

## **The Fatty Acid Composition of the Ethanolamine and Choline Phosphoglycerides of Ovine and Porcine Testes**

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### *Abstract*

The fatty acid composition of the ethanolamine and choline phosphoglycerides was examined in the testes of Merino sheep aged 2·5 years and lambs aged 2 weeks, and Large White crossbred pigs aged 3 years and young pigs aged 5 months. Only in the case of the choline phosphoglycerides was there a noticeable increase in the polyunsaturated fatty acid content with maturation, but the ethanolamine phosphoglycerides contained higher levels of polyunsaturated fatty acids throughout. Sheep testes were characterized by the absence of 4,7,10,13,16-docosapentaenoic acid, but contained high levels of 4,7,10,13,16,19-docosahexaenoic acid, particularly in the ethanolamine phosphoglycerides. This latter acid was also evident in the pig testes, but derivatives of linoleic acid, particularly arachidonic and 4,7,10,13,16-docosapentaenoic acids, were dominant.

### **Introduction**

The importance of particular fatty acids in animal reproductive tissues is emphasized by the high concentration of polyunsaturated fatty acids in these organs (Holman and Greenberg 1953) and the severe impairment to reproduction during periods of essential fatty acid deficiency (Holman 1970). In the testis, an increase in polyunsaturated fatty acid content is associated with the maturation of the germinal tissue (Johnson 1970), and most lipid studies on this organ have centred on the metabolism of polyunsaturated acids and their relative contribution to the total tissue lipids. Bieri and Prival (1965), for example, examined the testicular lipid content of eight monogastric species and suggested that animals could be grouped according to the relative proportions of 4,7,10,13,16-docosapentaenoic and 4,7,10,13,16,19-docosahexaenoic acids in the testis. Holman and Hofstetter (1965) found that the pig contains more polyunsaturated fatty acid in all neutral lipid classes and in the phospholipid of the testis than does the bull, whereas Scott and Setchell (1968) recorded no 4,7,10,13,16-docosapentaenoic acid in ram testis. Apparently there are major species differences in the regulatory mechanisms for elongation and desaturation reactions of linoleic and linolenic acids within the testis. One factor controlling the type and proportion of fatty acid synthesized may be the competition between the processes of desaturation and transacylation of fatty acids (Brenner and Peluffo 1969), and a measure of this competitive metabolism may be forthcoming from more detailed studies of the fatty acid composition within specific lipid classes.

A previous study on lipid metabolism in bull testis included an examination of the fatty acid content of individual lipid classes at various stages of development of the tissue (Neill and Masters 1974). The purpose of the present investigation was to

determine the fatty acid composition of the major testicular phosphoglyceride classes of a second ruminant species at two stages of development, and to compare the findings with those for the testis of a monogastric species of similar size and habitat.

## Methods

The fatty acid composition of the choline and ethanolamine phosphoglycerides was investigated in the testes of Merino sheep (*Ovis aries*) aged 2·5 years and lambs aged 2 weeks, and Large White crossbred pigs (*Sus scrofa*) aged 3 years and young pigs aged 5 months. Immediately following castration of the animals, the extraneous membranes, epididymis, dorsal pole and tunica albuginea were excised from each testis, and the germinal tissue was treated by the procedure of Bligh and Dyer (1959) to recover the testicular lipids. Phosphoglyceride classes were separated by thin-layer chromatography on 500- $\mu$ m-thick layers of activated (110°C, 1 h) silica gel G (Merck A-G, Darmstadt, Germany) with chloroform-methanol-water (65 : 25 : 4 v/v) as the initial developing solvent for 16 cm. After being dried under nitrogen the chromatograms were redeveloped for 20 cm in hexane-diethyl ether-acetic acid (80 : 20 : 1 v/v) to remove neutral lipid ahead of the initial solvent front. Individual components were located by spraying the plates with Rhodamine 6G (0·05% in ethanol) and viewing under ultraviolet light. Classes of phosphoglycerides were identified by comparison with the chromatographic behaviour of standard phospholipids (Applied Science Laboratories, State College, Pa, U.S.A.) under the same chromatographic conditions. Areas of silica gel containing the separated phosphoglycerides were removed from the plates and the methyl ester derivatives of the fatty acids were formed by the procedure of Metcalfe *et al.* (1966). The esters were analysed by gas-liquid chromatography at 190°C using 17% diethylene glycol succinate on Gas Chrom G (80-100 mesh, Applied Science Laboratories). Dimethyl acetals resulting from the methylation of plasmalogenic aldehydes within the phosphoglycerides were chromatographed with the esters and then recovered and analysed separately after removal of the esters by saponification. The contribution of acetals to the original gas-liquid chromatographic spectrum was then deducted to yield the correct proportions of fatty acids. This difference technique eliminated the problem of polyunsaturated fatty acid losses often encountered during saponification (cf. Darin-Bennett *et al.* 1973).

Histological examination of sections taken from the tissues used revealed active spermatogenesis in all testes except those from the 2-week-old lambs, in which a single layer of indifferent cells was present within small seminiferous tubules. The morphology of the pig testes at 5 months was consistent with the onset of maturation in this species at about this age (Mount and Ingram 1971).

