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EFFECTS OF PROTEIN SUPPLEMENTATION AND DEFICIENCY ON FLUORIDE-INDUCED TOXICITY IN REPRODUCTIVE ORGANS OF MALE MICE

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SUMMARY: Feeding a protein-deficient diet to male mice treated for 30 days with NaF (5, 10, 20 mg/kg body weight) caused a significant decrease in protein levels in testis, cauda epididymis, and vas deferens. The activity of testicular SDH and 3β- and 17β-HDS as well as ATPase in cauda epididymis and vas deferens also decreased as compared to controls fed a normal protein diet. The decrease was more significant in mice treated with 10 and 20 mg NaF/kg than with 5 mg/kg. By contrast, levels of cholesterol in testis and glycogen in the vas deferens were significantly enhanced as compared to controls. A protein-supplemented diet fed along with NaF in the same three doses did not cause any change in these parameters, which remained the same as the controls.

These results clearly indicate that protein supplementation is beneficial to overcome the toxic effects of fluoride on testicular steroidogenesis, protein, carbohydrate, and energy and oxidation metabolisms in the reproductive organs of male mice. Protein deficiency, on the other hand, aggravates fluoride toxicity. A protein-supplemented diet might therefore substantially mitigate certain fluoride-induced health hazards in humans living in endemic areas.

Keywords: Fluoride treatment, Male mice, Protein-deficient diet, Protein-supplemented diet, Reproductive organs.

INTRODUCTION

Fluoride occurs naturally in many foods and drinking water supplies and is universally present in the bodies of all higher animal species. The question has long been raised as to whether fluorine plays a physiological role or whether it is present in the tissues as an accidental constituent since it is ingested from food.[1] No human diet is entirely free from fluoride, and it is extremely difficult to prepare a diet for experimental animals which is very low in fluoride.

Excessive intake of fluoride results in dental and skeletal fluorosis, afflicting millions of people worldwide. Fluoride, under certain conditions can affect virtually every phase of human metabolism. It can readily penetrate cell membranes including those of erythrocytes and the fetus by simple diffusion and can cause adverse effects on tissue metabolism.[2]

Investigations carried out earlier in our laboratory revealed that fluoride interferes with the functional status of several tissues and organs, viz., endocrine glands, reproductive organs, liver, muscle, kidney, and blood in human populations of fluoride endemic areas.[3] Our studies in rodents have also revealed that ingestion of fluoride in concentrations higher than the permissible level interferes with reproduction in male and female rodents.[6-8]

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It is known that fluoride inhibits biosynthesis of protein *in vitro* and *in vivo*, due mainly to impairment of peptide chain initiation.[9] A decrease in protein levels has also been reported in the reproductive organs of rats, mice and rabbits treated with NaF.[7,8,10] Moreover, experiments in our laboratory have shown that the amino acids, glycine and/or glutamine are beneficial for recovery from fluoride-induced toxicity in uterine carbohydrate metabolism of mice and even produce an ameliorative effect.

The present work was undertaken to investigate the action of fluoride in the reproductive organs of male mice in relation to feeding protein-rich and protein-deficient diets.

MATERIALS AND METHODS

Animals: Healthy, adult male mice (*Mus musculus*) of Swiss strain were used for the experiments. The mice were obtained from Cadila Pharmaceuticals, Ghodasar, Ahmedabad and weighed between 25 and 35 g. They were kept in an air-conditioned animal house at a temperature at 26° ± 2°C and were exposed to 10 to 12 h of daylight/day. The mice were maintained on standard chow and water given *ad libitum*.

Exposures: The experimental protocol is presented in Table 1. The animals were divided into six groups. Sodium fluoride (Loba Chemie, Bombay, 99% purity) was administered to mice orally using a feeding tube attached to a hypodermic syringe. The NaF was mixed in water (0.2 ml) at a dose of 5, 10, or 20 mg/kg body weight. The dose was selected based on the LD_{50} value of fluoride, which is 54.4 mg F/kg body weight in male mice.[12] Oral administration was preferred since water is the main source of fluoride among human populations in endemic areas.

