# BIOCHEMICAL EFFECTS OF FLUORIDE ON LIPID METABOLISM IN THE REPRODUCTIVE ORGANS OF MALE RABBITS

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SUMMARY: The effect of fluoride on testicular lipid metabolism was assessed in male albino rabbits in experimental fluorosis. Fifty male albino rabbits were administered sodium fluoride (5, 10, 20, and 50 mg/kg body weight/day) subcutaneously for 100 days. The control animals were given 1 cc distilled water/kg body weight over the same period. Compared with controls, the experimental animals, especially those given 50 mg NaF/day/kg of body weight, showed abnormal accumulation of lipids in testes. Hyperphospholipidemia, hypertriglyceridemia, and hypercholesterolemia in testes indicate enhanced lipid biosynthesis in response to fluoride toxicosis. A progressive significant ($p<0.001$) increase in amount of free fatty acids was observed in testes of fluoridated animals. The increase of concentration of all lipid classes except free fatty acids in testes was directly correlated with the increase in dosage of fluoride administered.

Keywords: Albino rabbits; Cholesterol; Experimental fluorosis; Free fatty acids; Phospholipids; Testes; Total lipids; Triglycerides; Sodium fluoride.

## Introduction

Fluoride induces toxicological effects in reproductive organs in experimental animals. In mice, alterations in the reproductive organ structure and metabolism as well as reduction in fertility have been reported (1). Tokar (2) found an association between fluorosis and hypogonadism. In human beings, Tarinsky (3) recorded a 2-3 fold increase in symptoms of oligospermia and azoospermia in male workers suffering from industrial fluorosis. Several reports in the literature suggest a definite correlation between infertility and fluorosis (4-7). The present investigation is an attempt to elucidate the testicular lipid metabolism in experimental fluorosis.

## Materials and Methods

*Animals and treatment*

Fifty male albino rabbits weighing 400-650 gm were divided into five groups of ten animals each. They were administered fluoride (as NaF) subcutaneously in the dosage of 5, 10, 20, and 50 mg NaF/kg body weight/day for 100 days. The control animals were injected with 1 cc distilled water/kg body weight/day for the same period. All animals were maintained on standard laboratory chow; water was supplied *ad libitum*. After 100 days, the control and treated animals were sacrificed under ether anaesthesia, and testes were immediately removed, weighed, and kept in chloroform: methanol (2:1 v/v) for the extraction of lipids (8).

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*Separation of neutral lipids*

The silica-gel G thin-layer plates (20 x 20 cm) were prepared for thin-layer chromatography by employing the method of Freeman and West (9) with slight modifications. The dried plates, activated at 110° C for 90 minutes, were developed in *n*-hexane:diethyl ether:acetic acid glacial (90:10:1 v/v). The chromatograms were air dried and stained with iodine vapour in sealed chambers to provide yellow spots. These spots were identified, marked, and taken into extracting solvent (*n*-hexane:diethyl ether: 1:1 v/v). Pooled extracts evaporated to dryness under reduced pressure were taken up in a known volume of chloroform:methanol (1:1 v/v). Extracts containing different lipids fractions were used for spectrophotometric analysis.

*Triglycerides*

Triglycerides in the testes of control and treated rabbits were determined by the method of Van Handel and Zilversmit (10).

*Phospholipids*

Quantitative analysis of phospholipids was done according to the method of Ames (11).

*Cholesterol*

The estimation of cholesterol in testes of control and treated rabbits was carried out by the method of Stadtman (12).

*Free fatty acids*

The free fatty acids were assayed by the method of Chakrabarty *et al* (13).

*Statistical analysis*

Results are shown as mean ±SD. Significance was determined by Student's t-test.

## Results

The Table clearly shows that administration of fluoride to rabbits profoundly enhanced the synthesis of lipids in the testes.

Figure 1 shows the mean total lipid and triglyceride concentration in the testes of fluoridated and control animals. Total lipid content of the testes in all treated groups of animals was significantly elevated ($p < 0.001$) over the controls. Similarly, triglyceride levels in testes of treated animals were profoundly enhanced compared to the controls. The differences were statistically significant ($p < 0.001$). Values for treated groups reached a maximum 168.1 ±5.73 mg/g of tissue vs 7.7 ±0.18 in the controls.

