VITAMIN A IN TESTICULAR TISSUE OF THE BOAR AND INTERSEX PIG

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In mammals suffering from vitamin A deficiency, the testes show various degrees of atrophy associated with a reduction in spermatogenic activity (Moustgaard, 1969). Coward, Howell, Pitt & Thompson (1966) and Palludan (1966a) demonstrated that this effect of vitamin A deficiency is not related to an endocrine deficiency, and Palludan (1966b) in the boar and Ahluwalia & Bieri (1971) in the rat showed that it is due to a direct effect on the process of spermatogenesis. The same investigators observed that in animals whose rate of growth and health were maintained on retinoic acid (vitamin A acid), testicular lesions still occurred. Injections of retinol (vitamin A alcohol), or retinaldehyde (vitamin A aldehyde) made directly into the testes restored spermatogenesis at the site of injection. Little information is available on the levels of endogenous vitamin A in testis in relation to the development and maintenance of spermatogenesis, and the purpose of this communication is to present such data obtained from studies on boar testis and testicular tissue of the intersex pig.

Gonads were obtained within 30 min of slaughter from a number of boars and intersex pigs of different ages (see Tables 1 and 2). Samples were fixed in Bouin's fluid for histology, and the paraffin wax-embedded sections were stained with Delafield's haematoxylin and chromatrope 2R; the remainder of the tissue was frozen over solid CO₂ until required for the assay of vitamin A and steroids. For this purpose, a maximum of 50 g testicular tissue was taken and homogenized for 5 min in water (100 ml). The homogenate was added to 70% aqueous ethanol (200 ml) and the mixture was left overnight at +4°C. After centrifugation (22,902 *g*) for 20 min at +4°C, the supernatant was reduced to 20 ml under reduced pressure and then extracted with redistilled diethyl ether (3 × 60 ml). The combined ether extracts contained some vitamin A and free steroids and the aqueous phase contained steroid conjugates (an account of the steroid investigations is in preparation). The ether extract was evaporated to dryness, combined with the tissue pellet remaining after centrifugation and subjected to alkaline hydrolysis according to Mervyn & Morton (1959). The reaction mixture was extracted with redistilled diethyl ether (5 × 250 ml), washed with water to about pH 7, dried over anhydrous Na₂SO₄ and the ether was removed under reduced pressure. The dry material was dissolved in a small quantity of light petroleum (40 to 60 b.p.) and lipids were removed by 'freezing

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out' at -20°C . After centrifugation (26,838 *g*) for 20 min at -10°C , the supernatant was brought to 20 ml with further solvent and extracted with 83% aqueous methanol (6×20 ml). The combined methanol extracts were evaporated to dryness and submitted to thin-layer chromatography using silica gel impregnated with fluorescein; the solvent systems used by Gower (1964) were found suitable for separating retinol from C_{19} steroids (see Plate 1). Retinol was

Table 1. Vitamin A in boar testes

Age	Breed	Boars (testes assayed)	Total wt of testes per animal (g)	Vitamin A (i.u./100 g of testes)*	Stage of spermatogenesis
At birth	LW $\times$ Esx	Pooled (12)†	0.41‡	Trace	Gonocytes
6 weeks	LW $\times$ Esx	Pooled (12)†	7.44‡	22.5‡	Gonocytes
12 weeks	LW $\times$ Esx	Pooled (4)†	19.2‡	37.7‡	Spermatogonia
18 weeks	LW $\times$ Esx	C11	57.6	113	Few spermatozoa
	LW $\times$ Esx	C13	125	82.6	
24 weeks	LW $\times$ Esx	A39L	305	76.7	Abundant spermatozoa
	LW $\times$ Esx	B50C	354	79.3	
30 weeks	Ldr	340	477	75.5	
	LW	230G	542	61.1	
	LW $\times$ Esx	B36M	533	107	
	LW $\times$ Esx	B37M	427	61.0	
36 weeks	LW $\times$ Esx	R	465	63.0	
	LW $\times$ Esx	G	478	47.7	
2 years	LW $\times$ Ldr	A	1004	54.3	
	LW $\times$ Esx	B	935	92.0	

LW, Large White; Esx, Essex; Ldr, Landrace.