Diets: The control protein diet, the protein-rich, and the protein-deficient diets were prepared according to the protocol of the National Institute of Occupational Health (NIOH), Ahmedabad. The control protein diet contained 20% protein, the protein-deficient diet contained 5% protein, and the protein-rich diet contained 40% protein. Other ingredients as follows:

- The control diet contained 23.53% casein, 63.47% food starch powder, 4% salt mixture, 2% vitamin mixture, and 7% groundnut oil.
- The protein-deficient diet contained 5.88% casein, 81.12% starch powder, 4% salt mixture, 2% vitamin mixture and 7% groundnut oil.
- The protein-rich diet contained 47.06% casein, 39.94% starch powder, 4% salt mixture, 2% vitamin mixture and 7% groundnut oil.

Data collection: The control and treated groups of animals were weighed on an animal weighing balance (Ohaus, USA) and sacrificed by cervical dislocation after the respective treatments. The testis, cauda epididymis, and vas deferens were dissected out carefully, blotted free of blood, weighed on a torsion balance (Roller Smith, USA) to the nearest milligram, and used for carrying out biochemical tests.

Table 1. Experimental Protocol

Group	Diet and Treatment	Days of treatment	Day of autopsy	No. of mice
I	Control (20% protein)	–	*	10
IIA	Control + NaF (5 mg NaF/kg/animal/day)	30	31st	10
B	Control + NaF (10 mg NaF/kg /animal/day)	30	31st	10
C	Control + NaF (20 mg NaF/kg/animal/day)	30	31st	10
III	Protein-deficient (5% protein)	30	31st	10
IVA	Protein-deficient + NaF (5 mg/kg/animal/day)	30	31st	10
B	Protein-deficient + NaF (10 mg/kg/animal/day)	30	31st	10
C	Protein-deficient + NaF (20 mg/kg/animal/day)	30	31st	10
V	Protein-rich (40% protein)	30	31st	10
VIA	Protein-rich + NaF (5 mg/kg/animal/day)	30	31st	10
B	Protein-rich + NaF (10 mg/kg/animal/day)	30	31st	10
C	Protein-rich + NaF (20 mg/kg/animal/day)	30	31st	10

*Sacrificed with treated groups

Biochemical Study:

Protein levels in the testis, cauda epididymis, and vas deferens of control and all treated animals were determined by the method of Lowry *et al*[13] and expressed as mg/100mg fresh tissue weight.

Succinate dehydrogenase (SDH) (E.C.1.3.99.1) activity in the testis of control and all treated mice was determined by the modified tetrazolium reduction method of Beatty *et al*[14] and expressed as µg formazan formed/mg protein.

Cholesterol concentrations were estimated in the testis of control and all treated mice by the procedure of Zlatkis *et al*[15] and expressed as mg/100 mg fresh tissue weight.

3β- and 17β-Hydroxysteroid dehydrogenase (HSD) (E.C.1.1.1.53) activities were assayed in testis of control and treated mice by the method of Talalay[16] and expressed as nanomoles of androstenedione formed/mg protein/minute.

Adenosine triphosphatase (ATPase) (E.C.3.6.1.3) activity was assayed in cauda epididymis of control and treated mice by the method of Quinn and White[17] and expressed as µmoles of ip released/mg protein/hour.

Glycogen levels were determined in vas deferens of control and treated mice by the method of Seifter *et al.*[18] The concentrations were expressed as µg glycogen/100 mg fresh tissue weight.

Phosphorylase (E.C.2.4.1.1) activity in vas deferens of control and treated mice was assayed by the method of Cori *et al*[19] and inorganic phosphorus released by the method of Fiske and Subba Row.[20] The activity was expressed as mg phosphorus released/mg protein/15 min.

