LETTER TO THE EDITOR

PERMEABILITY OF LIPID BILAYER MEMBRANES TO MOLECULAR HF AND THE FLUORIDE ION F^-

Dear Editor

Using the data, in bold in the following table from Gutknecht and Walter, I calculated the time for molecular hydrogen fluoride (HF) and the fluoride ion (F^-) to pass through a biomembrane or lipid bilayer membrane.[1]

Table. Permeability coefficients for solutes to pass through membranes

Solute	Membrane composition	Permeability coefficient ($cm \cdot sec^{-1}$)
HNO_3	Lecithin+cholesterol	$(4.4 \pm 0.8) \cdot 10^{-4}$
HNO_3	Lecithin	$(9.2 \pm 1.5) \cdot 10^{-4}$
HF	**Lecithin+cholesterol**	$\mathbf{(1.4 \pm 0.3) \cdot 10^{-4}}$
HF	Lecithin	$(3.1 \pm 1.4) \cdot 10^{-4}$
NO_3^-	Lecithin+cholesterol	$(1.3 \pm 0.6) \cdot 10^{-10}$
$\mathbf{F^-}$	**Lecithin+cholesterol**	$\mathbf{(4.9 \pm 2.3) \cdot 10^{-11}}$
H^+	Lecithin+cholesterol	$(1.7 \pm 0.8) \cdot 10^{-9}$

I used metres (m) as the unit of distance. At first I could not believe the results of my calculations. I found that HF permeates the membrane in only 0.005 sec whereas for F^- it took approximately 4 hr.

$$\text{Permeability speed of HF} = 1.4 \cdot 10^{-4}\ cm \cdot sec^{-1} = 1.4 \cdot 10^{-6}\ m \cdot sec^{-1}$$

$$\text{Permeability speed of } F^- = 4.9 \cdot 10^{-11}\ cm \cdot sec^{-1} = 4.9 \cdot 10^{-13}\ m \cdot sec^{-1}$$

$$\frac{\text{Permeability speed of } F^-}{\text{Permeability speed of HF}} = \frac{1.4 \cdot 10^{-6}}{4.9 \cdot 10^{-13}} = 2.9 \cdot 10^{6} = 2{,}900{,}000 \approx 3{,}000{,}000$$

Thus, the permeability of biomembrane to HF is approximately 3 million-fold higher than the permeability to F^-.

According to Lodish et al. the thickness of biomembrane is 3–7 nm.[2] I took the thickness of the biomembrane as 7 nm = $7 \cdot 10^{-9}$ m

$$\text{Permeation time for HF to permeate through the biomembrane} = \frac{7 \cdot 10^{-9}\ m}{1.4 \cdot 10^{-6}\ m \cdot sec^{-1}} = 5 \times 10^{-3}\ sec = 0.005\ sec$$

$$\text{Permeation time for } F^- \text{ to permeate through the biomembrane} = \frac{7 \cdot 10^{-9}\ m}{4.9 \cdot 10^{-13}\ m \cdot sec^{-1}} = 1.43 \cdot 10^{4} = 14{,}300\ sec = 238\ min = 3.97\ hr \approx 4\ hr$$

Thus, HF can permeate a biomembrane 200 times in 1 second (1000÷5) while F^- takes 4 hr to permeate the biomembrane. This information is relevant to understanding how the element fluorine permeates (i) the cell and nuclear

membranes to damage DNA, (ii) the blood brain barrier to damage the central nervous system, and (iii) the placenta to damage the fetal central nervous system.

In the 1991 *Review of fluoride: risks and benefits* it is noted that:

"During the last 60 years, fluorides have been studied widely in biological systems. In many of the earlier studies, deficiencies, combined with the inability to measure fluorides accurately, led to a number of misunderstandings and faulty premises about fluoride metabolism. Today, however, the basic mechanics of fluoride absorption, distribution, biotransformation, and excretion are fairly well understood, and the basic mechanisms underlying many of fluoride's actions in the body are known. ... Results of numerous *in vitro* and *in situ* animal studies have shown that the undissociated molecule hydrogen fluoride (HF) is the principal permeating moiety in a number of cellular membranes and epithelia. ... Ionic fluoride (F^-) has been shown to be virtually incapable of such penetration, have a transmembrane permeability coefficient more than 10^6-fold less than that for HF. ... purportedly because of its ionic charge and large hydrated radius ... ."[3]

I understand what the writer of the above sentences wanted to say but doubt if it is widely known that the toxicity of fluorine biologically is the toxicity of HF rather than F^-. An implication of the different permeabilities of HF and F^- is that HF rather than sodium fluoride (NaF) should be used in *in vitro* studies. If this information was known by fluoride researchers 60 years ago fluoridation may not have been used to prevent dental caries. In Japan, NaF is available as a powerful drug but HF is legally classified as a deadly poison. Fluoride researchers, all over the world, should repeat previous *in vitro* studies on fluoride toxicity using HF rather than NaF.

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REFERENCES

1 Gutknecht J, Walter A. Hydrofluoric and nitric acid transport through lipid bilayer membranes. Biochim Biophys Acta 1981;644(1):153-6.

