Proceedings of the Iowa Academy of Science

Volume 42 | Annual Issue Article 40

1935

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EFFECT OF FLUORIDES ON SOME ORGANIC CONSTITUENTS OF BLOOD AND PHYSICAL MEASUREMENTS OF BONE

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The effects of excessive amounts of fluorine on the animal organism have attracted the attention of a large number of investigators recently. This interest in fluorine is associated with the fact that mottled enamel has been correlated with the presence of excessive amounts of fluorides in drinking water and, furthermore, by the fact that certain phosphatic mineral supplements are known to contain fluorine. The authors have been interested in the fluorine problem, since the discovery by Ostrem, Nelson, Greenwood, and Wilhelm (1) of mottled enamel in Iowa, due to the presence of excessive fluorides in the drinking waters is certain localities in this state. Since Iowa is such an important live stock state, the fluorine problem becomes of added interest because of the possible use of mineral supplements containing fluorine, both as feed and fertilizer. Some work from this laboratory has been reported on the relation of fluorides to blood and respiration, and studies have also been made on the removal of fluorine from drinking water.

The data in this paper relate to the effect of fluorine on blood sugar, nitrogenous constituents of blood, and to some physical measurements of bone. So far as we are aware little or no work has been done on the changes in organic substances of the blood of animals receiving fluorides. Forbes and Schulz (2) did some very important work on the effects of mineral supplements on the development of swine. They found that precipitated calcium carbonate and steamed bone produced relatively dense and strong bones; while, on the other hand, rock phosphate produced bones very little more dense than — and actually not so strong as — did the unsupplemented ration. Reed and Huffman (3) demonstrated that the feeding of rock phosphate resulted in a thickening of the jaw and the metatarsal bones of dairy cattle. Velu (4) has studied the effect of fluorine on the teeth and bones of sheep in northern Africa. Kick, Bethke, and Edgington (5) have reported the results of their studies on the effect of sodium fluoride and rock phosphate on the bones of swine. They state: "The femurs of the pigs were characterized by an increase in the diameter of the shaft, a loss of normal color and luster, the presence of exostoses, and a decreased breaking strength. These changes became more pronounced as the fluorine content of the ration increased." They found, for instance, that the breaking strength of the femurs from pigs receiving no fluorine was 1013 pounds; whereas, the breaking strength of the same bones of pigs receiving 0.058 per cent fluorine was 550 pounds. The fluorine content of their rations varied from traces to 0.097 per cent.

Moller and Gudjonsson (6) have described the symptoms of men working in a factory engaged in the crushing and refining of cryolite. Radiographs of the bones of some of the men revealed distinct increase in opaqueness, and the contours of the bones lacked in sharpness. The calcification changes were accompanied by stiffness of the limbs and impaired motility of the spinal column. The workers complained of rheumatism.

Experimental

Three dogs were employed in the study of the effect of fluorine (as NaF) on the organic constituents of blood, and the data are given in Table I. These dogs were about fifteen months of age when the experiments were performed. The dogs were raised in the laboratory under controlled conditions. The fluoride was added to 180 cc. of fresh whole milk, which the dogs received daily. The milk, containing the fluoride, was fed to the dogs when they were hungry, to insure complete consumption of the fluoride. Dog 1 had received 13.57 mg. of fluorine (as NaF) for four and one-half months prior to the time the tests were made. This dog received a normal ration for one year before it received any fluorine. Dog 9 received 4.52 mg. of fluorine (residue from Ankeny City drinking water) for twelve months prior to the time tests were made. Dog 3 received a normal ration for 13 months before analyses of the blood were made.

Blood sugar determinations in the sugar tolerance tests were made by the method of Folin and Malmros (7). The fasting period preceding the feeding of the test meal was twelve hours. The test meal consisted of 1.75 g. of glucose per kilo of body weight. Total nitrogen, non-protein nitrogen, protein nitrogen, preformed creatinine, and creatine plus creatinine were determined by the methods outlined by Hawk and Bergeim (8), urea nitrogen by the method of Karr (9), amino acid nitrogen by Folin's method (10), and uric acid nitrogen by the method of Benedict

Table I—Effect of Fluorine (as NaF) on Some Organic Constituents in Dog's Blood

CONSTITUENTS	Mg. Per 100 cc. Blood			
	Dog 3 No F Added to Ration *	Dog 1 13.57 Mg. F Daily Per Kilo Body Weight	Dog 9 4.52 Mg. F Daily Per Kilo Body Weight	Values for Normal Dogs **
Blood sugar fasting level	98.5	94.2	107.8	35-152
Blood sugar ½ hour after feeding of test meal	142.3	134.8		
Blood sugar 1 hour after feeding of test meal	116.9	124.6		
Blood sugar 2 hours after feeding of test meal	109.7	108.9		
Blood sugar 3 hours after feeding of test meal	103.9	108.3		
Total nitrogen	900.0	845.0	910	
Non-protein nitrogen	28.2	27.2	29.2	16.1-78
Protein nitrogen	871.8	817.8	880.8	
Urea nitrogen	13.0	14.0	15.3	5.1-42.1
Amino acid—nitrogen	11.3	11.5	11.3	2 -14.6
Creatine + Creatinine	3.6	3.4	3.4	
Creatinine Preformed	1.6	1.6	1.6	0.5-1.5
Creatine	2.0	1.8	1.8	
Uric Acid	1.6	2.1	1.7	1.1-2.8

