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TESTICULAR LIPIDS

IV. EFFECT OF UNILATERAL AND BILATERAL CRYPTORCHIDISM ON THE FATTY ACIDS OF ESTERIFIED CHOLESTEROL IN THE RAT AND RABBIT

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Summary. Scrotal testes from control and unilaterally cryptorchid rats and rabbits and abdominal testes from animals unilaterally or bilaterally cryptorchid for 6 days were used in this study. Total cholesterol rose ($P<0{\cdot}05$) in all abdominal testes as a result of increased ($P<0{\cdot}05$) levels of esterified cholesterol. Fatty acids of the scrotal testes from unilaterally cryptorchid animals were only slightly changed by treatment. Changes in fatty acid esters in abdominal testes of unilaterally cryptorchid animals of both species were similar. These changes consisted of increases in most fatty acids with a chain length of 14, 16 and 18, both saturated and unsaturated. Abdominal testes of bilaterally cryptorchid rabbits differed from those of unilateral cryptorchids in that proportions of long chain fatty acids were more consistently increased in the former. Abdominal testes of the bilaterally cryptorchid rats also differed from the unilateral cryptorchids but less than in the rabbit. Differences in hormonal levels in the unilateral and bilateral cryptorchids are suggested as the most probable cause of difference in the abdominal testes of these groups.

INTRODUCTION

Various treatments which alter the structural, metabolic and physiological patterns of the testis also result in alterations in testicular lipids of several species (Davis & Coniglio, 1967; Fleeger, Bishop, Gomes & VanDemark, 1968a, b; Johnson, Gomes & VanDemark, 1969; Johnson, 1970). Among other lipid changes, impairment of spermatogenesis consistently resulted in increased concentrations of esterified cholesterol in the testes, regardless of the anti-spermatogenic treatment employed (Johnson, VanDemark, Gomes, Butler & Hodgen, 1967; see Johnson, 1970).

Studies *in vitro* using testis tissue from rams subjected to elevated ambient temperature revealed that increases in cholesterol esters were attributable to quantitative changes in synthesis and esterification of cholesterol (Johnson *et al.*,

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1969). Whether this increase in cholesterol esters is accompanied by qualitative changes in the fatty acids esterified to cholesterol, however, has not yet been determined.

The present study was undertaken to determine the levels of esterified cholesterol in testes, the fatty acid composition of these esters and to ascertain whether these factors are altered by short-term cryptorchidism.

MATERIALS AND METHODS

Mature male rabbits of the giant breeds and mature male Wistar rats were randomly assigned to the control group or to unilateral or bilateral cryptorchid groups. In the latter cases, artificial cryptorchidism was produced as described by Fleeger *et al.* (1968a). All animals were killed 6 days after testes were translocated to the abdominal cavity. The testes were removed, trimmed, weighed, and frozen in liquid nitrogen.

Lipids were extracted from the testes and analysed for total, free, and esterified cholesterol (Fleeger *et al.*, 1968a). A portion of the lipid extract was dissolved in chloroform:methanol (1:1, v/v) and applied to thin layer plates. After chromatography in the diethyl ether:hexane system (6:94, v/v; Freeman & West, 1966), the cholesterol ester band was scraped from the plate and eluted from the silica gel with chloroform:methanol (2:1, v/v, 3 × 10 ml) and methanol (1 × 10 ml). The fatty acid methyl esters were prepared, extracted and identified using the method outlined by Fleeger *et al.* (1968b).

Differences between treatments were determined using Duncan's new multiple range test as outlined by Steel & Torrie (1960).

RESULTS

Rabbits

The majority of the changes in total cholesterol concentration (mg/g dry weight) of abdominal testes was accounted for by changes in esterified cholesterol (Table 1). While total and esterified cholesterol of the scrotal testis of the unilateral cryptorchid were unaffected by the treatment, both cholesterol fractions were significantly ($P<0{\cdot}01$) increased in its abdominal partner. The levels of esterified and total cholesterol were also increased ($P<0{\cdot}05$) in the testes of bilateral cryptorchids, but not to the extent observed in the abdominal testes of the unilaterally cryptorchid rabbits. This was similar to the finding previously reported (Johnson *et al.*, 1967).

The fatty acid methyl esters derived from esterified cholesterol of the testes of control rabbits (Table 1) showed a predominance of oleate (18:1) followed by stearate (18:0) and behenate (22:0). The dimethyl acetals of palmitaldehyde (16A) and stearaldehyde (18A) occurred in relatively high proportions as previously shown for the triglyceride and phospholipid fractions of the testis (Fleeger *et al.*, 1968b).

