the serous cells (S).

Fig. 5. Male pseudohermaphrodite, A17M, with a 38XX/XY chromosome complement. Note the preponderance of serous cells (S).

Fig. 6. Same pig as in Fig. 3. Note the strong activity in the ducts (d), and serous cells (S).

Table 1. Anatomical and morphological characteristics in intersex pigs

	Pig						
	B58	D16B	D18B	B16M	A20B†	D17B	A17M†
Sex chromosome constitution	*	38XX	38XX	38XX	*	38XX	38XX/XY
Gonad	Ovotestis (left and right)	Ovary (left) Ovotestis (right)	Ovary (left) Ovotestis (right)	Ovary (left) Ovotestis (right)	Ovotestis (left) Testis (right)	Testis (left and right)	Testis (left and right)‡
Total wt of testicular tissue/animal (g)	18.6	23.5	43.5	56.1	28.5	171	209
Uterus	Well developed				Immature Vestigial		
Prostate and seminal vesicles	Absent				Present Present		
Total wt of submaxillary glands (g)	47.6	44.3	40.5	61.0	70.1	64.2	80.3
% Seros cells in submaxillary glands	*	39.0	36.3	37.8	53.4	37.9	51.6
3 β -Hydroxy-5-ene-steroid dehydrogenase in serous cells§	*	+	+	*	*	++	+++

* No observations made.

† Distinct male secondary sexual characteristics present e.g. coarse hair, tusks and profuse salivation.

‡ Scrotal testis; other testes were intra-abdominal.

§ Activity: +++, strong; ++, moderate; +, weak.

Steroids in testicular tissue and submaxillary glands

A number of C₁₉ steroids including 16-androstenes were determined in the testicular tissue and submaxillary glands of the intersex pig (see Table 2); small quantities of testosterone and 5 α -dihydro-testosterone (<2 μ g/100 g) were also found in some of the submaxillary glands. Pooled samples of 16-androstenes gave similar colour reactions and R_f values to authentic compounds on thin-layer chromatography, and all C₁₉ steroid samples gave similar retention times to authentic compounds on gas-liquid chromatography as described previously (Booth, 1975). Small amounts of 3 β -andro-stadienol and androstadienone which had been extracted from testicular tissue, and 3 β -andro-stadienol from submaxillary glands, were conclusively identified by combined gas-liquid chromatography-mass spectrometry (for details of mass spectra, see Booth, 1975). Androstadienone was not detected in submaxillary gland extracts. Analytical losses of steroids were determined using trace amounts of tritium-labelled steroids which were added initially to the glandular homogenates and subsequently recovered before gas-liquid chromatography. The recovery values are given in Table 2 and are similar to those previously found in studies on the boar (Booth, 1975). One labelled 16-androstene, 5 α -androstene, was available for a few recovery estimations; a mean $\pm$ S.D. of 17.6 $\pm$ 4.7% was obtained. The recovery of 5 α -androstene from all thin-layer chromatography steps was 36%, and the recovery of other 16-androstenes ranged from 24 to 29%; the 16-androstene values have been corrected for a relative recovery in relation to the recovery of 5 α -androstene from thin-layer chromatography.

Discussion

A number of reports have indicated that most intersex pigs are male pseudohermaphrodites (see Breeuwsma, 1970). However, of 28 intersex pigs studied by Krishnamurthy *et al.* (1971), only two were male pseudohermaphrodites. In the present study male pseudohermaphrodites were similarly in

Table 2. The content of C₁₉ steroids, including 16-androstenes, in testicular tissue and submaxillary glands of intersex pigs

