EFFECT OF SODIUM FLUORIDE ON TISSUE PROTEIN IN RABBITS

by

A. Kathpalia and A.K. Susheela
New Delhi, India

SUMMARY: Rabbits were administered sodium fluoride, 50 mg/kg body weight, through intragastric route at 24 hour intervals. The protein content of soft tissues was estimated using a spectrophotometric method. These estimations were carried out on day 158, 165, 225 and 265 after fluoride administration. A reduction in the protein content of the tissues which were studied, ranged from 10 to 46 percent. The current study also revealed three different patterns of tissue protein response to fluoride ions. Fluoride ions exert an inhibitory influence on the majority of the tissues. Whether similar changes take place in humans after excessive ingestion of sodium fluoride is not known.

Excessive ingestion of fluoride ions results in fluorosis. The clinical manifestations of this disease are fairly well documented (1-5). Although, fluorosis has been regarded in the past as a disease affecting only bone and teeth, recent studies have given ample evidence that fluoride toxicity affects adversely soft tissues such as skeletal muscle (6-8), thyroid gland (9), kidneys, pancreas and adrenal glands (10,11). The histopathology of these tissues has been adequately described, but little information is available on the extent and the mode of involvement of various body tissues in the disease process. The current report therefore presents the results of a pilot study on how fluoride ions affect tissue protein in the experimental animal.

Material and Method

Adult, healthy rabbits whose body weight ranged from 1 to 1.9 kg were given sodium fluoride (50 mg/kg body weight) through intragastric route at 24 hour intervals. Control animals were pair fed but deprived of sodium fluoride. The animals were maintained under laboratory conditions and sacrificed at specified intervals, namely on day 158, 165, 225, and 265. The following tissues were chosen for protein estimation: Adrenal gland, cardiac muscle, kidney, liver, lung, nerve, pancreas, serum, skeletal muscle, spleen, spinal cord, stomach (fundic region) and testes. Blood samples were collected and sera separated.

From the Department of Anatomy, All India Institute of Medical Sciences, New Delhi-110016, India.

Total Protein Estimation: A known weight of the tissue samples were homogenized and a known volume of the homogenate was taken for the precipitation of protein with trichloracetic acid. The total protein content was estimated by the method of Lowry et al. (12) by means of the Folin Phenol reagent. Bovine albumin was used as standard protein. The optical density measurements were carried out at 660 mu by means of a Zeiss PMQ II Spectrophotometer. The results are expressed as mg protein present in 100 mg wet tissue and gm protein present in 100 ml sera.

Results and Discussion

The total protein contents of the various tissues are given in Table 1. In the control rabbits the protein content of nerve tissue is

Table 1
Protein Content of Rabbit Tissues Before and After NaF Administration

Tissue	Control Mean + S.E.	NaF Treated Mean + S.E.	Percentage Reduction
Adrenal gland	14.93 ± 1.36	13.25 ± 0.93	11
Cardiac muscle	9.16 ± 0.74	6.64 ± 0.72	27
Kidney	9.80 ± 0.36	6.85 ± 0.85	30
Liver	11.69 ± 1.24	7.66 ± 0.52	34
Lung	10.14 ± 1.47	9.10 ± 1.03	10
Nerve	7.24 ± 1.49	4.86 ± 1.08	32
Pancreas	10.99 ± 1.15	6.15 ± 1.06	44
Skeletal muscle	12.38 ± 1.05	7.58 ± 0.95	38
Spinal cord	8.20 ± 1.24	6.17 ± 1.65	24
Spleen	12.94 ± 1.24	8.43 ± 1.45	34
Stomach	10.32 ± 0.87	5.55 ± 1.62	46
Testes	8.85 ± 0.65	5.93 ± 1.24	32
Serum*	2.21 ± 0.55	2.94 ± 0.23	33 (+)

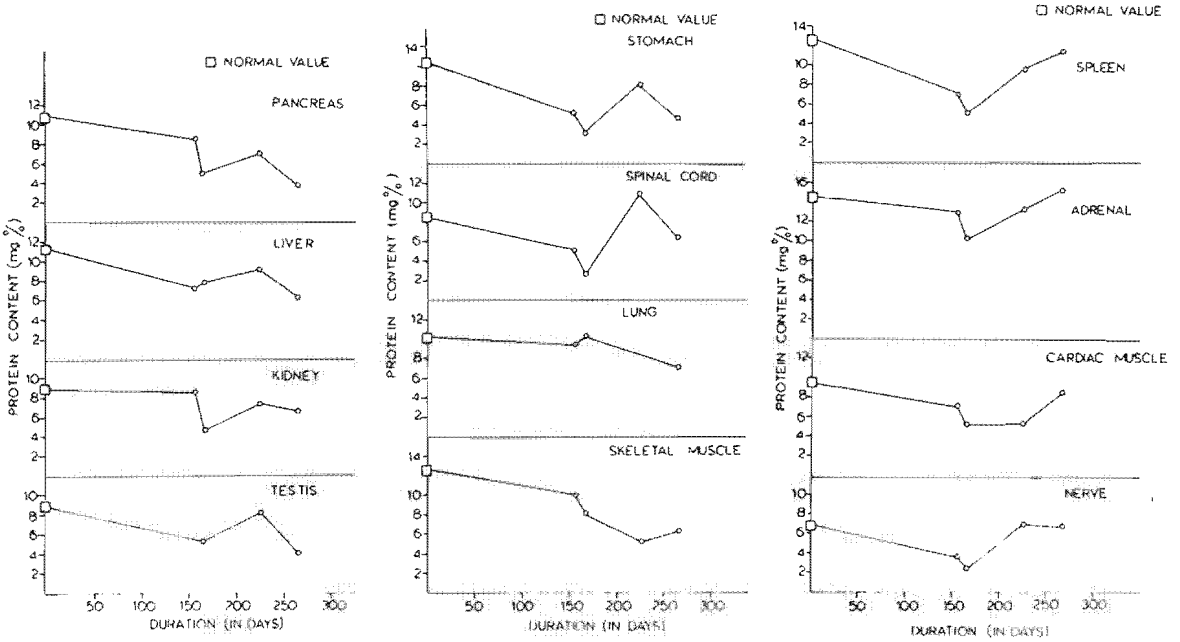
* The unit is gm percent, in all other tissues mg percent. The values are the mean of 3 to 4 experiments.

