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KENETICS OF FLUORIDE PENETRATION IN LIVER AND BRAIN

by

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SUMMARY: Our results suggest that orally administered sodium fluoride enters liver and brain. The blood-brain barrier fails to exclude the fluoride ion from nerve tissue. That fluoride ions also readily pass the placental barrier has been repeatedly demonstrated (9). Fluoride levels in brain reach a maximum approximately two hours after it has been administered, whereas accumulation in liver continues for at least three hours.

KEY WORDS: Fluoride penetration; Liver; Brain.

Introduction

To determine the effect of fluoride on brain and liver enzymes and the pharmacodynamics of the effect of fluoride, the following three questions should be answered: 1] Does fluoride penetrate into liver cells and does it pass the blood-brain barrier? 2]. What length of time is required for the fluoride concentration to reach a maximum in tissues? 3] What is the most suitable method for fluoride analysis of numerous samples within a wide range of concentrations?

Zipkin and Likins (1) showed that, in the rat, nearly 50% of the ingested fluoride is absorbed from the gastrointestinal tract within 30 minutes. A "plateau" is reached after 2 hours. Armstrong and Singer (2) studied the distribution of fluorides in muscle, liver and tendon; they observed that, after two hours, a maximum was reached which itself lasted for at least two hours. The work of Carlsson (3), on the pentration of fluoride into the brain, suggests the existence of an effective blood-brain barrier against fluoride in nervous tissue. On the other hand, Appelgren et al. (4) demonstrated (using autoradiography) that F^- penetrates into the central nervous system of the mouse.

Whereas ion-selective electrodes have been used widely for determining fluoride concentrations (5), we find that long adaptation times (up to 30 minutes) of the electrode and a lack of accuracy make this procedure unsuitable for our purposes. Therefore, the gas chromatographic method of Fresen et al. (6) for quantitative determination of fluoride in biological materials was adapted for measuring fluoride in brain and liver samples.

Materials and Methods

Male Wistar rats (250-300 g) were fed standard laboratory food (A.04 from U.A.R., Villemoisson-sur-Orge, France) and tap water. The fluoride content of

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food, as determined after $HClO_4$ digestion (5) was 10 mg/kg. The fluoride content of the water ranged between 0.0 and 0.4 mg per liter. Food was removed 24 hours prior to experiments. Rats were administered (by stomach intubation) a dose of 10, 20, 30, or 50 mg NaF/kg body weight dissolved in 0.9% NaCl. The animals were anesthetized with sodium pentobarbital (Nembutal®) i.p. at 45, 60, 90, 120, 150 and 180 min after the fluoride load (4 rats/concentration/timepoint). Blood was taken by heart puncture in heparinized tubes and the plasma was obtained by centrifugation. The animals were killed by decapitation and the brains and livers were removed and weighed. The tissues were homogenized with a Potter-Elvehjem homogenizor as 25% (w/v) suspensions in a 0.05 M TRIS-HCl buffer, pH 7.4.

Fluoride determination: 1. Gas Chromatograph: Hewlett-Packard 5710 A; Column: 20% of silicone oil DC 200/50 on Gas Chrom Q; Injection temperature: 150°C; Column temperature at start: 55°C; gradient: +5°C/min; final: 80°C for 5 min.; Detection temperature: 150°C; Carrier flow (nitrogen): 10 ml/min (as determined by the van Deemter equation); Detector: Flame ionization; Stock solution NaF: 0.221 g NaF/100 ml (equals 1 mg F^- per ml); Working solutions: 0.1-10 µg NaF/ml; Derivative and extraction solution (DES): 0.6 mg TCMS (Pierce Chemical Co., Rockford, Il, USA) + 6.1 µg isopentane (internal standard) per ml benzene; HCl 25%. 2. Procedure: 2 ml of the sample (homogenized tissue) were added to 1 ml HCl and 1 ml DES. Because of the low boiling point of the trimethylfluorosilane (TMFS) formed (16.4°C) and of the internal standard (28°C) the reaction was performed at 4°C. The tubes were mechanically shaken for 30 min, the two layers were separated by gentle centrifugation (5 min at 500 g) and 1 to 5 µl of the organic phase were injected into the GC. A set of standard solutions and a blank were analyzed at the same time.