In the scrotal testis of the unilateral cryptorchid, linolenate (18:3) and arachidate (20:0) were significantly increased ($P<0{\cdot}05$) over controls. Abdominal testes contained significantly ($P<0{\cdot}05$) higher proportions of myristate

(14:0), myristoleate (14:1), palmitate (16:0) and palmitoleate (16:1), whereas significant decreases ($P<0.05$) in linoleate (18:2) and nonadecanoate (19:0) were noted. The testes of bilaterally cryptorchid rabbits exhibited increases ($P<0.05$) in 18:3, arachidonate (20:4) and docosatetraenoate (22:4) and significant ($P<0.05$) reductions in 19:0 and *cis*-eicos-5-enoate (20:1). Regardless of treatment, 18:0 and 18:1 appeared to be the most prevalent fatty acids in rabbit testes.

Examination of the differences between unilaterally and bilaterally abdominal

TABLE 1

ESTERIFIED CHOLESTEROL AND ITS FATTY ACID FRACTION IN THE RABBIT TESTIS FOLLOWING CRYPTORCHIDISM

	Control	*Unilateral cryptorchid*		*Bilateral cryptorchid*
		Scrotal	*Abdominal*	
No. of testes	3	2	2	5
Total cholesterol concentration†	19·9	19·1	37·7**	25·9*
Esterified cholesterol concentration†	10·2	8·4	23·8**	13·4*
Fatty acids, % of total				
14:0	0·7	1·1	2·2*	0·4
14:1	1·7	0·4	2·2*	0·7
15:0	2·0	1·4	1·2	0·4
16A	3·1	1·2	1·5	1·6
16:0	5·4	7·3	12·3**	7·2
16:1	2·1	1·5	4·8*	2·1
17:0	5·6	7·1	3·2	3·4
18A	5·8	3·0	2·3	2·4
18:0	10·6	9·3	12·6	15·5
18:1	13·6	11·5	18·4	16·3
18:2	7·5	3·6	2·5*	4·1
18:3	4·7	8·8*	9·8*	13·4*
19:0	8·2	8·2	2·0*	4·3*
20:0	5·0	10·0*	2·7	1·3*
20:1	6·6	9·3	3·3	0·7*
20:4	3·3	3·2	4·4	12·7**
22:0	10·5	5·7	9·8	6·0*
22:4	0·5	2·8	1·2	5·3*
22:5	3·1	4·6	3·6	2·2

† mg/g dry weight.
* Significantly different from controls ($P<0.05$).
** Significantly different from controls ($P<0.01$).

testes revealed significant ($P<0.05$) reductions in 14:0, 14:1 and 20:1 in the testes of the bilateral cryptorchid with concurrent increases in 20:4 and 22:4.

Rats

As shown in Table 2, no significant differences existed in total or esterified cholesterol levels between scrotal testes of control and unilaterally cryptorchid rats. There was, however, a significant increase ($P<0.05$) in both cholesterol fractions in the abdominal testes of the unilateral and bilateral cryptorchids. Unlike the rabbit, however, no difference existed in the cholesterol levels of unilaterally and bilaterally cryptorchid rat testes.

The predominant fatty acid of the esterified cholesterol of the control rat testis was 18:1 followed by 18:0. In the scrotal testes of the unilateral cryptorchids, 14:1 and heptadecanoate (17:0) increased significantly ($P<0.05$), whereas abdominal testes of the unilateral cryptorchid exhibited significant increases in 14:0, 14:1, 16:1 ($P<0.01$) and 18:2 ($P<0.05$) and decreases in 18:3, 19:0 and 22:4 ($P<0.05$). Unilaterally cryptorchid testes contained greater proportions of 17:0 ($P<0.01$) and clupanodonate (22:5; $P<0.05$) and less 19:0 ($P<0.05$) than controls.