	Pig						
	B58	D16B	A20B	D18B	B16M	D17B	A17M
Total wt of testes/animal (g)	18.6	23.5	28.5	43.5	56.1	171	209
C ₁₉ steroids in testes							
Testosterone	2.34	3.10	4.23	7.14	Trace	8.93	13.0
Androstenedione	N.D.	1.84	2.30	1.20	2.36	3.49	22.4
DHA	2.92	3.53	5.45	2.10	5.63	12.3	45.1
DHA sulphate	0.92	3.52	2.64	1.79	Trace	Trace	221
5-Androstenediol	17.3 (5.10)	14.2 (4.60)	26.6 (10.6)	23.9 (10.2)	22.0 (10.3)	98.6 (41.0)	91.0 (43.5)
5-Androstenediol sulphate	3.50	4.60	6.20	2.60	17.8	47.2	150
16-Androstenes in testes							
3 α -Androstenol	71.7	33.6	112	386	595	1,672	957
3 β -Androstenol	162	154	460	824	1,452	2,482	1,897
3 β -Androstadienol	17.8	1.11	8.44	32.8	Trace	Trace	N.D.
5 α -Androstenone	2.60	0.60	11.2	14.9	4.50	75.2	64.4
Androstadienone	2.99	Trace	1.87	163	3.64	22.9	8.33
Total wt of submaxillary glands/animals (g)	47.6	44.3	70.1	40.5	61.0	64.0	80.3
16-Androstenes in submaxillary glands							
3 α -Androstenol	9.71	67.3	381	33.9	376	729	665
3 β -Androstenol	*	19.0	48.5	7.90	56.0	173	31.4
3 β -Androstadienol	0.91	Trace	6.73	1.00	1.61	27.0	N.D.
5 α -Androstenone	0.53	4.16	12.3	1.46	14.6	11.3	36.0

Steroid values are expressed in $\mu\text{g/gland/animal}$. The values other than 5-androstenediol sulphate and 16-androstenes have been corrected for total losses. 5-Androstenediol sulphate values can be compared with uncorrected values for the free steroid (shown in parentheses). % Recoveries (mean $\pm$ S.D.): testosterone, 27.1 $\pm$ 4.0; androstenedione, 13.6 $\pm$ 4.2; DHA, 24.7 $\pm$ 8.7; DHA sulphate, 11.0 $\pm$ 4.6; 5-androstenediol, 40.1 $\pm$ 6.9.

* Contaminant prevented determination on gas-liquid chromatography.

the minority. These discrepancies are probably due to a biased selection. Those animals that are significantly masculinized, and therefore usually male pseudohermaphrodites, would not be supplied mistakenly for gilts by pig breeders as often as true hermaphrodites.

The observation that four of the five pigs were genetic females with the 38XX sex chromosome constitution is in agreement with the findings of Breeuwsma (1970) and Melander *et al.* (1971). It is possible that in these pigs, as in intersex goats (Hamerton *et al.*, 1969), an autosomal modifier may override the normal controlling function of the heterochromatic X-chromosome and de-repress the male-determining genes (Hamerton, 1968). Alternatively, as suggested by Ferguson-Smith (1966), the Y-chromosome of a male conceptus may be incorporated into the X-chromosome by 'crossing over'. The finding of the sex chromosome complement XX/XY in one of the male pseudohermaphrodites (A17M) is a rare occurrence in pigs (Breeuwsma, 1970), as it is in goats (Hamerton *et al.*, 1969). The XX/XY mixoploidy in cattle is associated with freemartinism (Ohno, 1969; Short *et al.*, 1969), and there is evidence that goat intersexes with XX/XY mixoploidy may also be freemartins (Hamerton *et al.*, 1969). If the XX/XY mixoploidy was confined to the blood leucocytes in pig A17M, then this would have provided substantial evidence for freemartinism; the chromosome constitution in cells from other tissues, however, was not investigated. Vascular anastomosis of placentae is considered to be a necessary prerequisite for freemartinism, but evidence for this in pigs is limited (Hughes, 1929). Breeuwsma (1970) and Crombie (1972) did not find vascular anas-

tomosomes between pig embryos, and the aetiology of XX/XY mixoploidy in the pig therefore remains speculative; a number of other explanations for this condition has been reviewed by Biggers (1968).

