TESTICULAR LIPIDS

I. EFFECT OF UNILATERAL CRYPTORCHIDISM ON LIPID CLASSES

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Summary. Experiments designed to determine the effects of short-term unilateral cryptorchidism on testicular lipids have been carried out using twenty-four mature male rabbits. Testes from unoperated control rabbits and rabbits rendered unilaterally cryptorchid for 2, 4 and 6 days were analysed for wet and dry weight, total lipid, total, free and esterified cholesterol, total carboxyl ester, lipid phosphorus and triglyceride.

The translocated testes decreased in wet and dry weight ($P < 0.001$) and total lipid content ($P < 0.001$) 6 days after surgery. Lipid concentration increased ($P < 0.01$) in translocated testes, as did concentrations of total cholesterol ($P < 0.005$) and esterified cholesterol ($P < 0.005$). Total carboxyl ester ($P < 0.001$) and triglyceride ($P < 0.01$) concentrations also increased in translocated testes, but lipid phosphorus concentration was unchanged.

The contralateral scrotal testes increased in total lipid concentration ($P < 0.01$), esterified cholesterol concentration ($P < 0.05$), total carboxyl ester ($P < 0.001$) and triglyceride ($P < 0.01$) concentrations.

INTRODUCTION

Accumulation of lipids has been reported following several treatments which impair spermatogenesis. Increased total lipid concentration has been detected by histochemical techniques in 80- and 200-day cryptorchid testes of rats (McEnery & Nelson, 1953) and naturally cryptorchid swine testes (Hanes & Rosenbloom, 1911). Korman, Harkonen & Kontineu (1964) reported that lipids accumulate in the atrophied seminiferous tubules of cryptorchid rat testes. Hoffman (1960) reported that testicular cholesterol exhibits a rapid rise following translocation of one rat testis to the body cavity. The contralateral scrotal testis responded in a similar manner, but returned to normal more rapidly than the translocated testis.

This study was undertaken to examine the various lipid classes present in rabbit testes during short-term unilateral cryptorchidism.

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MATERIALS AND METHODS

Twenty-four mature male rabbits were randomly assigned to four experimental groups: (1) unoperated controls, (2) 2-day unilateral cryptorchidism, (3) 4-day unilateral cryptorchidism, and (4) 6-day unilateral cryptorchidism.

Unilateral cryptorchidism was produced by translocating one testis to the abdominal cavity by the following method. Rabbits were anaesthetized with a single intravenous dose of pentobarbital sodium. The scrotum, tunica vaginalis and gubernaculum testis were incised. The freed testis was bathed with saline, passed through the inguinal canal and the canal sutured. Sulphanilamide was placed in the scrotum and the incision was closed with wound clips. In three animals of each treatment group, the left testes were translocated and in the remaining three the right testes were translocated.

After a period of 2, 4 or 6 days, the rabbit was again anaesthetized. The linea alba was incised and the abdominal aorta perfused with saline to remove blood lipids from the testes. Both testes were removed, freed from the epididymides and weighed. Duplicate dry weight samples were taken and the remaining tissue was frozen on solid carbon dioxide and stored at -20°C until prepared for assay.

Each testis was homogenized with 20 parts chloroform:methanol (2:1 v/v) to 1 part testis. Following a 1-hr period of extraction the homogenate was filtered through a column of glass wool. The filtrate was purified according to the procedure of Folch, Lees & Sloane-Stanley (1957). Total lipid was determined gravimetrically. Lipid phosphorus was determined by the method of Bartlett (1959), ester linkage as outlined by Rapport & Alonzo (1955) and total and free cholesterol according to the procedure of Zak, Dickenman, White, Burnett & Cherney (1954). Esterified cholesterol was calculated as the difference between total and free cholesterol, and triglyceride was calculated from the ester linkage not accounted for by cholesterol esters and phospholipid.