Differences in the level of phospholipids in testes of fluorotic groups of animals and control were highly significant (Figure 2). The levels of significance were between $p < 0.001$ and 0.02.

The concentration of cholesterol in testes registered a moderate elevation (33.3%) in animals treated with 5 mg of NaF. In subsequent experimental groups of animals, the amount of cholesterol was highly elevated, the maximum being in animals treated with 50 mg of NaF (Figure 2).

**TABLE**

Lipid profile of rabbit testis during experimental fluorosis (Data are mean ± SD)

| Treatment NaF mg/kg body weight | Total lipids | Phospho-lipids | Triglycerides | Cholesterol | Free fatty acids |
|---|---|---|---|---|---|
| 1 cc distilled water (control) | 25.1 ±0.75 | 9.5 ±3.04 | 7.7 ±0.18 | 2.1 ±0.08 | 5.1 ±0.09 |
| 5 | 44.9 ±1.75[a] (+79) | 14.1 ±0.40[a] (+48) | 15.4 ±0.20[a] (+104) | 2.8 ±0.73[c] (+33) | 13.6 ±0.07[a] (+167) |
| 10 | 113.0 ±9.78[a,d] (+350) | 29.6 ±1.60[a,d] (+212) | 27.7 ±2.46[a,d] (+260) | 4.4 ±0.3[a,d] (+110) | 14.1 ±0.13[a,d] (176) |
| 20 | 151.7 ±16.08[a,e] (+504) | 37.5 ±1.28[a,d] (+295) | 32.3 ±0.41[a,e] (+319) | 4.5 ±0.29[a,NS] (+114) | 15.6 ±0.12[a,d] (+206) |
| 50 | 247.2 ±19.67[a,d] (+884) | 48.5 ±0.62[a,d] (+410) | 168.1 ±5.73[a,d] (+2083) | 7.4 ±1.02[a,d] (+252) | 9.1 ±0.51[a,d] (+78) |

Results are expressed as mg/g w.w. of tissues.
P values compared to the control: [a] $p < 0.001$; [b] $p < 0.02$; [c] $p < 0.05$.
Significant values in 5 mg vs 10 mg $F^-$ group, 10 mg vs 20 mg $F^-$ group and 20 mg vs 50 mg $F^-$ group are: [d] $p < 0.001$; [e] $p < 0.01$; [NS] non-significant.
Figures in parentheses indicate percent change.

