Under the microscope the localizations of the stain are found to be of two sorts. In the brain stem, the macroscopically visible blue color is due to an accumulation of dye in the walls (especially the endothelial nuclei), and to some small extent in the plasma, of the capillaries. The number of these which are open is enormously greater than in the control. No perivascular, pericellular or meningeal invasion with wandering cells has occurred, except in the medulla where some small pericellular infiltrates are visible. The same phenomenon is found in the spinal cord. In addition, some, but not more than half, of the anterior and posterior horn cells are found to be stained blue, usually with the dye collected in granules at or near the periphery and in some cases within the nucleus. The distribution of the stained cells is curiously uneven: it is neither symmetrical nor uniform at different levels; moreover stained and unstained cells are to be found side by side in the same section. Although no nerve-cell staining is seen outside the spinal cord, the microscopic appearance of some of the cells in the brainstem, particularly the medulla, is not entirely normal. The experiments indicate that the earliest secondary reaction in the central nervous system to invasion by virus is simple hyperemia, beginning about the third day and reaching a high degree by the sixth day of the incubationary period. This reaction precedes general invasion of the pericellular, perivascular and meningeal spaces by wandering cells of the microglial, leucocytic and lymphocytic series. It may, moreover, be completely absent, as in the olfactory bulbs, where virus first appears and is known to be continuously present in high concentration throughout the period of invasion.

8836 C

Effect of Low Levels of Fluorine Intake on Bones and Teeth.

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A large number of investigations with different species are in agreement that the toxicity of fluorine, as measured by growth and certain physical and chemical changes in the bones, varies with the form of fluorine fed. A recent study by Smith and Leverton[1] with rats, while substantiating this conclusion as regards levels necessary

[1] Smith, M. C., and Leverton, R. M., *Ind. Eng. Chem.*, 1934, **26**, 791.

to retard growth or to cause death, shows that various compounds fed at a much lower fluorine level, namely 14 ppm., were all equally effective in causing enamel pigment changes in the incisors of rats. These observations raise the question as to why the results should be different at different levels and as to the significance of the pigment changes in terms of bone and tooth structure. We therefore undertook a study at the lowest levels used by Smith and Leverton, which included bone and teeth analyses for fluorine as well as observations of pigment changes. For this study, sodium fluoride, generally reported among the most toxic sources, and bone meal, a natural product containing a relatively small amount of fluorine in a complex insoluble form, were selected.

In view of the recent reports of the appreciable and variable occurrence of fluorine in common foods, it was deemed desirable to use a basal diet of known fluorine content. After considerable study the Sherman diet used by Smith and Leverton, consisting of two-thirds wheat and one-third whole milk with 1.3% of salt, was selected as the lowest one found. This diet, as made up by us, contained approximately 3 ppm. of fluorine. The bone meal used contained 380 ppm. Our fluorine analyses of the foods, bone and teeth were made by ashing with calcium oxide as a fixative at 500°C., distillation with perchloric acid at 135°C., as outlined by Willard and Winter[2] and by the determination of fluorine in the distillate according to the method of Sanchis.[3] The incisors were examined at frequent intervals for the pigment changes and graded according to the degree of lightening of the pigment observed.

Growing rats which had been fed the basal diet from weaning until placed on experiment were used. One group continued to receive the basal diet alone, while a second received the same diet plus sodium fluoride and a third, bone meal, the supplements being added at levels which would provide the fluorine intake under study. In order to keep the calcium and phosphorus relations the same, appropriate amounts of calcium oxide and monocalcium phosphate were introduced into the basal diet and the sodium fluoride diet to balance the minerals added by the bone meal. These calcium and phosphorus salts were especially prepared to be as low as possible in fluorine and according to our analyses were sufficiently low that they did not add materially to the fluorine content of the rations in which they were introduced.

In the first experiment rats 53 days of age were fed the 3 diets

[2] Willard, H. H., and Winter, O. B., *Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 7.

[3] Sanchis, J. M., *Ind. Eng. Chem., Anal. Ed.*, 1934, **6**, 134.

in which the fluorine sources were included to provide an added fluorine level of 14 ppm. After 62 days on the diets the animals were killed and their leg bones dissected for analyses. Food intake records were kept and it was found that without restricting the intake for any group they were substantially alike on the 3 diets. The results of the teeth and bone examinations are given in Table I.

TABLE I.
Comparison of Sodium Fluoride and Bone Meal at a Fluorine Level of 14 ppm.

