Skeletal Lesions Following Ingestion of Fluoridated Water

Clinical and experimental trials have disclosed that administration of fluorides may give rise to lesions of various skeletal and dental tissues. Mainly in the form of fluorapatite[1,2], the fluorine apparently accumulates in the osseous tissues, and the spinal column is one of the first places where it is found. It has long been known that intake of fluorine in large amounts – either added to drinking water or by inhalation of fluoride-containing dust – induces a characteristic disease entity which is readily diagnosed clinically and resembles spondylosis. In the skeletal system, this condition is manifested as osteosclerosis[3] and in the teeth as so-called mottled enamel. According to a team of U. S. investigators, the production of gross skeletal lesions by fluoridated drinking water requires of a dose exceeding 4 mg of fluorine/l[4]. Judging by animal experiments, however, it seems as though these osseous lesions set in long before they can be diagnosed clinically[2]. Moreover, there is a dearth of evidence regarding the action on the skeleton of prolonged administration of small doses of fluorine.

As a rule, skeletal lesions have been induced in experimental animals by administration of fluorine in large amounts over a short period, typically the very high dosage of 50 mg/kg body weight daily[5]. The most recent findings with respect to the effect upon growing bone of fluorine would seen to be contained in a Japanese report dated 1959[6], where it is claimed that skeletal growth is inhibited by high doses and promoted by moderate doses of fluorine.

Experimental. Male and female albino rats of the same strain were used for the experiments. Two groups originally comprising 10 rats were, from birth, given exclusively distilled water to drink which contained respectively 20 and 40 mg of fluorides/l. They were allowed to drink this water *ad libitum* and were otherwise fed a standardized, fluoride-free rat bread. A group of 10 controls were fed the same bread and plain distilled water. At approximately quarterly intervals one rat from each group was sacrificed and samples for analysis were taken from teeth, mandible, lumbar spine, thigh, and one hind leg. The offspring of the 2nd and 3rd generation were similarly treated.

The bone specimens were embedded in methyl methacrylate and sawn into pieces convenient for preparing plane-parallel sections of great planeness an smoothness[7]. These sections were placed on a photographic emulsion (Kodak Spectroscopic Plate No. 649) and exposed to X-rays in the wavelength band around 3 Ångström units (the so-called K absorption edge of calcium is situated at 3 Å). The transmittancy of the organic matrix is very high at this wavelength and so is the absorption of calcium. Consequently, the density of the blackened film will be roughly inversely proportional to the calcium content of the specimen. The white areas of the film represent calcium deposits and hence the dark ones correspond to less mineralized portions. The X-rays were generated in a Matchlett Type OEG-50A tube.

With the degree of water fluoridation adopted, no unmistakeable gross radiographic lesions were apparent. The microradiograms nevertheless disclosed distinct differences between the groups receiving fluoridated water and the control group in the degree of skeletal mineralization. However, such dissimilarities were apparent only in sections from the spine and not in those from other parts of the skeleton. Normally osseous tissue includes Haversian systems with varying degrees of mineralization (Fig. 1), as was the case in the controls. The corresponding sites in the rats given fluoridated water were much more uniformly mineralized, and low-mineralized osteons were exceedingly rare or absent altogether in these groups. In other words, X-ray microscopically visible osteosclerosis would seem to have set in (Fig. 2).

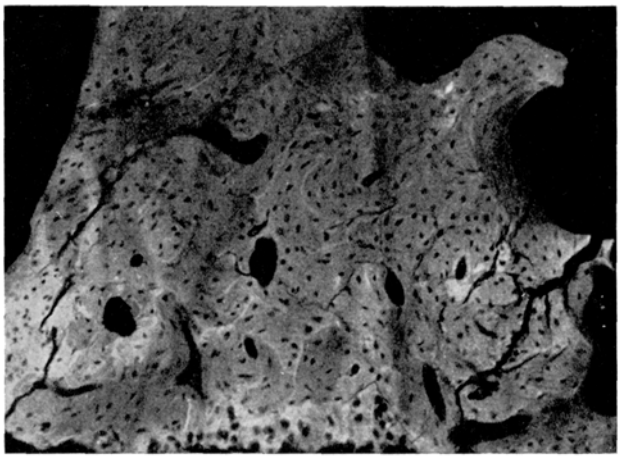

Fig. 1. – Microradiogram showing unequally mineralized Haversian systems in the spine from a control rat

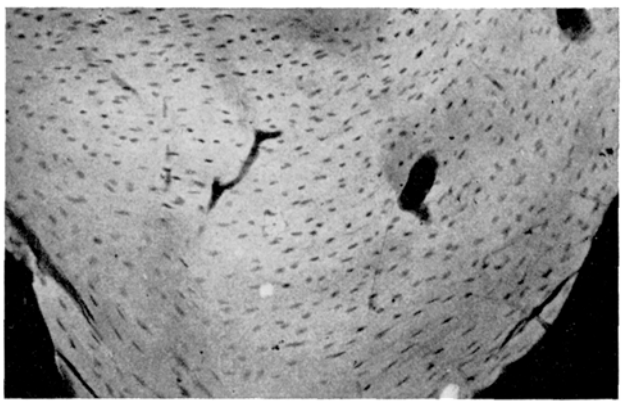

Fig. 2. – Microradiogram presenting distinctly osteosclerotic appearances in the spine from rat on fluoridated water

[1] P. A. Bishop, Amer. J. Röntgenol. *35*, 577 (1936).

[2] W. F. Neuman, M. W. Neuman, E. R. Main, J. O Leary, and F. A. Smith, J. biol. Chem. *187*, 655 (1950).