TABLE 2

EFFECT OF CRYPTORCHIDISM ON ESTERIFIED CHOLESTEROL AND ITS FATTY ACID COMPONENTS IN THE RAT TESTIS

	Control	*Unilateral cryptorchid*		*Bilateral cryptorchid*
		Scrotal	*Abdominal*	
No. of testes	7	2	2	3
Total cholesterol concentration†	16·6	16·2	25·3*	29·4**
Esterified cholesterol concentration†	8·4	10·3	15·2*	17·1*
Fatty acids, % of total				
14:0	1·3	0·7	3·4**	0·4
14:1	0·5	2·7*	2·2**	0·3
15:0	1·3	2·0	1·7	0·6
16A	1·6	1·7	0·7	0·3
16:0	7·4	8·3	12·8	9·4
16:1	5·2	4·4	28·1**	1·3
17:0	1·7	5·8*	1·2	8·4**
18A	4·1	6·5	3·6	2·9
18:0	13·5	6·8	7·5	9·9
18:1	16·3	12·6	15·2	18·7
18:2	0·9	5·3	11·1*	1·1
18:3	9·2	8·7	3·5*	10·5
19:0	9·8	4·3	2·7*	2·1*
20:0	4·3	5·0	1·5	11·3*
20:1	3·4	0·0	0·0	1·6
20:4	2·9	3·7	1·5	4·2
22:0	7·8	11·5	2·9	8·4
22:4	5·9	6·8	0·4*	2·6
22:5	2·9	3·2	0·0	6·1*

† mg/g dry weight.
* Significantly different from controls ($P<0.05$).
** Significantly different from controls ($P<0.01$).

Comparison of unilateral and bilateral abdominal testes revealed that the latter contained lower proportions of 14:0, 14:1, 16:1 ($P<0.01$) and 18:2 ($P<0.05$) and higher levels of 17:0, 18:3, 20:0 and 22:5 ($P<0.05$; Table 2).

DISCUSSION

Oleate was the predominant fatty acid found in the study followed by stearate in both species and most treatments. Neither of these varied significantly with treatment. It has been previously found that oleate was the most prevalent fatty

acid in the triglyceride fraction of testicular lipids, whereas palmitate was the most abundant in the phospholipid fraction (Fleeger *et al.*, 1968b).

Fatty acid changes in the scrotal testis of the unilateral cryptorchid in the present study were few as they were in the triglyceride and phospholipid fractions reported by Fleeger *et al.* (1968b). Some workers have found quantitative lipid (Fleeger *et al.*, 1968a) and histological (Clegg, 1965) changes in the scrotal testis of the unilateral cryptorchid while others have found neither of these changes (Johnson, Gomes, Free & VanDemark, 1968). The changes in fatty acid in the present study represent significant increases above control, but they are not the same as changes in the abdominal partner. That alterations did occur, however, suggests that qualitative changes, albeit minor, may occur in the scrotal testis of the unilateral cryptorchid.

Significant fatty acid changes in the abdominal testes of the unilateral cryptorchid animal were quite similar in the two species studied. Most changes occurred in the proportions of the even numbered fatty acids suggesting alterations in the pathways of fatty acid metabolism as a direct result of the elevated temperature or of the hormonal changes which apparently occur in cryptorchid animals (Johnson *et al.*, 1968). The temperature might reduce the activity of specific enzymes while the hormonal change could alter fatty acid biosynthesis, transport, transformation and other metabolic functions.

The fatty acids of cholesterol ester from the testes of bilaterally cryptorchid rabbits appear different from those found in the abdominal testes of the unilaterally cryptorchid animals. Rather than the alteration in levels of the relatively shorter chained fatty acids, marked increases were noted in the unsaturated long chain fatty acids (18:3, 20:4 and 22:4) with reduction of the saturated fatty acids of comparable chain length (19:0, 20:0 and 22:0). This change suggests a shift from the pathway normally involved in synthesis of fatty acids to lipolysis of dietary lipid. It further suggests a blockage of the new pathway in that there is little synthesis of the long chain (longer than 18) fatty acids and an almost complete reliance on dietary lipid resulting in the unsaturated compounds. If the bilateral cryptorchid has a reduced steroid output (Llaurado & Dominguez, 1963), then this implies that a high dependence on androgens in the implementation of the new pathway for the synthesis of fatty acids may exist in the testis.

As suggested by the quantification of esterified cholesterol, less difference exists between the unilaterally and bilaterally cryptorchid rat than in the rabbit. Qualitatively, this also appears to be the case since changes in bilateral abdominal testes appear to be more similar to the testes of the unilateral cryptorchid, suggesting that the mechanisms altering specific cholesterol esters may be similar in both conditions.

It is evident from the foregoing that there are conditions in the unilateral cryptorchid which can qualitatively alter a limited number of fatty acids of the scrotal testis. The abdominal testis of the unilateral cryptorchid, however, appears to be more profoundly affected. In both the rat and rabbit, pathways appear to be markedly altered and the testes of the bilateral cryptorchid are also changed; however, these differ with each species. Hormonal changes resulting from the different treatments may be responsible for the changes in

the abundance of fatty acid which result in alterations in the fatty acid components of esterified cholesterol of the testis.

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