Diet	Age when killed, days	Wt. when killed, gm.	F in bones, dry basis, ppm.	Tooth pigment changes
Control	115	300	91	0
		280	141	0
		212	156	0
		288	95	0
		292	112	0
Aver.			119	
Control plus NaF (14 ppm. F)	115	284	370	lightening
		334	400	"
		292	430	"
Aver.			400	
Control plus Bone Meal (14 ppm. F)	115	280	347	"
		322	361	"
		312	400	"
Aver.			369	

These data show the entire absence of pigment changes in the teeth of the control animals, in contrast to the distinct and similar changes on the diets containing sodium fluoride and bone meal. Particularly striking are the marked and similar increases in the fluorine content of the bones where the supplements were added, as compared to the results with the basal diet.

A second experiment was carried out in which the fluorine sources were added at a level of 8 and 12 ppm. The animals were started at 60 days of age. Some of each group were killed for bone examination after 56 days on experiment and the others at 168 days, in order to observe any progressive changes with age. The results are shown in Table II. While the number of animals for a given treatment are few, the data are sufficiently uniform to make certain conclusions clear.

It is noted that for each diet and level of feeding there is a marked increase in the fluorine content of the bones after 168 days as compared with the content at 56 days. This presumably reflects an increase which normally occurs with age. The data for both 56 and 168 days, however, clearly show that a fluorine addendum as low as 8 ppm. results in a marked increase in the fluorine content of the bones, compared to the content for animals of the same age on

TABLE II.
Comparison of Sodium Fluoride and Bone Meal at Fluorine Levels of 8 and 12 ppm.

Diets	Days on diet	Age when killed, days	Wt. when killed, gm.	F in bones, dry basis, ppm.	Lightening of tooth pigment
No. 4					
Control	56	118	212	94	0
			236	85	0
			187	96	0
Aver.				92	
No. 4					
Control	168	230	322	176	0
			320	135	0
			237	150	0
Aver.				154	
No. 5					
Control plus	56	118	148	226	0
NaF (8 ppm.)			246	257	slight
			213	296	0
			210	282	very slight
Aver.				265	
No. 5					
Control plus	168	230	350	374	" "
NaF (8 ppm.)			348	364	" "
Aver.				369	
No. 6					
Control plus	56	118	244	248	lightening
Bone meal			232	292	very slight
(8 ppm.)			185	267	" "
Aver.				269	
No. 6					
Control plus	168	230	213	368	" "
Bone meal			376	352	" "
(8 ppm.)			228	376	lightening
Aver.				365	
No. 7					
Control plus	56	118	220	257	very slight
NaF (12 ppm.)			212	235	lightening
			170	386	slight
			208	350	lightening
Aver.				307	
No. 7					
Control plus	168	230	388	657	lightening
NaF (12 ppm.)			248	456	very slight
Aver.				556	
No. 8					
Control plus	56	118	213	345	" "
Bone meal			166	369	" "
(12 ppm.)			206	323	slight
Aver.				346	

the basal diet. The response to bone meal and to sodium fluoride appears to be identical, in agreement with the results in Table I. The limited data for the level of 12 ppm. also present a similar picture and it is noted that for a given period on experiment the fluorine content is uniformly higher than at the level of 8 ppm.

The data for pigment changes in the teeth are in agreement with

the bone analyses in showing an effect at the level of 8 ppm. The variable data reveal no differences between the 2 sources of fluorine, nor for the different lengths of time on experiment. The teeth of the animals from the groups killed at 56 days were combined and analyzed for fluorine with the following results:

	Fluorine, ppm.
Controls	81
Sodium fluoride, 8 ppm	228
Bone meal, 8 ppm	220
Sodium fluoride, 12 ppm	246
Bone meal, 12 ppm	276

These data confirm the results of the bone analyses in showing the distinct effect of the small fluorine additions, in revealing no differences between the two supplements, and in showing a greater effect at the higher level.

It cannot be stated, without further study, whether the pigment changes and increases in fluorine content obtained in these experiments represent a definite structural injury. The diet fed our stock rats has regularly contained one per cent of bone meal. In a recent examination of our entire colony of mature animals most of them showed a slight lightening of the enamel, the others showing no detectable change. Analyses of the bones of animals approximately 500 days of age gave an average fluorine value of 335 ppm.

Summary. The data here reported show clearly that fluorine additions of 8 to 14 ppm. to a basal diet containing 3 ppm. results in growing rats in increases in the fluorine content of the bones and teeth, which are marked and roughly proportional to the level fed. They show sodium fluoride and bone meal to be equally effective in causing these changes. They suggest that analysis for fluorine is a more sensitive measure than the enamel pigment changes which are also noted at these low levels. The data indicate that the fluorine content of the bones and teeth increases with age during growth.

8837 C

Purification of Tetanic Toxin.

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The methods used in the purification of tetanic toxin were based on the general principles outlined in a previous paper on the purifi-