[3] P. F. Möller and S. V. Gudjonsson, Acta radiol. scand. *13*, 268 (1932).

[4] C. A. Stevenson and A. R. Watson, Amer. J. Röntgenol. *78*, 13 (1957).

[5] E. J. Largent, W. Machle, and I. F. Ferneau, J. indust. Hyg. Toxicol. *25*, 396 (1943).

[6] K. Abe, Shikoku Acta medica *14*, 61 (1959).

[7] O. Hallén and H. Röckert, Nature *182*, 1255 (1958).

[8] A. Roholm, *Fluorine Intoxication. A Clinical-Hygienic Study* (Lewis & Co., Ltd., London 1937).

[9] W. F. Ramseyer, C. A. H. Smith, and C. M. McCay, J. Geront. *12*, 14 (1957).

In the rats given 20 mg/l, as well as in those given 40 mg/l, these lesions initially appeared at the age of 9 or 12 months. Specimens were obtained from three generations in both groups on fluoridated water. Trials with lower dosages are in progress, and in some of these the total amount of ingested fluorides will be assayed.

The fact that lesions could be demonstrated only in the spine of the rats on fluoridated water is well in line with observations reported by previous investigators. For example, in chronic fluorine intoxication gross lesions have been noted on radiograms of the spine and pelvis[1,3], sites at which the lesions tend to be incapacitating[8]. It seems not unlikely that, in analogy with other animal experiments[9], our experiments might disclose involvement of skeletal portions other than the spine when they have been going on for a comparatively long period. Accordingly the initial clinical signs of excessive fluorine administration must be anticipated from the spine. It is too early yet to say whether microscopic lesions (corresponding to those encountered in the present investigation) may eventually give rise to clinical manifestations. Mild lumbar disorders are not susceptible to diagnosis in the rat; and findings in rats cannot be directly translated to human beings unless it has definitely been established that every relevant condition is comparable in the two species.

Incidentally, we would draw attention to the suitability of X-ray microscopy as a tool enabling quantitative changes in chemical composition to be coordinated with morphological appearances.

H. RÖCKERT and H. SUNZEL

Departments of Histology and Surgery, University of Gothenburg (Sweden), December 17, 1959.

Zusammenfassung

Mit Hilfe der Röntgenmikroskopie wurde bei Ratten, die während 9–12 Monaten Wasser mit einem Fluorgehalt von 20–40 mg/l getrunken hatten, Veränderungen in der Mineralisierung der Wirbelsäule nachgewiesen. Die Veränderungen sind mit makroskopischer Röntgenuntersuchung nicht nachweisbar.

Electroshock, Brain Serotonin, and Barbiturate Narcosis

In previous papers[1,2] an increase of brain serotonin has been reported in rats submitted to electroshock. Table I shows the different increases in brain serotonin with different methods of dosage, suggesting that other components of the brain are affected by electroshock.

An investigation using the spectrofluorimetric method has been carried out on different parts of the rat's brain to observe a possible difference in the variation of serotonin content 15 min after electroshock[7].

Other experiments concern the brain serotonin content at different times after electroshock. The figures obtained are summarized in Table II.

Other kinds of convulsant treatments have been studied. The most important variations (increase of 30% in whole brain, $p < 0{\cdot}001$) have been observed after metrazol administration (70 mg/kg i. p.), while strychnine (1·5 mg/kg i. p.) and hyperoxia induce only a small rise of brain serotonin (respectively 21 and 19% – $0{\cdot}02 > p > 0{\cdot}001$) and amphetamine (15 mg/kg i.p.) or anoxia are practically inactive[7].

Treatments with barbiturates (phenobarbital 120 mg/kg i.p., pentobarbital 35 mg/kg i.o. or hexobarbital 80 mg/kg i. p.) or diphenylhydantoine (100 mg/kg i.p.) or succinylcholine (1 mg/kg i.p.) are unable to antagonize the increase in brain serotonin induced by electroshock. These data indicate that the variation of brain serotonin observed after electroshock could not be related to the convulsive state[8]. This point has also been confirmed by using electroshocks with different intensity, unable to induce typical convulsions, as reported in Table IV.

[1] S. GARATTINI *et al.*, Exper. *13*, 330 (1957).
[2] S. GARATTINI *et al.*, in *Psychotropic Drugs* (ed. S. GARATTINI and V. GHETTI, Elsevier Publ. Co., Amsterdam 1957), p. 428.
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[4] J. D. GARVEN, Brit. J. Pharmacol. *11*, 66 (1956).
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[7] S. GARATTINI *et al.*, Atti Soc. Lomb. Sci. Med. Biol. *13*, 300 (1958).
[8] M. BISIANI *et al.*, Atti Soc. Lomb. Sci. Med. Biol. *13*, 345 (1958).

Table I

Method employed	Brain serotonin (γ/g)		Factor of Increase
	Controls	Electroshock*	
Isolated colon of rat[3]	0·013 ± 4 (27)	0·360 ± 7 (27)	× 27
Isolated uterus of rat with destruction of norepinephrine according to GARVEN[4]	0·25 (4)	0·56 (4)	× 2·2
Spectrophotometer (275 mμ)[5]	0·97 ± 0·004 (64)	3·02 ± 0·28 (28)	× 3·1
Spectrophotofluorimeter[6]	0·47 ± 0·003 (40)	0·78 ± 0·021 (36)	× 1·6

* Electroshock was always supramaximal (110 V; 0·2 s) and it was obtained by applying electrodes to the ears. In brackets the number of determinations is reported