Contrary to the observations of van der Horst & Sybesma (1969) and Breeuwsma (1970), the testicular tissue of all intersex pigs in the present study contained appreciable quantities of testosterone and its immediate precursors. These results are comparable to those found for the intersex goat (Hamerton *et al.*, 1969; Zlotnik, 1973) and in the bovine freemartin (Short *et al.*, 1969) insofar as the concentrations of steroid were similar in the testes of hermaphrodites and normal males. The observations of Navratil *et al.* (1968) indicated that less androgen is produced in the intersex pig than the boar, one factor presumably being the smaller mass of testicular tissue. However, Liptrap & Raeside (1970) found similar levels of DHA and oestrogens in the urine of the cryptorchid and normal boar, suggesting that the intra-abdominal testis of the pig can be as endocrinologically active as the scrotal testis. When a comparison is made between the steroid values for the intersex pig obtained in this study with those for the boar (Booth, 1975), it is apparent that the intra-abdominal testicular tissue of intersex pigs is at least as endocrinologically competent as the testis of the 6-month-old boar; the relative concentrations of 16-androstenes, i.e. 3β -androstanol $>$ 3α -androstanol $\geq$ 3β -androstadienol and 5α -androsthenone $>$ androstadienone, found in the testicular tissue of intersex pigs and postpubertal boars particularly support this aspect. High levels of 5-androstenediol in relation to DHA, were found in the testis of the boar after the onset of spermatogenesis (Booth, 1975), and it is possible that the seminiferous tubules might be involved in the metabolism of DHA to 5-androstenediol as previously shown in the rat (Richards & Neville, 1973). If this is the case in the pig, then the similar findings in the intersex pig in which germinal cells are absent in postnatal testicular tissue suggests that the Sertoli cells might be involved in this metabolism. The significance of 5-androstenediol as a possible precursor for testosterone synthesis in the pig has been discussed recently (Booth, 1975). The high levels of 16-androstene found in the submaxillary glands of intersex pigs compared with the very low levels in female pigs (W. D. Booth, unpublished) indicates that these steroids are being secreted by the intersex gonad in sufficient quantities to cause taint in the carcass, supporting the earlier suggestion by Bishop (1969) that intra-abdominal gonads may cause taint in the pig.

The degree of masculinization of the genital tract was related to the amount of testicular tissue which predominated over ovarian tissue. The effect was minimal in the true hermaphrodites and most apparent in the male pseudohermaphrodites; the amounts of steroid in the testicular tissue provided quantitative data to support the endocrine aspect of these observations. The experiments of Jost (see review 1972) have shown that the fetal testis produces an apparently non-steroidal factor which inhibits the development of the Müllerian duct, while testicular androgens stimulate the development of the Wolffian duct; these two effects were clearly illustrated in the intersex pigs. In addition to the presence of ovarian tissue in true hermaphrodites, the presence of XX sex chromosomes in both true and male pseudohermaphrodites probably limits to a variable degree the masculinizing effect of androgens, as shown in relation to certain characteristics in the submaxillary glands. The activity of 3β -hydroxy-5-ene-steroid dehydrogenase was greatest in the serous cells of the submaxillary gland of male pseudohermaphrodites, but there was no serous cell hypertrophy in the male pseudohermaphrodite with the 38XX sex chromosome constitution, suggesting that, in the absence of a Y-chromosome, the serous cell may have a higher threshold response to androgen. Steroid dehydrogenase activity has been observed in the ducts of all submaxillary gland preparations in the pig and may be of general anabolic significance (Booth *et al.*, 1973). The small quantities of testosterone and 5α -dihydrotestosterone that were found in submaxillary glands of some intersex pigs were similar to those found in boars before 36 weeks of age (Booth, 1972). The amounts were unrelated to the abundance of serous cells and in all cases there was more testosterone than 5α -dihydrotestosterone, indicating limited 5α -reductase activity in the gland. The intersex pigs did not salivate as readily as boars, which was further evidence to suggest that the serous cells in the submaxillary gland of intersex pigs may be refractory to androgen stimulation. The exception was the pig with XX/XY mixoploidy; this animal salivated profusely when sexually excited and caused oestrous gilts to adopt the mating stance, presumably due to the release of the pheromones, 16-androstenes, in the saliva.

The authors 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. Thanks are also due to Dr Mary F. Hay for help with histology, Mr K. Elsome for the chromosome preparations, Mr R. Patman for the photography and Professor T. R. R. Mann for helpful suggestions in the preparation of the manuscript.