## Results and Discussion

There was a general distribution of palmitic, stearic and oleic acids within the ethanolamine phosphoglycerides of the testes, with proportions of each acid ranging between 8 and 17% of the total fatty acids. Palmitic acid predominated (30-40%) in the choline phosphoglycerides except in lamb testes, in which there was an equivalent amount of oleic acid (35%); the amount of oleic acid normally varied from 16 to 20% and that of stearic acid from 7 to 10%.

The proportions of the major polyunsaturated components of the ethanolamine and choline phosphoglycerides of the testes are presented in Table 1. In general, the ethanolamine phosphoglycerides contained more C<sub>20</sub> and C<sub>22</sub> acids than the choline phosphoglycerides, whereas the latter contained more linoleic acid. Such generalizations appear to be characteristic of these phosphoglycerides, irrespective of the tissue and species from which they are obtained (Gray and Macfarlane 1961), but the spermatozoon is a known exception to this generalization as the C<sub>22</sub> polyunsaturated acids occur in a higher proportion in the choline phosphoglycerides (Johnson *et al.* 1969; Neill and Masters 1973). This difference in the spermatozoon, the end product of the germinal processes in the testis, warrants consideration with the present findings, for there was an increase with age in the polyunsaturated fatty acid content of the

testicular choline phosphoglycerides in each species. This feature is thought to be associated with the increase in the number of germinal elements of the testis during maturation (Johnson 1970) and was accentuated by the differences between the sheep testes in the present instance. In contrast to these findings, however, the proportion of individual polyunsaturated fatty acids in the testicular ethanolamine phosphoglycerides was fairly comparable in tissues of the same species, irrespective of age. The choline phosphoglycerides, therefore, would appear to have a markedly different role from that of the ethanolamine phosphoglycerides in spermatogenesis, at least in relation to their fatty acid components.

**Table 1. Polyunsaturated fatty acid content of testicular ethanolamine and choline phosphoglycerides**  
Boar 1 was 3 years and boar 2 was 5 months old. Fatty acid composition is given relative to total fatty acid content

Fatty acid <sup>A</sup>	Equivalent chain length <sup>B</sup>	Fatty acid composition (%):							
		Ethanolamine phosphoglycerides <sup>C</sup>				Choline phosphoglycerides <sup>C</sup>			
		Ram	Lamb	Boar 1	Boar 2	Ram	Lamb	Boar 1	Boar 2
18:2, <i>n</i> -6	19·35	2·3	1·6	4·5	2·7	4·1	4·8	11·6	5·8
18:3, <i>n</i> -3	20·50	7·3	0·7	0·2	1·7	4·1	0·6	0·3	0·6
20:3, <i>n</i> -6	21·92	—	1·5	3·4	2·7	2·3	0·2	10·2	4·9
20:3, <i>n</i> -9	21·62	—	2·8	—	—	—	—	—	—
20:4, <i>n</i> -6	22·64	13·4	18·5	24·7	23·6	11·9	5·0	13·3	11·7
20:5, <i>n</i> -3	23·44	—	3·5	0·2	—	—	0·1	0·3	—
22:3, <i>n</i> -9	23·77	—	—	0·1	—	—	—	0·3	—
22:4, <i>n</i> -6	24·38	—	—	2·3	4·3	—	—	0·5	—
22:5, <i>n</i> -3	25·40	—	3·5	0·4	—	—	—	—	—
22:5, <i>n</i> -6	24·96	—	—	14·1	14·7	—	—	4·4	3·3
22:6, <i>n</i> -3	26·03	37·5	30·0	8·5	7·3	17·0	3·6	4·6	1·1
24:4, <i>n</i> -6	26·30	—	—	—	3·7	—	—	—	—

<sup>A</sup> Designations in accordance with the tentative rules and recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature.

<sup>B</sup> Determined with diethyl glycol succinate as liquid phase.

<sup>C</sup> Include diacyl, alk-1-enyl acyl and alkyl acyl components.

The results in Table 1 suggest that in sheep testis the desaturation and elongation of linoleic acid is controlled at the point of arachidonic acid synthesis, but small amounts of C<sub>22</sub> derivatives can apparently be formed in association with specific lipids such as the cholesteryl esters and minor acidic phospholipids (Neill 1972). The virtual absence of 4,7,10,13,16-docosapentaenoic acid in sheep testis was in marked contrast to the high proportions of 4,7,10,13,16,19-docosahexaenoic acid, particularly in the ethanolamine phosphoglycerides. Whereas traces of the higher metabolites of oleic acid were found in all testes, 5,8,11-eicosatrienoic acid occurred in a measurable proportion in the ethanolamine phosphoglycerides of lamb testis only. The amount present was comparable to that observed in the liver phospholipids of unweaned lambs (Noble *et al.* 1970).

In the pig testes, linoleic acid derivatives formed the major polyunsaturated class. Arachidonic acid was the predominant derivative, but marked levels of 4,7,10,13,16-docosapentaenoic acid were present also, together with a component tentatively identified as 8,11,14-eicosatrienoic acid. This latter component occurred in similar proportions to arachidonic acid in the choline phosphoglycerides of the adult boar

testis. Other polyunsaturated metabolites of linoleic acid included 7,10,13,16-docosatetraenoic acid and a component tentatively identified as 9,12,15,18-tetra-cosatetraenoic acid. These acids were most noticeable in the ethanolamine phosphoglycerides, but only the young boar testis contained a significant quantity of the latter acid.

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