Results and Discussion

A. THE GAS CHROMATOGRAPH (GC) DETERMINATION OF FLUORIDE as described by Fresen et al. (6) is based on the work of Bock and Semmler (7) and involves two reactions:

$$R_3\text{-SiCl} + H_2O \longrightarrow R_3\text{-SiOH} + HCl$$
$$R_3SiOH + H^+ + F^- \longrightarrow R_3\text{-SiF} + H_2O$$
$$R_3\text{-SiCl} + H^+ + F^- \longrightarrow R_3\text{-SiF} + HCl$$

(in our case: R_3SiCl = trimethylchlorosilane = TMCS). Thus, the alkylsilane is first converted by water into the corresponding silanol which in turn reacts selectively with fluoride to form fluorosilane. This compound can be extracted from the acidified reaction medium with benzene. The extracted fluorosilane is then determined quantitatively by GC.

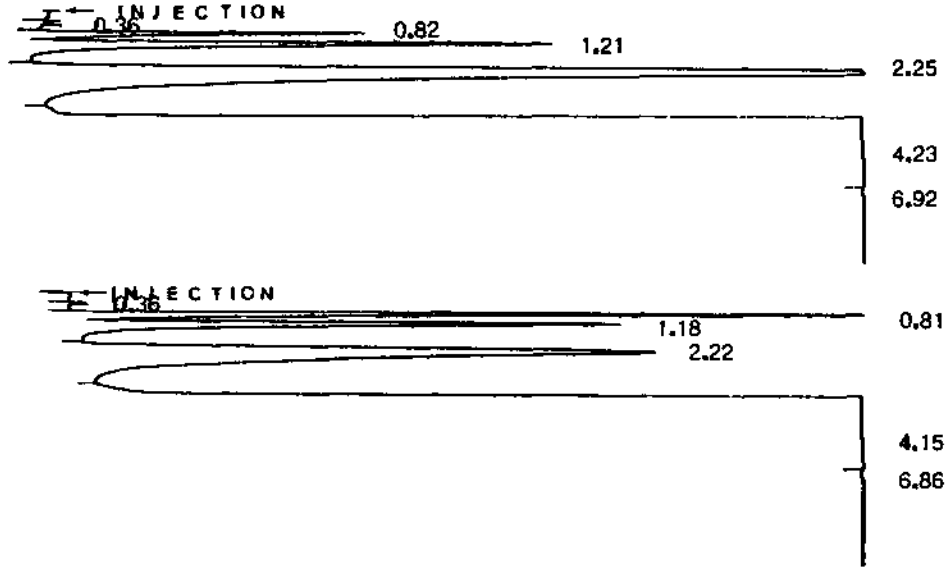
The standard solutions and other aqueous samples (serum, saliva) could be analyzed as described by Fresen et al. (6) without any further treatment. However, the high protein content of brain and liver samples disturbed the adequate centrifugal separation of the organic and the aqueous layers by forming a thick floating mass. Elimination of the proteins by trichloroacetic or perchloric acid results in an important and variable loss of F^-. Therefore, in these samples, these proteins were digested with trypsin (30 mg/sample; incubation at 37°C for 2 hrs) before the DES was added. The addition of the digestion step

broadens the application range of the method. The presence and the action of trypsin has no effect on the linearity of the F^- determination.

Figures 1a and 1b represent respectively the gas chromatograms obtained with a standard NaF solution (1 µg/ml) and for the plasma of a fluoride-treated rat (10 mg/kg). Peak 1 corresponds to the TMFS formed by the substitution in TMCS of Cl^- by F^- present in the sample. Peak 2 is the internal standard (isopentane), while peak 3 is the excess of TMCS. The solvent (benzene) eluates hereafter as a broad peak and does not interfere with the analysis.

Fig. 1.a. and 1.b.

GC chromatograms



(1.a.) Standard NaF solution (1 µg/ml). (1.b.) Plasma sample of NaF-treated rat.

Table I

Amount Added (µg F^-)	Liver + F^- mean (µg)	Liver + F^- recovery (%)	Brain + F^- mean (µg)	Brain + F^- recovery (%)
0.00	0.000		0.000	
0.05	0.046	92.0	0.045	90.0
0.20	0.190	95.0	0.193	96.5
0.50	0.481	96.2	0.493	98.0
1	1.030	103.0	0.970	97.0
2	1.990	99.5	1.985	99.2
5	4.830	96.6	4.980	98.0
10	9.700	97.0	9.650	96.5