Statistics: For all biochemical parameters, a minimum of 5-6 replicates were made, and the data were subjected to statistical analysis by ANOVA and Student's 't' test.

RESULTS

Protein in testis: The 5, 10, and 20 mg/kg NaF treatment administered to mice fed a control protein diet (Groups IIA,B,C) caused a significant ($p<0.001$) reduction in protein levels in the testis as compared to mice fed just the control protein diet (Group I). The reduction was highly significant with the 20 mg NaF treatment (Group IIC) (Table 2).

In Group III, wherein a protein-deficient diet alone was fed to mice, the testis protein was significantly ($p<0.001$) decreased compared to Group I (Table 2). NaF treatment along with the protein-deficient diet (Groups IVA,B,C) also resulted in a significant ($p<0.001$) decrease of testis protein. The 20 mg NaF treatment (Group IVC) caused the most significant decline ($p<0.001$) as compared to the 5 and 10 mg NaF treatment (Table 2).

Group V mice were fed a protein-rich diet which caused no change in testis protein as compared to Group I. In Groups VIA,B,C administered 5, 10, and 20 mg NaF along with the protein-rich diet, the testis protein levels were almost the same as in control Group I, but increased significantly ($p<0.001$) compared to those of Group IIA,B,C (NaF treatment) (Table 2).

The protein levels in cauda epididymis and vas deferens of mice showed the same trend as in the testis (Table 2).

Table 2. Protein levels (mg/100 mg tissue wt) in testis, cauda epididymis, and vas deferens of control and treated mice of groups I to VI

Group	Diet and Treatment	Testis	Cauda epididymis	Vas deferens
I	Control (20% protein)	14.29 ± 0.15	14.35 ± 0.12	16.47 ± 0.30
IIA	Control + NaF (*5* mg)	12.02 ± 0.18†	11.94 ± 0.19†	14.09 ± 0.25†
B	Control + NaF (10 mg)	10.88 ± 0.12†	10.76 ± 0.11†	12.87 ± 0.15†
C	Control + NaF (20 mg)	9.49 ± 0.13†	9.53 ± 0.14†	11.67 ± 0.15†
III	Protein-deficient (5%)	9.52 ± 0.30†	10.22 ± 0.27†	10.85 ± 0.36†
IVA	Protein-deficient + NaF (5 mg)	7.61 ± 0.26†	8.75 ± 0.30†	8.96 ± 0.15†
B	Protein-deficient + NaF (10 mg)	5.64 ± 0.22†	7.68 ± 0.15†	7.75 ± 0.16†
C	Protein-deficient + NaF (20 mg)	4.89 ± 0.21†	6.26 ± 0.28†	5.96 ± 0.25†
V	Protein-rich (40%)	14.57 ± 0.30^{ns}	14.79 ± 0.32^{ns}	16.68 ± 0.17^{ns}
VIA	Protein-rich + NaF (5 mg)	14.26 ± 0.23†	14.08 ± 0.90*	16.37 ± 0.22†
B	Protein-rich + NaF (10 mg)	13.75 ± 0.26†	14.12 ± 0.19†	16.22 ± 0.18†
C	Protein-rich + NaF (20 mg)	13.09 ± 0.22†	14.01 ± 0.21†	16.12 ± 0.18†

Values are mean ± S.E. *$p<0.05$ $^{\dagger}p<0.001$ ns=not significant

For p values, comparison done between Groups:

I and IIA,B,C IIA and IVA, VIA IIC and IVC, VIC

I and III, V IIB and IVB, VIB

Table 2a. Protein ANOVA (Testis)

Source of Variation	SS	df	MSS	F(crit)	F(tab)
Groups	13.88	11	1.26	3.30	1.98
Residual	22.89	60	0.3815		

SS Sum of Squares, df degree of freedom, MS Mean of Squares

Table 2b. Protein ANOVA (Cauda epididymis)