RESULTS

Testis weight

As shown in Table 1, neither wet weight nor dry weight of testes differed significantly from unoperated controls after 2 or 4 days of translocation, nor did translocated testes differ from their contralateral scrotal partners during this time period. Six days after surgery, both the wet and dry weight of the translocated testes were significantly lower ($P<0.001$) than the control testes or the scrotal testes of unilaterally cryptorchid rabbits. No differences existed in weight when scrotal testes of unilaterally cryptorchid animals were compared with testes from unoperated control rabbits.

Total lipids

Neither the total lipid in the scrotal testes of unilateral cryptorchids (Table 1) nor the lipid levels in abdominal testes which were translocated for 2 or 4 days were significantly different from controls. After 6 days of translocation, however, total lipid levels had decreased significantly ($P<0.001$). Total lipid concentration (mg lipid/g dry weight) increased ($P<0.01$) in both the scrotal and

translocated testes of treated rabbits as compared with unoperated controls (Table 1).

TABLE 1
TESTIS WEIGHT AND TOTAL LIPID EXTRACT IN TESTES FROM UNILATERALLY CRYPTORCHID RABBITS*

Days	Testis weight		Total lipid extract	
	Wet wt (g)	Dry wt (mg)	mg/testis	mg/g dry wt
CONTROL				
0	2.44 ± 0.13†	338.0 ± 13.4	58.1 ± 3.3	171.6 ± 4.1
SCROTAL‡				
2	2.56 ± 0.26	345.6 ± 42.5	68.1 ± 7.7	201.0 ± 13.3
4	2.24 ± 0.14	301.5 ± 13.5	66.2 ± 5.7	219.8 ± 16.2
6	2.23 ± 0.24	298.1 ± 44.1	59.6 ± 5.8	207.3 ± 9.3
TRANSLOCATED‡				
2	2.43 ± 0.16	301.0 ± 36.1	60.9 ± 6.2	204.7 ± 9.3
4	2.38 ± 0.23	292.1 ± 29.9	67.0 ± 10.9	223.6 ± 12.7
6	1.19 ± 0.09	165.3 ± 17.2	33.4 ± 3.5	203.1 ± 12.8

* Testes from six rabbits in each group.

† Mean ± standard error.

‡ Scrotal and translocated testis of unilaterally cryptorchid rabbits.

Cholesterol

Total cholesterol (mg/testis) levels in the scrotal testes of treated rabbits were not significantly different from the control value (Table 2). Two and 4 days following translocation, abdominal testes were not different from controls, but 6 days after surgery the total cholesterol level in translocated testes was lower ($P < 0.01$) than that in control testes, but not significantly different from the level in the contralateral scrotal testes of the same animals.

TABLE 2
CHOLESTEROL, LIPID PHOSPHORUS, TOTAL CARBOXYL ESTER AND TRIGLYCERIDE LEVELS FOLLOWING UNILATERAL CRYPTORCHIDISM*

Days	Cholesterol Levels (mg/testis)			Lipid phosphorus (mg/testis)	Total carboxyl ester (μ-equiv./testis)	Triglyceride† (μ-equiv./testis)
	Total	Free	Esterified			
CONTROL						
0	7.1 ± 0.3‡	5.0 ± 0.2	2.1 ± 0.1	1.4 ± 0.1	95.7 ± 6.0	11.4 ± 1.5
SCROTAL§						
2	7.8 ± 0.8	5.4 ± 0.6	2.4 ± 0.4	1.4 ± 0.1	132.9 ± 17.6	23.9 ± 4.9
4	6.8 ± 0.5	4.6 ± 0.3	2.1 ± 0.2	1.4 ± 0.1	135.5 ± 19.4	25.0 ± 7.4
6	6.3 ± 0.8	4.2 ± 0.3	2.0 ± 0.2	1.2 ± 0.2	115.9 ± 11.6	20.4 ± 4.8
TRANSLOCATED§						
2	7.4 ± 0.9	4.9 ± 0.5	2.4 ± 0.3	1.2 ± 0.1	112.4 ± 12.1	19.3 ± 2.3
4	7.4 ± 0.7	4.7 ± 0.4	2.7 ± 0.4	1.2 ± 0.2	144.8 ± 30.6	29.7 ± 8.1
6	5.4 ± 0.7	2.8 ± 0.4	2.6 ± 0.4	0.6 ± 0.1	60.1 ± 5.1	9.3 ± 1.8

* Testes from six rabbits in each group.

