PARADOXICAL DOSE-RESPONSE EFFECTS OF FLUORIDE

In dealing with concentration or dose-response effects in biological systems, one usually expects to find fairly regular monotonic changes, especially at low concentrations of a promoting or an inhibiting reagent. Oftentimes, however, competing reactions and/or interfering species produce anomalous "paradoxical" effects. These occur when the rate of a reaction or a response at first increases with increasing concentration of a reagent and then shows a *decrease* followed again by an increase at still higher concentrations. Alternatively, the effect may at first decrease and then *increase* with increasing concentrations and then again decrease with further increases in concentration. A stimulatory or beneficial effect of a subinhibitory concentration of a toxic substance is also known as hormesis.

In many cases a paradoxical effect may go unnoticed or be too small to be statistically significant, even though it is reproducible. In other cases it is statistically significant but not readily explained. In still other cases, in view of what is known about the system, plausible explanations or mechanisms can be proposed and investigated.

As far back as 1964 special attention was drawn to paradoxical concentration and dose-response effects in biological systems in a first-ever survey[1] and also in a review of examples involving fluoride.[2] Later research on the phenomenon, focusing mainly on hormesis aspects, does not appear to have attracted much interest. No doubt this neglect reflects the general lack of treatment of the topic in standard textbooks of biochemistry and toxicology, thereby creating the impression among teachers and students that paradoxical effects may not be important or may not even exist. In fact, the latter became the focus of a dispute a few years ago in *Fluoride*.[3] Nevertheless, as discussed below, research reports in this issue and in other recent issues of *Fluoride* indicate that both *in vitro* and *in vivo* paradoxical dose-response effects of fluoride are not uncommon.

In an investigation of *in vitro* effects of fluoride on enzyme activities in ram semen, Zakrzewska *et al* (pages 153-160) found that activities of acid phosphatase (ACP), lactate dehydrogenase (LDH), and γ-glutamyl transferase (γ-GT-10S) displayed marked decreases with 20-200 μM NaF (0.38-3.8 ppm F), but the activity of aspartate transaminase (AspAT) nearly doubled. At the much higher concentration of 0.1 M NaF (1900 ppm F) the activity of ACP increased almost to that of the control without fluoride, whereas the activity of γ-GT-10S slightly exceeded that of the control. On the other hand, the activity of LDH, after a nine-fold decrease at 20-200 μM increased at 0.1 M NaF to nearly 40% above that of the control. Even more striking, the activity of AspAT nearly doubled at 200 μM NaF but at 0.1 M NaF was only 35% higher than that of the control. Because of the large number of

rams (25) that were sampled, these results were all statistically significant ($p \leq 0.05$).

An *in vivo* study by Shivarajashankara *et al* (pages 197-203) of increased oxidative stress in brain tissue of fluoride-intoxicated rats revealed that malondialdehyde as a marker of lipid peroxidation was elevated in young rats exposed to 100 ppm F but not to 30 ppm F in their drinking water for 10 weeks after birth. On the other hand, levels of total glutathione, reduced glutathione (GSH), and ascorbic acid were more elevated in the rats exposed to 30 ppm F but were lower in the 100 ppm F group. In the case of ascorbic acid the level was 15% higher in the 30 ppm F group than in the controls but was nearly 10% lower in the 100 ppm F group. Moreover, whereas the activity of glutathione peroxidase (GSH-Px) and of glutathione S-transferase (GST) was significantly elevated in both the 30 and 100 ppm F exposed rats, the elevation of GST activity compared to the controls was much greater in the 30 ppm F rats (143% higher) than in the 100 ppm F group (21% higher). In this work, overriding adaptive response mechanisms appeared to be operating at the higher F intake. Again, these paradoxical effects were also statistically significant ($p < 0.001$).

In another *in vivo* investigation, Mysłiwiec *et al* (pages 168-175), found that of 5 and 10 µg of selenium/kg bw/24 hr over a period of 3 months in the diet of fluoride-intoxicated rats showed similar anomalous effects. For example, the enhanced activity of serum alkaline phosphatase was decreased more by the higher dose of Se, but in the case of γ-glutamyl transferase the lower dose of Se was significantly more effective in countering the F-induced activity increase of the enzyme than the higher dose. Likewise, although not statistically significant, the 5 µg level of Se reduced the F-induced elevation of bilirubin, whereas the 10 µg level caused it to increase in the same manner seen in the non-F exposed rats. Similarly for the triglyceride and high-density lipoprotein levels, the higher dose of Se was less effective than the lower dose. As noted by the authors, the greater protective effect of the smaller dose of Se can be easily offset by the toxic effect of the higher dose.

And what about other research reports of paradoxical effects in recent issues of *Fluoride*? Last year Bohatyrewicz[4] recorded a higher compressive bone strength after six weeks in rats drinking water with 8 ppm F than with 0, 30, or 60 ppm F. By contrast, an epidemiological study by Alarcón-Herrera *et al*[5] of deleterious dental and skeletal effects of well water fluoride in the Guadiana Valley of Mexico revealed that spontaneous (nontrauma) bone fractures among adults, which usually reflect decreased bone tensile strength, were proportionately greater at 6 ppm F in the water than with lower or higher F concentrations. On the other hand, dental fluorosis, which

increased in severity with increased levels of F in the water, correlated directly with bone fracture incidence in both children and adults.