Source of Variation	SS	Df	MSS	F(crit)	F(tab)
Groups	8821.74	11	801.97	5.7382	1.95
Residual	576.007	60	8.228		

SS Sum of Squares, df degree of freedom MS Mean of Squares

Table 2c. Protein ANOVA (vas deferens)

Source of Variation	SS	df	MSS	F(crit)	F(tab)
Groups	831.87	11	75.62	40.71	2.15
Residual	120.53	60	1.854		

SS Sum of Squares, df degree of freedom, MS Mean of Squares

Succinate dehydrogenase (SDH): The SDH activity in testis of Group II animals was decreased depending on the dose of NaF administered along with the control diet. The decrease was most significant ($p<0.001$) in Group IIC as compared to Group I (Table 3).

In Group III wherein a protein-deficient diet was fed to mice, the SDH activity decreased ($p<0.001$) in comparison to Group I (Table 3). In Groups IVA,B,C, the SDH activity declined ($p<0.001$) as compared to those of Group IIA,B,C (Table 3). On the other hand, the SDH activity was almost same in Groups V, and VIA,B,C as compared to Group I mice (Table 3).

ATPase activity in cauda epididymis revealed almost the same changes as for SDH described above (Table 3).

Table 3. SDH activity in testis and ATPase activity in cauda epididymis of control and treated mice of groups I to VI

Group	Diet and Treatment	SDH (Testis)[a]	ATPase[b]
I	Control (20% protein)	10.53 ± 0.09	1.91 ± 0.04
IIA	Control + NaF (5 mg)	9.17 ± 0.15*	0.96 ± 0.02*
B	Control + NaF (10 mg)	8.29 ± 0.05*	0.86 ± 0.02*
C	Control + NaF (20 mg)	7.52 ± 0.20*	0.72 ± 0.007*
III	Protein-deficient (5%)	8.46 ± 0.27*	0.87 ± 0.016*
IVA	Protein-deficient + NaF (5 mg)	6.68 ± 0.30*	0.75 ± 0.025*
B	Protein-deficient + NaF (10 mg)	5.38 ± 0.14*	0.65 ± 0.027*
C	Protein-deficient + NaF (20 mg)	4.51 ± 0.13*	0.52 ± 0.009*
V	Protein-rich (40%)	11.13 ± 0.26[ns]	1.91 ± 0.08[ns]
VIA	Protein-rich + NaF (5 mg)	11.01 ± 0.29*	1.79 ± 0.09*
B	Protein-rich + NaF (10 mg)	10.54 ± 0.09*	1.71 ± 0.11*
C	Protein-rich + NaF (20 mg)	10.34 ± 0.14*	1.48 ± 0.09*

[a](µg formazan formed/mg protein [b](µmoles of ip released/mg protein)

Values are mean ± S.E. *p<0.001; ns=not significant

For p values comparison done between Group:

I and IIA,B,C IIA and IVA,VIA IIC and IVC,VIC

I and III,V, IIB and IVB,VIB

Table 3a. Testis SDH ANOVA

Source of Variation	SS	df	MSS	F(crit)	F(tab)
Groups	971.93	11	88.35	4.019	1.98
Residual	1319.25	60	21.98		

SS Sum of Squares, df degree of freedom, MS Mean of Squares

Table 3b. Cauda epididymal ATPase ANOVA

Source of Variation	SS	df	MSS	F(crit)	F(tab)
Groups	172.13	11	28.68	12	1.98
Residual	153.3	60	2.39		

SS Sum of Squares, df degree of freedom, MS Mean of Squares

Cholesterol: The levels of cholesterol were not affected in Group IIA mice as compared to Group I. However, a significant accumulation of cholesterol ($p<0.001$) occurred in Groups IIB,C (Table 4). In Group III, the increase was less significant ($p<0.02$), whereas it was not significant in Group IVA as compared to Group IIA. However, a significant ($p<0.001$) increase was obtained in testis cholesterol of Groups IVB,C in comparison with the corresponding Groups IIB,C.