† μ-equiv. triglyceride = $\frac{\mu\text{-equiv. carboxyl ester} - 2(\mu\text{-equiv. P} - \mu\text{-equiv. esterified cholesterol})}{3}$

‡ Mean ± standard error.

§ Scrotal and translocated testis of unilaterally cryptorchid rabbits.

When total cholesterol was compared on a concentration basis (mg cholesterol/g dry weight) as shown in Table 3, significant increases ($P<0.005$) were demonstrated in translocated, but not in contralateral scrotal testes.

Free cholesterol levels in scrotal testes (Table 2) were not significantly different from controls. Free cholesterol in translocated testes was unchanged at 2 and 4 days, but declined ($P<0.005$) 6 days after surgery. Concentration of free cholesterol (mg/g dry weight) was not significantly different in translocated testes or their scrotal partners when compared to unoperated controls (Table 3), but the 6-day translocated testes had greater ($P<0.05$) concentrations of free cholesterol than the contralateral scrotal testes from the same animals.

Esterified cholesterol levels (Table 2) in scrotal testes from treated animals were not different from control levels, but esterified cholesterol increased

TABLE 3
TESTICULAR LIPID CONCENTRATIONS FOLLOWING UNILATERAL CRYPTORCHIDISM*

Days	Cholesterol levels (mg/g dry wt)			Lipid phosphorus (mg/g dry wt)	Total carboxyl ester (μ -equiv./g dry wt)	Triglyceride (μ -equiv./g dry wt)
	Total	Free	Esterified			
CONTROL						
0	21.3 $\pm$ 0.3†	15.0 $\pm$ 1.2	6.3 $\pm$ 0.4	4.2 $\pm$ 0.1	262.0 $\pm$ 24.4	33.7 $\pm$ 4.9
SCROTAL‡						
2	22.8 $\pm$ 0.5	15.9 $\pm$ 1.1	6.9 $\pm$ 0.9	4.2 $\pm$ 0.3	390.0 $\pm$ 36.8	69.3 $\pm$ 13.8
4	22.8 $\pm$ 0.8	15.6 $\pm$ 0.6	7.2 $\pm$ 0.4	4.6 $\pm$ 0.2	450.7 $\pm$ 68.4	83.1 $\pm$ 21.4
6	21.5 $\pm$ 0.7	14.3 $\pm$ 0.4	7.2 $\pm$ 0.4	4.2 $\pm$ 0.2	416.3 $\pm$ 56.7	68.6 $\pm$ 21.4
TRANSLOCATED‡						
2	24.6 $\pm$ 0.5	16.5 $\pm$ 0.7	8.1 $\pm$ 0.2	4.2 $\pm$ 0.2	378.9 $\pm$ 28.7	64.0 $\pm$ 8.8
4	25.8 $\pm$ 1.1	16.4 $\pm$ 1.5	9.4 $\pm$ 0.5	4.2 $\pm$ 0.3	474.0 $\pm$ 57.5	101.7 $\pm$ 23.2
6	32.6 $\pm$ 1.0	16.6 $\pm$ 0.8	16.0 $\pm$ 2.0	3.9 $\pm$ 0.2	372.7 $\pm$ 33.5	56.4 $\pm$ 11.2

* Testes from six rabbits in each group.

† Mean $\pm$ standard error.

‡ Scrotal and translocated testis of unilaterally cryptorchid rabbits.

($P<0.025$) in the translocated testes. Concentration of esterified cholesterol (Table 3) increased ($P<0.05$) on Days 4 and 6 for the contralateral scrotal testes. All translocated testes had increased ($P<0.001$) esterified cholesterol concentrations.