Figure 1
Testicular total lipid and triglyceride
in experimental fluorosis

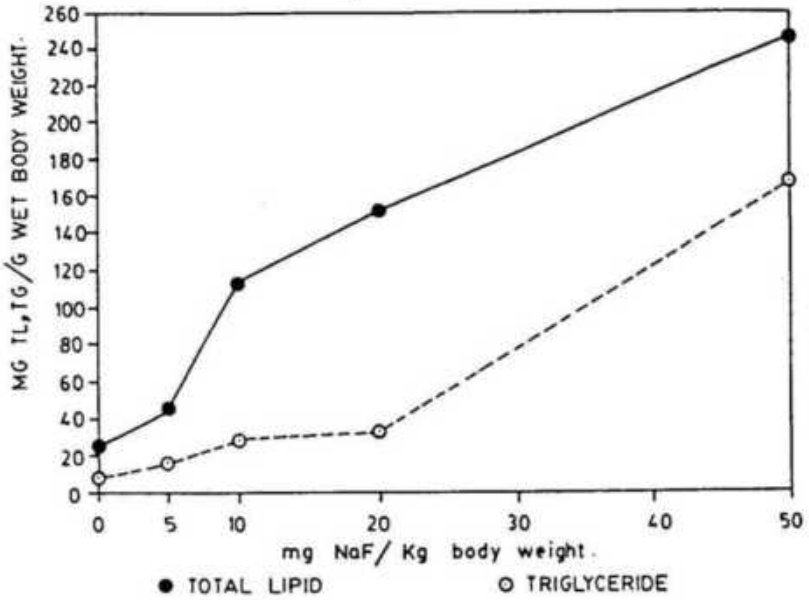
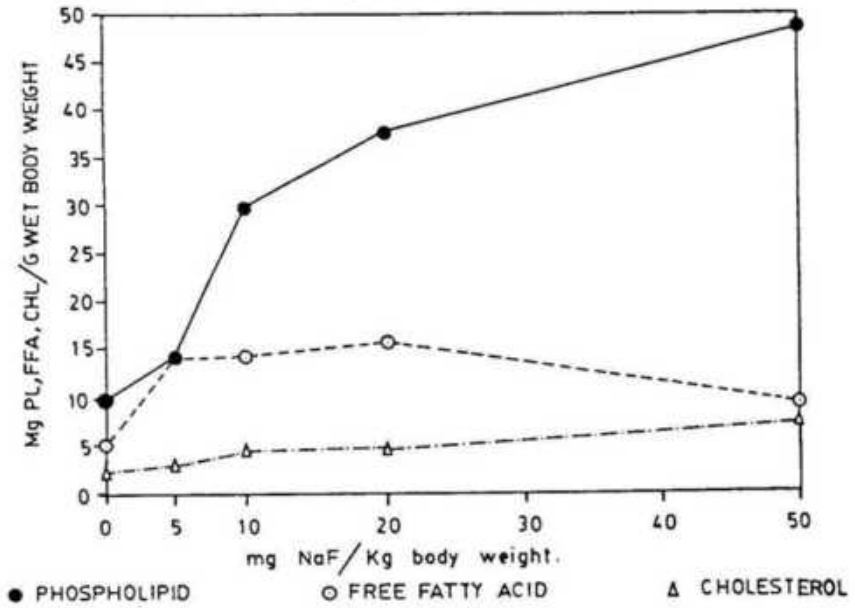



Figure 2
Testicular phospholipid, free fatty acid and cholesterol
in experimental fluorosis



The experimental group of rabbits showed significant ($p < 0.001$) increase in free fatty acid content of testes (Figure 2). There was significant elevation ($p < 0.001$) in the amount of free fatty acids in the 5 mg vs 10 mg NaF group and the 10 mg vs 20 mg NaF group and a decrease ($p < 0.001$) in the 20 mg vs 50 mg NaF group.

## Discussion

In the fluoridated animals abnormal quantities of lipids, phospholipids, triglycerides, cholesterol and free fatty acids accumulated in the testes. As shown in Table 1, the amount of these lipids deposited in the testes increased in direct relation to the increase in the dosage of fluoride administered. The maximum increase in lipids level was seen in animals of the highest fluoride group (50 mg NaF).

The results obtained from this study, which confirm our earlier reports, suggest a strong association between fluorosis and alteration in lipid metabolism in rabbits (14-16).

The high levels of lipids in the testes of experimental animals in response to fluoride toxicosis strongly indicate an imbalance between the synthesis and breakdown of the lipid in the testes. Fluoride is known to inhibit hormone sensitive lipase, thus not only reducing the release of free fatty acids but of glycerol as well and results in enhanced lipogenesis (17). The increased fatty acid levels suggest increased triglyceride synthesis, decreased fatty acid oxidation, and increased cholesterol synthesis. Similar hypercholesterolemic effects in the serum of experimental animals after exposure to fluoride have been reported (18,19). However, Chinoy and Sequeira (1) found no significant changes in testes cholesterol in fluoride-treated mice.

Hyperlipidemia, hyperphospholipidemia, hypertriglyceridemia also indicate excessive mobilization of fat (20). The noted degenerative changes in spermatocytes, Leydig cells, and sertoli cells (7) reflect disturbances in the synthetic processes in the testes of rabbits in experimental fluorosis. Since the various stages of spermatogenesis are controlled by different hormones, it appears likely that testosterone, the male sex hormone required for the maintenance of spermatogenesis (21), is lowered in fluoride intoxication (1), reflecting hormonal imbalance in the body (22).

## Conclusions

1. This investigation demonstrates a significant elevation in all lipid classes, thereby indicating enhanced lipogenesis in testes of rabbits in response to fluoride toxicosis.
2. The quantity of testicular lipids in different fluoridated groups of animals is influenced by the dosage of fluoride administered.

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