References

- BERRY, K.E. & SINK, J.D. (1971) Isolation and identification of 3 α -hydroxy-5 α -androst-16-ene and 5 α -androst-16-en-3-one from porcine adipose tissue. *J. Endocr.* **51**, 223-224.
- BIGGERS, J.D. (1968) Aspects of intersexuality in domestic mammals. *Proc. 6th Int. Congr. Anim. Reprod. & A.I., Paris* pp. 841-870.
- BISHOP, M.W.H. (1969) Possible explanations for the aberrant occurrence of boar taint in female and castrated pigs. In *Meat Production from Entire Male Animals*, pp. 297-301. Ed. D. N. Rhodes. J. & A. Churchill Ltd, London.
- 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. (1975) Changes with age in the occurrence of C₁₉ steroids in the testis and submaxillary gland of the boar. *J. Reprod. Fert.* **42**, 459-472.
- 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.
- BREEUWSMA, A.J. (1970) *Studies on Intersexuality in Pigs*. Diss., Drukkerij Bronder-Offset N.V., Rotterdam.
- CROMBIE, P.R. (1972) The occurrence of 'end-to-end' fusion between adjacent chorioallantoic sacs in the early pig embryo. *J. Reprod. Fert.* **29**, 127-129.
- FERGUSON-SMITH, M.A. (1966) X-Y chromosomal interchange in the aetiology of true hermaphroditism and of Klinefelter's syndrome. *Lancet* **ii**, 475-476.
- GOWER, D.B. (1972) 16-Unsaturated C₁₉ steroids, a review of their chemistry, biochemistry and possible physiological role. *J. Steroid Biochem.* **3**, 45-103.
- HAMERTON, J.L. (1968) Significance of sex chromosome derived heterochromatin in mammals. *Nature, Lond.* **219**, 910-914.
- HAMERTON, J.L., DICKSON, J.M., POLLARD, C.E., GRIEVES, S.A. & SHORT, R.V. (1969) Genetic intersexuality in goats. *J. Reprod. Fert., Suppl.* **7**, 25-51.
- HUGHES, W. (1929) The freemartin condition in swine. *Anat. Rec.* **41**, 213-245.
- Hsu, T.C. & BENIRSCHKE, K. (1967) *An Atlas of Mammalian Chromosomes*, Vol. 1. Springer, Berlin.
- JOST, A. (1972) A new look at the mechanisms controlling sex differentiation in mammals. *Johns Hopkins med. J.* **130**, 38-53.
- KRISHNAMURTHY, S., MACPHERSON, J.W. & KING, G.J. (1971) Intersexuality in Ontario swine. *Can. J. Anim. Sci.* **51**, 807-809.
- LIPTRAP, R.M. & RAESIDE, J.I. (1970) Urinary steroid excretion in cryptorchidism in the pig. *J. Reprod. Fert.* **21**, 293-301.
- McFEE, A.F., BANNER, M.W. & MURPHREE, R.I. (1965) Chromosome analysis of peripheral leucocytes of the sheep. *J. Anim. Sci.* **24**, 551-554.
- MELANDER, Y., HANSEN-MELANDER, E., HOLM, L. & SOMLEV, B. (1971) Seven swine intersexes with XX chromosome constitution. *Hereditas* **69**, 51-58.
- NAVRAJIL, S., HRONOVA, B. & LOJDA, L. (1968) Die endokrinologische Studie von erblich bedingten Fällen der Intersexualität bei Schweinen. *Proc. 6th Int. Congr. Anim. Reprod. & A.I., Paris*, pp. 913-916.
- OHNO, S. (1969) The problem of the bovine freemartin. *J. Reprod. Fert., Suppl.* **7**, 53-61.
- PATTERSON, R.L.S. (1968) 5 α -Androst-16-ene-3-one:—compound responsible for taint in boar fat. *J. Sci. Fd Agric.* **19**, 31-38.
- RICHARDS, G. & NEVILLE, A. (1973) Androgen metabolism in rat interstitial tissue and seminiferous tubules. *Nature, Lond.* **244**, 359-361.
- SHORT, R.V., SMITH, J., MANN, T., EVANS, E.P., HALLET, J., FRYER, A. & HAMERTON, J.L. (1969) Cytogenetic and endocrine studies of a freemartin heifer and its bull co-twin. *Cytogenetics* **8**, 369-388.
- VAN DER HORST, C.J.G. & SYBESMA, W. (1969) A preliminary investigation into the occurrence of steroids and hypotaurine in the reproductive organs of pig intersexes. *Tijdschr. Diergeneesk.* **94**, 1270-1276.
- ZLOTNIK, G. (1973) Testosterone levels in intersex goats. *J. Reprod. Fert.* **32**, 287-290.

Received 15 March 1975