Carboxyl ester and triglycerides

Total carboxyl ester per testis (Table 2) increased in the contralateral scrotal testes on Days 2 and 4 ($P<0.05$) and decreased on Day 6 to a level no different from control testes. The translocated testes were not significantly different from controls at Day 2, but were higher ($P<0.05$) at 4 days and decreased at 6 days to a level of carboxyl ester lower ($P<0.001$) than that found for controls. When compared on a dry weight basis (Table 3), total carboxyl ester concentration increased ($P<0.001$) above control levels in both contralateral scrotal and translocated testes and remained higher throughout the experimental period.

The calculated triglyceride per testis (Table 2) was higher than controls in all scrotal testes of the treated groups ($P<0.001$). In translocated testes, triglyceride rose at 2 and 4 days ($P<0.001$), then fell at 6 days to a level no different from controls, but lower ($P<0.001$) than the contralateral scrotal testes. Triglyceride

concentration (μ -equiv./g dry weight) increased ($P<0.01$) in both scrotal and translocated testes of treated rabbits and remained above control levels throughout the experiment (Table 3).

Phospholipids

The total lipid phosphorus in scrotal testes (Table 2) was unchanged by treatment. Translocated testis levels were not different from controls on Days 2 or 4, but decreased ($P<0.005$) on Day 6. When expressed on a dry weight basis (Table 3), lipid phosphorus concentration was higher ($P<0.05$) in contralateral scrotal testes after 4 days, but no other value differed from controls.

Composition of total lipid

Total cholesterol, expressed as a percentage of total lipid extract (mg cholesterol/100 mg lipid) did not change significantly in the contralateral scrotal testes nor in the testes translocated for 2 or 4 days (Table 4). However, the con-

TABLE 4
CHARACTERISTICS OF THE LIPID EXTRACT FROM TESTES FOLLOWING UNILATERAL CRYPTORCHIDISM*

Days	Cholesterol levels (mg/100 mg lipid)			Lipid phosphorus (mg/100 mg lipid)	Total carboxyl ester (μ -equiv./100 mg lipid)	Triglyceride (μ -equiv./100 mg lipid)
	Total	Free	Esterified			
CONTROL 0	12.4 $\pm$ 0.4†	8.8 $\pm$ 0.2	3.6 $\pm$ 0.3	2.5 $\pm$ 0.1	163.8 $\pm$ 3.6	19.6 $\pm$ 2.7
SCROTAL‡						
2	11.5 $\pm$ 0.6	8.0 $\pm$ 0.6	3.5 $\pm$ 0.3	2.1 $\pm$ 0.1	193.8 $\pm$ 12.8	35.1 $\pm$ 6.9
4	10.6 $\pm$ 0.7	7.2 $\pm$ 0.5	3.4 $\pm$ 0.3	2.2 $\pm$ 0.2	200.2 $\pm$ 15.3	37.8 $\pm$ 9.1
6	10.5 $\pm$ 0.7	7.1 $\pm$ 0.6	3.4 $\pm$ 0.2	2.1 $\pm$ 0.2	196.7 $\pm$ 16.4	34.3 $\pm$ 8.7
TRANSLOCATED‡						
2	12.4 $\pm$ 0.7	8.2 $\pm$ 0.5	4.2 $\pm$ 0.5	2.1 $\pm$ 0.1	183.5 $\pm$ 8.6	31.7 $\pm$ 3.6
4	11.8 $\pm$ 1.0	7.6 $\pm$ 0.9	4.2 $\pm$ 0.2	1.9 $\pm$ 0.2	209.0 $\pm$ 17.4	44.4 $\pm$ 9.5
6	16.2 $\pm$ 1.0	8.4 $\pm$ 0.7	7.8 $\pm$ 0.9	1.9 $\pm$ 0.1	182.2 $\pm$ 8.6	28.0 $\pm$ 4.6

* Testes from six rabbits in each group.

† Mean $\pm$ standard error.