In Groups V and VIA, cholesterol levels were unaffected as compared to Groups I and IIA, respectively (Table 4). On the contrary, cholesterol levels in Groups VIB and VIC were almost the same as in Group I but significantly less ($p<0.001$) than in Groups IIB and IIC) (Table 4).

Table 4. Levels of cholesterol, activities of 3β- and 17β-hydroxysteroid dehydrogenase (HSD) in testis of control and treated mice of groups I to VI

Group	Diet and Treatment	Cholesterol[a]	3β HSD[b]	17β HSD[c]
I	Control (20% protein)	0.495 ± 0.009	0.174 ± 0.003	0.057 ± 0.0007
IIA	Control + NaF (5 mg)	0.535 ± 0.022^{ns}	0.145 ± 0.002**	0.037 ± 0.003**
B	Control + NaF (10 mg)	0.582 ± 0.018†	0.135 ± 0.001†	0.025 ± 0.001†
C	Control + NaF (20 mg)	0.615 ± 0.013†	0.126 ± 0.0007†	0.018 ± 0.0007†
III	Protein-deficient (5%)	0.554 ± 0.02*	0.141 ± 0.002†	0.026 ± 0.001†
IVA	Protein-deficient + NaF (5mg)	0.614 ± 0.009^{ns}	0.126 ± 0.002†	0.018 ± 0.0003†
B	Protein-deficient + NaF (10mg)	0.647 ± 0.004†	0.117 ± 0.0007†	0.0165 ± 0.0002†
C	Protein-deficient + NaF (20mg)	0.729 ± 0.014†	0.110 ± 0.002†	0.015 ± 0.0001†
V	Protein-rich (40%)	0.480 ± 0.015^{ns}	0.191 ± 0.005^{ns}	0.059 ± 0.0009^{ns}
VIA	Protein-rich + NaF (5 mg)	0.506 ± 0.02^{ns}	0.186 ± 0.002†	0.055 ± 0.0007†
B	Protein-rich + NaF (10 mg)	0.493 ± 0.016†	0.179 ± 0.002†	0.054 ± 0.0003†
C	Protein-rich + NaF (20mg)	0.497 ± 0.022†	0.176 ± 0.002†	0.053 ± 0.0007†

Values are mean ± S.E. *$p<0.02$ $^{\dagger}p<0.001$ ns=not significant
For p values, comparisons are the same as in Table 2.
[a](mg cholesterol/100 mg fresh tissue wt)
[b](nanomoles of androstenedione formed/mg protein/min)
[c](nanomoles of androstenedione formed/mg protein/min)

3β-HSD: The activity of 3β-HSD was significantly ($p<0.001$) decreased in Groups IIA,B,C and III: IVA,B,C as compared to respective groups shown in Table 4.

In Group V the enzyme activity was insignificantly affected as compared to Group I, whereas, in Groups IVA,B,C the activity was almost the same as in Group I but significantly more ($p<0.001$) than in Groups IIA,B,C (Table 4).

17β-HSD: Alterations in the activity of 17β-HSD in testis of mice in the different groups were almost same as for 3β-HSD (Table 4).

Table 4a. Cholesterol ANOVA (Testis)

Source of Variation	SS	df	MSS	F(crit)	F(tab)
Groups	0.3854	11	0.077	4.3502	1.95
Residual	0.505	60	0.00701		

SS Sum of Squares, df degree of freedom, MS Mean of Squares

Table 4b. 3β HSD ANOVA (Testis)

Source of Variation	SS	df	MSS	F(crit)	F(tab)
Groups	0.0297	11	0.002708	0.3262	1.95
Residual	0.4980	60	0.00830		

SS Sum of Squares, df degree of freedom, MS Mean of Squares

Table 4c. 17β HSD ANOVA (Testis)

Source of Variation	SS	df	MSS	F(crit)	F(tab)
Groups	0.00339	11	0.00030	0.977	1.95
Residual	0.01891	60	0.000315		