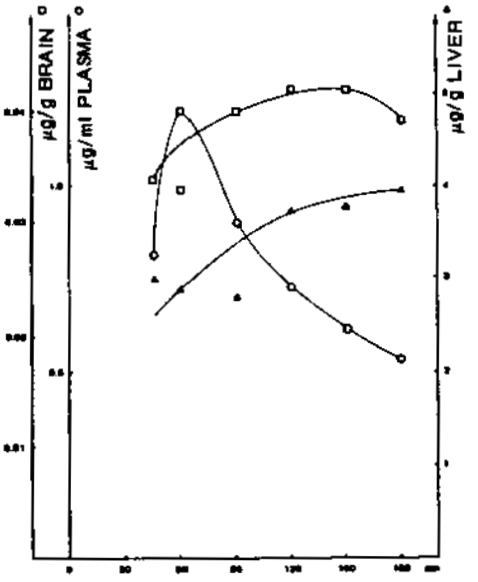
To check the recovery, different amounts of fluoride (ranging from 0.01 to 10 µg) were added to 5 rat liver and 5 rat brain samples before homogenation. These samples, together with a set of standards were carried through the entire procedure. The results of this experiment are shown in Table I.

Thus, the GC method of Fresen et al. for the quantitative determination of fluoride, once adapted for protein-rich samples by including an enzymatic digestion step, proved to be reproducible, sensible and accurate. Very recently, Retief et al. (13) showed, in a comparative study, the accuracy of the Fresen method.

B. Pharmacokinetics of F^-: The 50 mg/kg dose proved to be lethal for at least 75% of the animals within 30 minutes (range: 5-30 min). With 30 mg/kg respiration difficulties and convulsions were observed in all rats. Figure 2 represents the data obtained from rats treated with 10 mg NaF/kg body weight. In contrast to the results of Carlsson (3), the fluoride ion is able to cross the blood-brain barrier and to penetrate into the brain, where its concentration reaches a maximum two hours after ingestion. Penetration into the liver is slower, but greater amounts are taken up. Although the lower values at 60 min are not as marked for the the other doses, uptake in the brain and liver might be biphasic. The F^- concentration in the liver and in the brain rises with the dose of F^- administered (see Figure 3 and Table II).

Figure 2

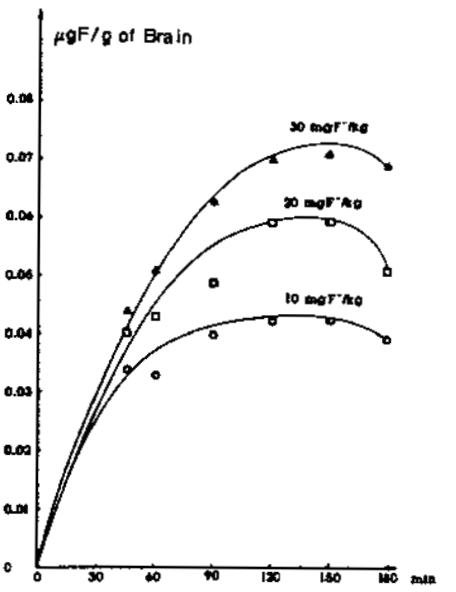
Kinetics of Penetration of F^- in Plasma, Liver and Brain



Rats treated with 10 mg NaF/kg.

Figure 3

Relationship between Dose and F^- Concentration in Brain.



Our data, for plasma samples, correspond with those of Patz et al. (8). In our experiments the absorption of fluoride by the liver is slower than that described by Armstrong and Singer (2). The sharp rise in plasma F^- concentra-

tions corresponds with previous observations obtained using ^{18}F (10). The levels in the plasma of fluoride-treated rats (10^{-5} M) are of the same order of magnitude as those for which in vitro activity of certain enzymes is significantly reduced (11,12).

Table II

Time	45 min			60 min			90 min		
Dose (mg/kg)	Plasma	Brain	Liver	Plasma	Brain	Liver	Plasma	Brain	Liver
10	0.80	0.034	3.00	1.20	0.033	2.90	0.90	0.040	2.80
20	1.40	0.040	5.60	1.80	0.043	5.60	1.45	0.49	6.00
30	1.60	0.044	7.10	2.20	0.051	7.90	2.10	0.063	9.30
	120 min			150 min			180 min		
	Plasma	Brain	Liver	Plasma	Brain	Liver	Plasma	Brain	Liver
10	0.74	0.042	3.75	0.62	0.042	3.80	0.54	0.039	3.97
20	1.20	0.056	6.20	1.04	0.056	6.50	0.79	0.051	6.64
30	1.90	0.70	9.60	1.60	0.071	10.12	1.40	0.069	11.36

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