lowest (7.2%) whereas in the adrenal gland it is highest (14.9%). On fluoride administration, all tissues investigated revealed a reduction in the protein content ranging from 10% in lung tissue to 46% in stomach. That the maximum reduction in the protein content occurred in the stomach is of interest since the stomach is the first target organ when sodium fluoride is administered through the intragastric route.

Considering the number of days that the animals have been exposed to sodium fluoride, the data reveal that the protein values follow three different patterns. The protein content of pancreas, liver, kidney, testes, stomach and spinal cord have shown a gradual reduction up to day 158-165 followed by a gradual rise and fall by day 265 (Fig. 1).

On the other hand, the protein content of lung and skeletal

Figures 1 - 3



Figures 1 - 3 reveal the protein content of various tissues, obtained on day 158, 165, 225 and 265 after fluoride administration. The protein values have been plotted against duration of exposure to NaF. The normal protein values have been indicated by the square.

muscle, unlike that of the other tissues, revealed another mode of response to fluoride ions. Their protein values continued to decline day by day following fluoride adminstration (Fig. 2).

In spleen tissue, adrenal gland, cardiac muscle and nerve the protein content declined up to day 165 after which a steady rise in protein was recorded (Fig. 3). This rise is strikingly different since on day 225 or day 265, the protein content was in the normal or above normal range.

The current investigation has revealed that, 1) soft tissues of the body are affected adversely by fluoride ions; 2) the response of the tissues to fluoride ions varies since three distinct patterns have been observed; 3) fluoride ions interfere with protein biosynthesis in all the tissues investigated exerting an inhibitory influence in a wide range of tissues. A few tissues such as spleen, adrenal gland, cardiac muscle and nerve develop a resistance to fluoride toxicity (toward very late stages). Whether similar changes take place in humans after excessive ingestion of sodium fluoride, is yet to be investigated.

Acknowledgement

The first author is grateful to the Academic Section of the All India Institute of Medical Sciences, New Delhi for the award of a Summer fellowship, during the tenure of which, this investigation was carried out. The authors are indebted to Shri. K. Ramachandran, Associate Professor of Biostatistics of the statistical evaluation of the data.

Bibliography

1. Siddiqui, J.L.: Endemic Fluorosis in Nalgonda District, Hyderabad, Deccan. Br. Med. J., 2:1408-1413, 1955.
2. Singh, A., Dass, R., Heyresh, S.S. and Jolly, S.S.: Skeletal Changes in Endemic Fluorosis. J. Bone and Joint Surg., 44B:806-815, 1962.
3. Singh, A., Jolly, S.S., Bansal, B.C. and Mathur, O.C.: Endemic Fluorosis: Epidemological, Clinical and Biochemical Study of Chronic Fluoride Intoxication in Punjab (India). Medicine (Baltimore), 42:229-246, 1963; 44:97, 1965.
4. Waldbott, G.L.: Fluoride and Optic Neuritis. Br. Med. J., 2:945, 1964.
5. Teotia, M., Teotia, S.P.S. and Kunwar, K.B.: Endemic Skeletal Fluorosis. Arch. Dis. Children, 46:686-691, 1971.
6. Kaul, R.D. and Susheela, A.K.: Evidence of Skeletal Muscle Degeneration in Rabbits Treated With Sodium Fluoride. Fluoride, 7:174-181, 1974.
7. Kaul, R.D.: Muscular and Neural Manifestations of Fluoride Toxicity in Rabbit and Man. Ph.D. Thesis, All India Institute of Medical Sciences, New Delhi, 1976.

8. Kaul, R.D., Keswani, N.H. and Susheela, A.K.: Histoenzymic and Ultrastructural Effects of Fluoride Toxicity. In Proceedings of the Symposium on Fluorosis, Indian Academy of Geoscience, Hyderabad, 1977, pp. 497-509.
9. Demole, V.: Toxic Effects on the Thyroid. In Fluorides and Human Health, WHO Monograph Series No. 59, 1970, p. 255.
10. Ogilivie, A.L.: Histopathologic Findings in the Kidney, Liver, Pancreas, Adrenal Gland and Thyroid of Rat Following NaF Administration. J. Dent. Res., 32:386-397, 1953.
11. Rao, K. and Susheela, A.K.: Sodium Fluoride Administration and Its Effects on Ascorbic Acid Content and delta 5,3beta- hydroxysteroid Dehydrogenase Activity of Adrenal Gland of Rabbit. In Proceedings of the 26th Annual Conference of the Anatomical Society of India, in press, 1977.
12. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein Measurement with Folin Phenol Reagent. J. Biol. Chem., 193: 265-275, 1951.

****