SS Sum of Squares, df degree of freedom, MS Mean of Squares

Glycogen: Levels of glycogen were significantly increased in vas deferens of Group IIA,B,C mice as compared to Group I (Table 5). A similar significant accumulation of glycogen was obtained in Group III as compared to Group I and in Group IVA,B,C as compared to Group IIA,B,C (Table 5). However, in Groups V and VIA the glycogen levels were almost same as in Group I but significantly less ($p<0.001$) than in Group IIA (Table 5). In Group VIB,C the levels were also significantly less ($p<0.001$) than in Group IVB,C.

Phosphorylase: The activity of phosphorylase declined significantly ($p<0.001$) in vas deferens of mice in Groups IIA,B,C and III as compared to Group I animals (Table 5). The activity further declined ($p<0.001$) in Group IVA,B,C mice as compared to Group IIA,B,C (Table 5). In Group V mice, the enzyme

activity was enhanced in comparison to Group I ($p<0.05$). On the other hand, the activity in Groups VIA,B,C was almost same as in control Group I but significantly more ($p<0.001$) than in Groups IIA,B,C (Table 5).

Table 5. Levels of glycogen and Phosphorylase activity in vas deferens of control and treated mice

Group	Diet	Glycogen[a]	Phosphorylase[b]
I	Control (20% protein)	688.62 ± 8.05	124.92 ± 2.05
IIA	Control + NaF (5 mg)	965.43 ± 8.67†	102.00 ± 0.99†
B	Control + NaF (10 mg)	1029.70 ± 23.57†	98.93 ± 0.52†
C	Control + NaF (20 mg)	1107.85 ± 13.30†	95.82 ± 0.24†
III	Protein-deficient (5%)	883.67 ± 19.21†	99.91 ± 0.37†
IVA	Protein-deficient + NaF (5 mg)	1056.71 ± 12.43†	82.65 ± 2.36†
B	Protein-deficient + NaF (10 mg)	1130.50 ± 8.89†	78.95 ± 0.34†
C	Protein-deficient + NaF (20 mg)	1218.75 ± 22.98†	75.11 ± 0.33†
V	Protein-rich (40%)	688.02 ± 9.64ns	133.42 ± 2.39*
VIA	Protein-rich + NaF (5 mg)	688.49 ± 14.50†	125.40 ± 1.94†
B	Protein-rich + NaF (10 mg)	703.26 ± 19.18†	124.76 ± 1.14†
C	Protein-rich diet + NaF (20 mg)	719.98 ± 27.84†	123.24 ± 1.43†

[a](µg/100 mg fresh tissue wt) [b](µg phosphorus released/mg protein/15 min)

Values are mean ± S.E. *$p<0.05$; †$p<0.001$; ns=not significant

For p values comparison done between Groups:

I and IIA,B,C IIA and IVA,VIA IIC and IVC,VIC

I and III, V IIB and IVB,VIB

Table 5a. Vas deferens glycogen ANOVA

Source of Variation	SS	Df	MSS	F(crit)	F(tab)
Groups	1444702.9	11	131336.6	2.017	1.95
Residual	3906854.5	60	65114.24		

SS Sum of Squares, df degree of freedom, MS Mean of Squares

Table 5b. Phosphorylase ANOVA

Source of Variation	SS	Df	MSS	F(crit)	F(tab)
Groups	27416.22	11	2492.38	170.36	1.95
Residual	877.93	60	14.63		

SS Sum of Squares, df degree of freedom, MS Mean of Squares

DISCUSSION

Recent data from our laboratory prompted us to determine if a protein-supplemented diet would reduce fluoride toxicity in mice. In the present study, the effects of sodium fluoride (NaF) were investigated on testis, cauda epididymis, and vas deferens of adult male mice. The mice were given NaF at

a dose of 5, 10, and 20 mg/kg body weight for 30 days and fed a control protein, a protein-deficient, or a protein-rich diet.