In experiments by Machalinski *et al,*[6] the cloning ability of umbilical cord blood hematopoietic progenitor CD34+ cells exposed *in vitro* to 0, 1, 10, and 50 mM NaF indicated a stimulatory (hormetic) effect in 7 of 8 sets of experimental conditions at 1 mM, but at the higher concentrations colony formation was depressed, especially at the 50 mM level. Although the paradoxical stimulatory effect was not significant statistically in each experiment, the fact that it occurred in 7 out of 8 sets of conditions indicates that it was probably real. In a related study on human bone marrow and umbilical cord blood hematopoietic CD34+ cells, these authors found that early stage apoptosis was mildly retarded at 1 and 10 mg NaF/L in cell cultures but was significantly increased at 50 mg NaF/L.[7] Again, this appears to be a nonlinear or "paradoxical" concentration effect.

As a final example, Nicolau and Leite,[8] in studying *in vitro* effects of fluoride on human salivary amylase activity, observed a small but not statistically significant 9% increase in starch hydrolysis on going from 0 mM F to 75 mM NaF followed by a decrease to 92% of the control at 500 mM NaF. An *in vivo* experiment with 30-second mouthrinses using 0.05% NaF (11.9 mM) showed a gradually declining initial stimulatory effect on the salivary amylase for up to an hour. In an earlier *in vivo* study by Hara and Yu,[9] these amylase activity promoting effects were not observed, although inhibition could be detected with as little as 50 mM NaF in their experiments, in contrast to a higher F level reported by Nicolau and Leite.

In view of the foregoing examples, the existence of paradoxical dose-response effects of fluoride can hardly be doubted. But how important are they? What they show is that, under certain circumstances, the inhibitory or stimulatory impact of fluoride can actually be greater at a lower level of intake than at a higher level. An impressive illustration of this fact is seen in the administration of fluoride as aluminum fluoride to rats. In both a 45-week study[10] and a confirmatory 52-week study,[11] the neuronal, cerebrovascular, and nephritic toxicity of AlF_3 at 0.5 ppm Al^{3+} (= 1 ppm F) in the drinking water was significantly greater than with AlF_3 at 5 or 50 ppm Al^{3+}. Sodium fluoride at equivalent concentrations of 1, 10, and 100 ppm F produced analogous but qualitatively different changes in the brain and kidneys. Clearly, like evidence for unanticipated supra-linear (paradoxical) toxic effects of low-level ionizing radiation,[12] these findings have important potential implications for human health, in this case with respect to the presence of certain critical levels of aluminum in combination with fluoride.[13]

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REFERENCES AND NOTES

1 Schatz A, Schalscha EB, Schatz V. Soil organic matter as a natural chelating material, Part 2: The occurrence and importance of paradoxical concentration effects in biological systems. Compost Sci 1964;5(1):26-30.

Professor Schatz (Personal communication, June 30, 2002) provided the following explanation of why this review was written. In 1960, after three years of graduate work, his wife Vivian had completed all her course work and all examination requirements and had passed her Ph.D. written preliminary examination in the Department of Microbiology in the College of Medicine at the University of Pennsylvania. She then spent two additional years doing research on the inhibitory effects of adenine on the growth of the bacterium *Salmonella typhimurium* LT-2 in the presence of thiamine (Schatz V. Adaptive growth of Salmonella typhimurium on adenine. Naturwissenschaften 1964;51:121). During this work she found that adenine paradoxically affected the growth of *S. typhimurium*, with an acceleration by adenine at 5 and 20 μg/mL but a strong inhibition at 10 μg/mL in the presence of thiamine at concentrations ranging from only 0.005 to 0.1 μg/mL. Her dissertation committee, however, doubted the validity of the paradoxical effect and required her to devote six months of additional research to replicate and verify her findings. Despite her doing that, her committee refused to grant her a Ph.D. and awarded her only a master's degree after she had successfully completed five years of graduate study. Together with her husband, she subsequently published a report on those findings (Schatz A, Schatz V. Paradoxical effect of adenine on Salmonella typhimurium. Can J Microbiol 1965;11:1029-31).

From this sad story, one is reminded of the similar experience of Svante Arrhenius (1859-1927), who won the Nobel Prize in Chemistry in 1903 for his seminal research on the electrolytic dissociation of ionic compounds in water. At his defense of his doctoral dissertation on the "the electrical conductivity of salts in solution" at the University of Uppsala in 1884, he was awarded a fourth class degree (*non sine laude approbatur* – "approved without praise"), which prevented him from becoming a docent. However, Professor Sven Petersson at the University of Stockholm recognized the value and importance of what Arrhenius had proposed, and, despite its rejection at the time, it was not long before it became widely accepted as Arrhenius went on to achieve the fame he deserved. Unfortunately, this has not proved to be the case with the discovery of paradoxical effects, at least in biology.

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7 Machalinska A, Machoy-Mokrzynska A, Marlicz W, Stecewiecz I, Machalinski B. NaF-induced apoptosis in human bone marrow and cord blood CD34 positive cells. Fluoride 2001;34:258-63.

8 Nicolau J, Leite MF. In vitro and in vivo effects of fluoride solutions on human salivary amylase. Fluoride 2001;34:55-60.

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10 Isaacson RL, Varner JA, Jensen KF. Toxin-induced blood vessel inclusions caused by chronic administration of aluminum and sodium fluoride and their implications for dementia. Ann NY Acad Sci 1997;825:152-66.

Along with detailed abstracts of this and the following paper in ref. 11, our Managing Editor Bruce Spittle published a cogent discussion of this research in Fluoride 1998;31:89-99.

11 Varner, JA Jensen KF, Horvath W, Isaacson RL. Chronic administration of aluminum-fluoride or sodium-fluoride to rats in drinking water: alterations in neuronal and cerebrovascular integrity. Brain Res 1998;784:284-98.

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