2 Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. Molecular cell biology. 4th ed. New York: WH Freeman; 2000. [see The Japanese Wikipedia 2017].

3 Subcommittee on Fluoride of the Committee to Coordinate Environmental Health and Related Programs, Public Health Service, Department of Health and Human Services, Public Health Service. Review of fluoride: benefits and risks. Washington, DC: Public Health Service, Department of Health and Human Services; 1991. pp. 2 and 3.

Editorial note: Dr Narita has made some valuable calculations of the permeability speeds in lipid bilayer biomembrane for molecular hydrogen fluoride (HF) and the fluoride ion (F^-), and of the time it takes for them to permeate through such a membrane. An aqueous solution of hydrogen fluoride (HF) is called hydrofluoric acid (HF). It is a weak acid with a dissociation constant (pK_a) of 3.17.[1] From the Henderson-Hasselbalch equation, at pH 3.17 the concentration of molecular HF will equal that of F^-. The range of the pH of gastric juice, containing hydrochloric acid (HCl), a strong acid, is 1.5–3.5. In the stomach, depending on the pH, up to 99.9% of ingested F^- will be protonated to produce HF.[2] As HF is neutral, it can readily penetrate the gut epithelial cell walls and enter the cell cytoplasm.[2] In the neutral pH environment of the cytoplasm, the HF dissociates into protons (H^+) and F^-. Any increase in F^- in the cell can cause interference with cellular function.[2] Other than in the stomach, where mucus and bicarbonate secreted by mucous cells create a pH gradient to maintain the epithelial cell surface at near neutral pH, cells are not subject to similar low pHs. For most cell types, if the pH is lowered from the physiological pH of 7.4 to 6.8, massive cell death results from "pH shock." At pH 6.8, almost all the F^- from NaF would still be in ionic form and not get into cells but

there would be some increase in HF molecules. The pH of various human organelles, cells, tissues, body fluids, and contents is shown in the Table.[4]

Table. pH of various human organelles, cells, tissues, body fluids, and contents[4]

Lyosomes	4.0–5.0	Cerebrospinal fluid (CFS)	7.35–7.45
Mitochondria	6.6	Saliva	6.7–7.4
Kupffer cells	6.4–6.5	Tears	7.4
Osteoblasts	9.0–10.0	Milk	6.9–7.0
Prostate	3.0–5.0	Bile	7.4–8.5
Blood	7.35–7.45	Gastric juice	1.0–2.0
Urine	5.0–7.5	Stool	7.0–7.4

Raising the stomach pH from 2 to 5 would dramatically lower the HF to F^- ratio but several studies have shown that fluorine is absorbed at basically similar rates throughout the course of the gastrointestinal tract. In the rat, approximately 25% of F absorption occurs in the stomach and 75% is from the small intestine.[3] Living cells can only be studied *in vitro* in an aqueous medium with a pH close to 7.4. If gaseous molecular HF was introduced into the cell bathing medium it would react with the water to form hydrofluoric acid which would dissociate into H^+ and F^- to an extent determined by the pH of the medium. At a pH of 3.17, the pKa for hydrofluoric acid, half of the fluorine would be in the form of molecular HF and half would be present as F^-. At a pH close to or equal to 7.40 almost all the hydrofluoric acid would be present as F^- and only an extremely tiny amount as HF. However, as calculated by Dr Narita, because of the permeability of biomembrane to HF being approximately 3 million-fold higher than the permeability to F^- some of the HF would rapidly permeate through the cell biomembrane in 0.005 sec. This HF would then be quickly replaced, because of the dynamic equilibrium, by more HF from the ions H+ and F^- associating again to form molecular HF. The cycle would be repeated by the newly formed HF again permeating through the cell membrane. Because it is not possible to have F^- in an aqueous medium at a pH at which cells are viable without at least a tiny amount of HF also being present, in essence it is not possible to study the *in vitro* effects of NaF or F^- on living cells without HF also being present. When NaF is added to an aqueous medium, some highly permeable HF is formed which then passes the biomembrane and changes back to being predominately F^- when inside the cell or in the extracellular fluid. While it is HF rather than F^- that passes the biomembrane, once the HF is through the biomembrane it can dissociate to H^+ and F^- and thus allow F^- to be toxic.

Bruce Spittle, Editor-in-Chief, *Fluoride*

REFERENCE

1 Harris DC. Quantitative chemical analysis. 8th International ed. New York: WH Freeman; 2010. p. AP14. Quoted in: Wikipedia. Hydrofluoric acid. Available from: https://en.wikipedia.org/wiki/Hydrofluoric_acid

2 Limeback H, Robinson C. Fluoride therapy. In: Limeback H, editor. Comprehensive preventive dentistry. Ames, Iowa, USA, and Chichester, West Sussex, UK: Wiley-Blackwell, an imprint of John Wiley & Sons; 2012. pp. 265.

3 Nopakun J. Messer HH, Voller V. Fluoride absorption from the gastrointestinal tract of rats. J Nutr 1989;119(10):1411-7.

4 Gupta PP. Textbook of biochemistry with biomedical significance for medical and dental students. 2nd ed. New Delhi, India: CBS Publishers and Distributors; 2013.