‡ Scrotal and translocated testis of unilaterally cryptorchid rabbits.

centration of total cholesterol in the lipid extract increased after 6 days of translocation ($P<0.01$). Free cholesterol concentration in the lipid extract decreased ($P<0.01$) in the scrotal testes, but did not differ significantly between control and translocated testes. Conversely, esterified cholesterol concentration was unchanged in the contralateral scrotal testes, but increased in the translocated testes after 6 days ($P<0.001$).

Total carboxyl ester concentration in the lipid extract increased in both scrotal ($P<0.05$) and translocated testes ($P<0.01$) and triglyceride levels/100 mg of lipid also increased in both groups ($P<0.005$). Lipid phosphorus decreased ($P<0.05$) in both scrotal and translocated testes.

DISCUSSION

The pattern of dry weight loss in the translocated testes found in this study was similar to that reported by others (Clegg, 1963; Zogg, Hays, VanDemark &

Johnson, 1968) and followed the time sequence of degenerative changes of the germinal epithelium reported by Clegg (1963). Zogg *et al.* (1968) reported wet weight increases of abdominal rabbit testes 2 days after translocation, suggesting oedema, but no similar changes were found in this study (Table 1).

Total lipid levels found in this study are slightly higher than those reported for rabbit testes by Bieri & Prival (1965). The increase in lipid concentration following surgery is in agreement with the data of Clegg (1960), Hanes & Rosenbloom (1911) and Moore (1924), who reported histological evidence indicating increased lipid concentrations in cryptorchid testes. An increase in lipid concentration has also been reported following oestrogen treatment (Lacy, 1960) or hypophysectomy (Lynch & Scott, 1951).

The effect of cryptorchidism on cholesterol concentration has been reported (Perlman, 1950). The present data support Perlman's report that total cholesterol in the testes decreases, but concentration increases, and an increase in the ester/free cholesterol ratio follows cryptorchidism. Hoffman (1960) reported that unilateral cryptorchidism in the rat causes an initial decline in cholesterol concentration of both testes, followed by a rapid increase until 15 days after surgery. In the rabbit, the present study did not demonstrate an early decline in cholesterol concentration, nor was an increase in cholesterol concentration in the contralateral scrotal testis shown. However, the translocated testes were found to increase in total cholesterol concentration as described by Hoffman, and both translocated and contralateral scrotal testes increased in esterified cholesterol concentration. Since a decrease in the enzymes necessary for androgen biosynthesis occurs following cryptorchidism (Llaurodo & Dominguez, 1963) it may be that the increased cholesterol concentration reflects a decreased utilization for androgen biosynthesis. The levels of cholesterol found for normal testes in this study are within the range found by others (Bieri & Prival, 1965; Ewing, Noble & Ebner, 1964) using rabbit testes.

The increase in total carboxyl ester in both testes can be attributed almost entirely to the increase in triglycerides, since the correlation coefficient between these two variables was 0.96 ($P < 0.001$). However, the reason for increased triglyceride levels in both tissues remains unresolved. The decreased lipid phosphorus levels found in this study were highly correlated with dry weight ($r = 0.72$; $P < 0.01$), suggesting phospholipid loss due to degeneration of structural tissues in the seminiferous tubules.

The indication from this study and that of Hoffman (1960) is that unilateral cryptorchidism affects lipid metabolism in both the abdominal testis and its contralateral scrotal partner. Clegg (1965) has reported that the scrotal testis of the unilaterally cryptorchid rat undergoes hypertrophy of the germ cells and hypertrophy and hyperplasia of the Leydig cells, and has postulated that these effects are the result of increased gonadotrophin secretion. However, other work has shown increased lipid concentration in the testes of animals following treatment which decreases gonadotrophin secretion, i.e. oestrogen treatment (Lacy, 1960) or hypophysectomy (Lynch & Scott, 1951). Thus, the mechanisms causing this effect are unknown, but it appears that endocrine factors are involved.

It is also possible that the effects on testicular lipids are caused by surgical

procedures even though this is not the case when histological measurements (Clegg, 1965), in-vitro respiration or in-vitro carbohydrate metabolism (Zogg *et al.*, 1968) are measured.

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