Fluoride is known to inhibit protein synthesis, mainly due to impairment of peptide chain initiation on ribosomes.[9] Shashi *et al*[21] found a significant decline in acidic, basic, and total proteins of the reticulocyte lysate system of rabbits treated with NaF for 100 days. A decrease in protein levels was also reported in the reproductive organs of male rats, mice, and rabbits treated with different doses of NaF.[10,22-24]

The results of the present study revealed a significant decline in protein levels of testis, cauda epididymis, and vas deferens in NaF-dosed mice fed a control protein diet or a protein-deficient diet. In the latter animals (Groups IVA,B,C), reproductive organ protein levels were significantly decreased, which was probably a reflection of changes in protein metabolism. However, in animals fed a protein-rich diet or a protein-rich diet with NaF, protein levels in all tissues investigated were maintained at almost the same level as in the control group. These results demonstrate that protein supplementation does suppress fluoride-induced effects on protein levels in tissues.

The activity of succinate dehydrogenase (SDH) in testis and ATPase in cauda epididymis also declined significantly in Groups IIB,C, III and IVA,B,C mice, whereas, a protein-rich diet + NaF nearly maintained the Group I control *status quo* in SDH and ATPase activity. These data again suggest that a protein-supplemented diet would be conducive for countering adverse effects of fluoride on SDH and ATPase.

SDH is primarily a mitochondrial enzyme, while ATPase is involved in energy metabolism. Any change in their activity would affect the oxidative and energy metabolisms of testis in treated mice, probably by disruption of mitochondrial structure as observed in the ovary of NaF-treated mice.[25]

The data on cholesterol levels in testis revealed significant increases especially in Groups IIB,C and IVB,C, whereas, in Groups VIB and VIC the levels were almost the same as those of control Group I. These results are correlated with a significant decline in the activities of 3β- and 17β-HSD particularly in Groups IIA,B,C, III, and IVA,B,C. The data show that steroidogenesis was affected more in these groups, whereas in mice fed the protein-rich diet + NaF it was not altered. Narayana and Chinoy[26] also reported an effect on steroidogenesis in NaF-treated rats. Susheela and Jethanandani[27] found a decrease in testosterone levels in men suffering from skeletal fluorosis.

In the present study, significant accumulation of glycogen levels in the vas deferens was observed after treatment in Groups IIA,B,C (control diet + NaF) and IVA,B,C (protein-deficient diet + NaF). The decline in phosphorylase activity in these groups could have led to a decrease in glycogen utilization. However, by feeding a protein-rich diet, the levels of glycogen and the activity of phosphorylase in vas deferens were not affected as compared to the control.

The results of the present study show that the toxic effects of fluoride are enhanced when administered to mice fed a protein-deficient diet. By contrast,

feeding a protein-rich diet definitely has a beneficial influence in reducing NaF-induced toxicity in testis, cauda epididymis, and vas deferens of mice.

Recently, Chinoy and Patel[11] reported that supplementation of amino acids (glycine and/or glutamine) alone and in combination ameliorated all NaF-induced effects in uterine carbohydrate metabolism in mice. The recovery was more pronounced when both glycine and glutamine were administered together. The present study supports epidemiological and experimental investigations which have shown that dietary factors such as proteins, amino acids, and vitamins could modify or influence the toxic effects of fluoride.

Sriranga, Reddy and Srikantia[28] have reported that, in experimentally produced fluorotic monkeys, administration of a low-protein diet appeared to accelerate the development of bone fragility, and a higher incidence of rarefaction was observed in these animals. These findings could be due to inadequate protein intake. The results obtained in the present study corroborate these findings and suggest that a protein-supplemented diet would be beneficial, while a protein-deficient diet would aggravate fluoride toxicity.

Clearly, these investigations have important implications, especially in developing countries where protein deficiency and the occurrence of fluorosis co-exist. More detailed studies in this direction are therefore solicited.

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