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.

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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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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

Table II

Part of the brain assayed	Serotonin content (γ/g)		Increase %	p
	Controls	Electroshock		
Whole brain	0·47 ± 0·003 (40)	0·78 ± 0·02 (36)	+66	< 0·001
Hemispheres	0·37 ± 0·012 (40)	0·53 ± 0·019 (36)	+43	< 0·001
Cerebellum*	0·11 ± 0·009 (40)	0·17 ± 0·03 (36)		N. S.
Diencephalic part	0·81 ± 0·02 (40)	1·28 ± 0·06 (36)	+58	< 0·001
Mesencephalic part	0·56 ± 0·03 (40)	1·08 ± 0·06 (36)	+93	< 0·001

* The figures obtained are at the minimum limit of sensitivity of the method

The different parts of the brain were prepared according to a method previously described[7]

Table III

Time after electroshock	Serotonin content (γ/g)								Sleeping time after pentobarbital*** (min ± S. E.)		*
	Whole brain	*	Hemispheres	*	Diencephalic part	*	Mesencephalic part	*			
Controls	0·47 ± 0·01	—	0·38 ± 0·01	—	0·78 ± 0·02	—	0·59 ± 0·02	—	65 ± 2·7	(55)	—
1 min	0·54 ± 0·01	+15	0·47 ± 0·02	−23	0·87 ± 0·03	+10	0·71 ± 0·04	+20	—	—	—
10 min	0·64 ± 0·09	+36**	0·55 ± 0·02	+45**	1·21 ± 0·06	+56	0·89 ± 0·05	+51	86 ± 5·4	(20)	+32**
20 min	0·69 ± 0·02	+47**	0·53 ± 0·01	+40**	1·28 ± 0·06	+61**	1·08 ± 0·06	+86**	116 ± 7·5	(15)	+80**
30 min	0·58 ± 0·01	+24**	0·51 ± 0·05	+34	1·09 ± 0·08	+39**	0·82 ± 0·07	+39	113 ± 6	(15)	+74**
1 h	0·52 ± 0·01	+11	0·56 ± 0·02	+42**	1·10 ± 0·02	+40**	0·86 ± 0·01	+46**	107 ± 7·0	(15)	+65**
4 h	0·54 ± 0·01	+15	0·48 ± 0·01	+18	1·00 ± 0·05	+28**	0·81 ± 0·04	+36	82 ± 7·0	(10)	+26
8 h	0·51 ± 0·03	+ 8	0·52 ± 0·01	+37	0·88 ± 0·03	+12	0·76 ± 0·03	+29	—	—	—
12 h	0·50 ± 0·07	+ 6	0·49 ± 0·02	+29	0·98 ± 0·01	+25**	0·72 ± 0·03	+24	73 ± 6·5	(10)	+22
24 h	0·45 ± 0·01	− 4	0·41 ± 0·01	+ 8	0·84 ± 0·04	+ 7	0·65 ± 0·04	+10	50 ± 7·5	(20)	−23
26 h	0·49 ± 0·01	+ 4	0·41 ± 0·01	+ 8	0·82 ± 0·06	+ 3	0·66 ± 0·09	+12	85 ± 4·8	(15)	+31
48 h	0·55 ± 0·02	+17**	0·37 ± 0·01	− 3	1·01 ± 0·01	+29**	0·73 ± 0·01	+25	91 ± 4·4	(20)	+40**
60 h	0·57 ± 0·01	+21**	0·48 ± 0·01	+26	1·07 ± 0·07	+37**	0·81 ± 0·02	+35	100 ± 5·2	(25)	+54**
72 h	0·56 ± 0·01	+19**	0·50 ± 0·09	+32**	1·11 ± 0·05	+41**	0·83 ± 0·02	+41**	98 ± 4·0	(20)	+46**
96 h	0·55 ± 0·01	+17**	0·48 ± 0·01	+26	1·12 ± 0·03	+43**	0·82 ± 0·01	+39**	76 ± 5·6	(10)	+17**
120 h	0·53 ± 0·01	+13	0·47 ± 0·01	+23	0·93 ± 0·03	+19	0·71 ± 0·03	+22	58 ±10·1	(10)	−11
144 h	0·54 ± 0·01	+15	0·46 ± 0·01	+21	0·92 ± 0·01	+18	0·77 ± 0·03	+31	58 ± 5	(10)	−11

* Percentual change respect to controls

** $p < 0·001$

*** Pentobarbital was injected i. p. at the concentration of 25 mg/kg. In brackets the number of animals used is reported

Each figure represents the average of at least 8 determinations for the whole brain, and the average of 4 determinations for the different encephalic parts

A further aspect of this investigation concerns a detailed analysis of the relationship between change in brain serotonin and duration of barbiturate sleeping-time after electroshock. The results obtained are summarized in the last column of the Table III.

Table IV

Number of rats	Voltage applied (time = 0·2 s)	Convulsions	Serotonin content (γ/g) mesencephalic part
16	—	—	0·59 ± 0·02
8	30	—	0·82 ± 0·01*
8	50	—	0·86 ± 0·02*
8	70	±	0·87 ± 0·05*
8	90	+	0·85 ± 0·02*
8	110	+ +	0·93 ± 0·03*
8	130	+ +	0·84 ± 0·03*
8	150	+ +	0·91 ± 0·03*

* $p < 0·01$

These data, showing a good parallelism between changes in brain serotonin and variations of sensitivity to barbiturate after electroshock, could be related to other investigations reporting an anticonvulsant effect exerted by monoamineoxidase inhibitors[9] and demonstrating an increase in brain serotonin after barbiturate treatment[10,11].

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Riassunto

Dopo elettroshock si osserva nell'encefalo di ratto un aumento di serotonina, particolarmente evidente nell'area mesencefalica e diencefalica, che si accompagna ad una maggior sensibilità al pentobarbital.

[9] D. J. Prockop *et al.*, Exper. *15*, 145 (1959).

[10] E. G. Anderson *et al.*, Abstr. Fall Meeting Amer. Soc. Pharmacol., Ann. Arbor (Mich.), 1958, p. 1.

[11] D. D. Bonnycastle *et al.*, Brit. J. Pharmacol. *12*, 228 (1957).