* Uncorrected for losses. † No. of animals. ‡ Mean.

Table 2. Vitamin A in the testicular tissue of intersex pigs

Pig no.	Age	Gonad*		Total wt of testicular tissue (g)	Vitamin A (i.u./100 g of testicular tissue)†
		Left	Right		
B58	36 to 52 weeks	Ovotestis	Ovotestis	18.6	7.70
D16B	36 to 52 weeks	Ovary	Ovotestis	23.5	Trace
A20B	1 year	Ovotestis	Testis	28.5	8.53
D18B	36 to 52 weeks	Ovary	Ovotestis	43.5	18.9
B16M	1 year	Ovary	Ovotestis	56.1	16.0
D17B	36 to 52 weeks	Testis	Testis	171	32.0
A17M	1 year	Testis	Testis	209	4.87

* Germinal cells absent in testicular portion of gonad.

† Uncorrected for losses.

located under u.v. light (254 nm) and subsequently eluted with chloroform: ether (1:1, v/v; 2×5 ml). The solvent was removed under reduced pressure and the extract taken up in chloroform (1 ml); 0.3-ml samples were taken in duplicate for the spectrophotometric determination of vitamin A (Glover, Goodwin & Morton, 1947). The recovery of the method was approximately 25%.

The results are presented in Tables 1 and 2. The testicular extract of boars at

The separation on thin-layer chromatography of authentic retinol (spot 4), C_{19} steroids and 16-unsaturated C_{19} steroids which were studied in pig testis. Solvent system toluene: ethyl acetate (9:1, v/v; $\times 2$) and benzene: ether (9:1, v/v) after Gower (1964). Compounds located by u.v. light (outlined) and ethanol: H_2SO_4 (1:1, v/v). (1) 5 α -Androst-16-en-3-one; (2) 4,16-androstadien-3-one; (3) 5 α -androst-16-en-3 α -ol; (4) vitamin A alcohol; (5) cholest-5-en-3 β -ol; (6) 5,16-androstadien-3 β -ol; (7) 5 α -androst-16-en-3 β -ol; (8) 3 β -hydroxy-5-androsten-17-one (DHA); (9) 4-androstene-3,17 dione; (10) 5-androstene-3 β ,17 β -diol; (11) 17 β -hydroxy-4-androsten-3-one (testosterone).

(Facing p. 220)

birth produced a faint fluorescence which corresponded to retinol on the thin-layer chromatogram, but the amounts were below the sensitivity of the assay method. Between 12 and 24 weeks of age, spermatogenesis became established and the concentration of vitamin A reached maximum levels, coincident with the appearance of spermatozoa; the high levels of vitamin A were maintained in older boars showing active spermatogenesis. The concentration of vitamin A in the testicular extracts of all intersex pigs was lower than that found in the testes of normal boars during or after puberty; furthermore, there was no obvious correlation between the levels of vitamin A and testicular weight or age in the intersex pigs. The right testis of intersex pig A17M was in the scrotal position; this testis had well-developed seminiferous tubules with large lumina. The left testis of pig A17M and the gonads of the other intersex pigs were intra-abdominal; the seminiferous tubules rarely had a lumen. Only Sertoli-like cells were present in the seminiferous tubules of the intersex gonad, a distinctive characteristic of such gonads (Breeuwsma, 1970).

The data presented here support the view that vitamin A is intimately associated with spermatogenesis. The difference between the levels of vitamin A in boar testis and in the testicular tissue of intersex pigs suggests that it might be the germinal cells which are responsible for an additional accumulation of vitamin in boar testis. It is unlikely that the lack of germinal cells in the testicular tissue of intersex pigs is a direct consequence of a vitamin A deficiency. A more likely explanation is the influence of genetic anomalies (Fechheimer, 1970) and the intra-abdominal location of the gonad (Waites & Setchell, 1969). Subsequent endocrine studies on the intersex pig in this laboratory have shown that the lower levels of vitamin A in the testis do not affect the steroid production of this tissue. The need for vitamin A in spermatogenesis is probably similar to that required by other proliferative tissues (Fell, 1969); the underlying mechanism perhaps involves a specific effect of vitamin A on cell membranes (Lucy, 1969) and lysosome stability (Roels, 1969).

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