* Rations contained trace of fluorine. Dogs Nos. 1 and 3 were litter mates.
** Haden, R. L. and Orr, T. G. (13).

(11). The determinations were made in duplicate and promptly after the blood was obtained from the external saphenous vein.

The data on physical measurements of bone were obtained from studies on thirteen dogs; all of the dogs were raised in the laboratory under controlled conditions. The rations employed were the same as those used by Greenwood, Hewitt, and Nelson (12). The femurs of the dogs were removed and cleaned of adhering flesh. Physical measurements were then made on the bones. The maximum length and smallest diameter were obtained by means of vernier micrometers. Breaking strength determinations were made with an Olsen dynamometer about one week after the bones were removed from the dogs. The distance between the two supports used in determining the breaking strength of the bones was 11.43 cm. throughout all of the experiments.

DISCUSSION

The data in Table I are for the most part self-explanatory. Dog 9 (Table I) showed mottled enamel due to the fact that she received fluorides during the period when her teeth were being formed. Dog 1 did not show mottled enamel, because this animal did not obtain the fluoride until her teeth were fully formed. In view of the fact that fluorides have such a detrimental effect on tooth structure, it is surprising that no significant changes occurred in the components of the blood. Dog 3 served as a control. The data indicate that glucose tolerance is not affected by the ingestion of as high as 13.57 mg. fluorine as NaF daily.

The results summarized in Table II reveal variations in the physical measurements of the femurs of the dogs used in these experiments. In general, variations are likely due to individual variations in the dogs rather than due to the fluorides which were ingested. The error involved in making breaking strength determinations on femurs from the same dog varied from 2 to 10 kilograms. Dogs 1 to 4 were litter mates and dogs 5 to 9 were also litter mates; and, likewise, dogs 10 to 13 were from the same litter. It is interesting to note that the breaking strength of the femur of dog No. 1, which received 13.57 mg. of fluorine as NaF per kilo of body weight daily for 4½ months, decreased. The smallest diameter of the femur increased. This case seems to agree with the results Kick, Bethke, and Edgington obtained on swine.

X-ray studies were made at frequent intervals of the head and front and hind legs of eight of the dogs used in this study. None of the dogs examined revealed any of the changes of the bones

Table II — Physical Measurements of the Femurs of Dogs Fed Fluorine as Sodium Fluoride

Dog No.	Sex	Mg. F Added to Ration Daily Per Kilo of Body Weight	Months on Ration	Weight in Cm.	Length in Cm.	Smallest Diameter in Cm.	Breaking Strength in Kg.	Age of Dog at End of Experiment Months
4	M	0.0 *	6.5	60	17.3	1.17	136.36	8
2	F	0.0 *	8.0	52	16.5	1.14	136.36	9.5
3	F	0.0 *	14.0	54	16.50	1.15	163.63	15.5
1	F	13.57 **	4.5	60	17.00	1.27	115.91	15.2
7	M	2.26	7.0	40	15.20	1.16	127.27	9
6	M	2.26	11.4	56	17.0	1.22	150.00	13.4
5	M	2.26	11.4	40	15.5	1.14	111.36	13.4
8	F	4.52 ***	9.4	58	17.3	1.15	127.27	11.4
9	F	4.52 ***	14.7	56	16.5	1.22	127.27	16.7
12	M	0.45	7.0	50	16.0	0.97	100.00	9.0
13	F	0.45	11.4	28	14.2	0.95	93.18	13.4
10	F	0.90 ****	9.4	38	16.0	0.98	95.45	11.4
11	F	0.90 ****	11.4	20	11.5	0.92	81.82	13.4

* Control rations contained a trace of fluorine.
** Received normal ration until it was about one year of age.
*** Received 2.26 mg. F per kilo of body weight daily for 2 months before they were changed to 4.52 level.
**** Received 0.45 mg. F per kilo of body weight daily for 2 months before they were changed to 0.90 level.

described by Moller and Gudjonsson. However, the amounts of fluorine fed in these experiments were possibly much lower than the amounts consumed by the workers in the cryolite plant. In addition, the industrial workers consumed the fluorine for a much longer period of time before bone changes occurred. The gross appearance of the bones of all of the dogs was normal.

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