Effects of co-administration of fluoride and aluminum on the metabolism of these two ions in the American Kestrel (*Falco sparverius*)

Jee Yan Chu

Department of Renewable Resources

Macdonald College of McGill University, Montreal

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ABSTRACT

In order to test the hypotheses: (1) that the digestive absorption of fluoride (F) is affected by the presence of aluminum (Al), and (2) that the distribution of F absorbed among organs/tissues is affected by Al, 36 American kestrels (Falco sparverius) were randomly divided into 6 groups of 6, and given oral doses daily for 30 days according to the following scheme: (1) deionized water only, (2) 30 mg/kg F^-, (3) 24 mg/kg F^-, (4) 24 mg/kg Al^{3+}, (5) 30 mg/kg F^- + 24 mg/kg Al^{3+}, (6) 24 mg/kg F^- + 24 mg/kg Al^{3+}. Excreta was collected every 24 hours between dosing. Femurae, kidneys, hearts, alimentary canals, skeletal muscle, and livers were obtained from all birds at the end of experiment. All samples were analyzed for F, Al, total phosphate (P) and calcium (Ca). F excretion was significantly higher in birds given 30 mg/kg F^- + 24 mg/kg Al^{3+} than in their counterparts which received the same amount of either F or Al alone ($p<0.05$). Excretion of Al was also elevated in all groups (except those only given water) from the level of excretion before the experiment commenced ($p<0.05$), despite the fact that 2 groups out of those 5 received F only. P and Ca contents of excreta were not affected by the oral dose. F contents in femurae from groups receiving both F and Al were significantly lower compared to those levels in those birds which were given F only ($p<0.05$). Significantly more Al was found in kidneys from the group receiving 30 mg/kg F^- + 24 mg/kg Al^{3+} than in those groups given only 1 of the 2 ions ($p<0.05$). Interactions were demonstrated between F and Al with respect to tissue absorption and distribution, and they appeared to correspond to the dosage of F.

RESUME

Afin de vérifier nos hypothèses selon lesquelles: (1) la présence d'aluminium (Al) dans le système digestif modifie l'absorption du fluor (F) et, (2) l'Al affecte la distribution du F absorbé par les tissus et les organes, 36 crécerelles d'Amérique (Falco sparverius) ont été assignées au hasard à 6 groupes de 6 oiseaux qui ont reçu des doses orales quotidiennes pendant 30 jours, selon le protocole suivant: (1) eau déionisée seulement, (2) 30 mg/kg de F^-, (3) 24 mg/kg de F^-, (4) 24 mg/kg d'Al^{3+}, (5) 30 mg/kg de F^- + 24 mg/kg d'Al^{3+}, (6) 24 mg/kg de F^- + 24 mg/kg d'Al^{3+}. Entre chaque scéance de traitement, les excreta étaient ramassés toutes les 24 heures. A la fin de l'expérience, les fémurs, les reins, les coeurs, les canaux alimentaires, le muscle squelettique et les foies de tous les oiseaux ont été prélevés. Le contenu en F, Al, phosphate total (P) et calcium (Ca) a été mesuré dans tous les échantillons. Les oiseaux qui ont reçu 30 mg/kg de F^- + 24 mg/kg d'Al^{3+} excrétaient significativement plus de F que ceux qui ont été traités avec la même dose de F et d'Al administrés séparément ($p<0.05$). Même si 2 groupes d'oiseaux sur 5 ont reçu seulement du F, tous les groupes d'oiseaux excrétaient plus d'Al que pendant la période pré-traitement ($p<0.05$), à l'exception des oiseaux témoins traités à l'eau déionisée seulement. Les doses orales n'ont pas changé les contenus en P et Ca des excréta. Dans les groupes traités au F et à l'Al simultanément, les fémurs contenaient significativement moins de F que ceux des groupes traités au F seulement ($p<0.05$). Les reins des oiseaux traités avec 30 mg/kg de F^- + 24 mg/kg d'Al^{3+} contenaient significativement plus d'Al que ceux des

groupes traités avec l'un des 2 ions seulement ($p<0.05$). Il est clair qu'il existe un synergisme entre le F et l'Al lors de l'absorption et de la distribution de ces ions dans les tissus et les organes, et il semble être relié à la dose de F.

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PREFACE

This thesis deals with the absorption ard excretion of fluoride under the influence of aluminum in the body of the American kestrel (Falco sparverius). The first chapter of the thesis is a collection of the findings of most of the research that has been done on fluoride and aluminum on various organisms, with regards to their effects on various body functions, as well as their chemical properties. Unfortunately, the combined action of fluoride and aluminum has been studied by few scientists, and their findings have been included in this literature review to the best of my knowledge. The scientific names of certain animals in the studies cited, e.g. albino rats, mice and rabbits, were not supplied by the original authors, and so they have been omitted from the text and tables of this thesis.

The second chapter of the thesis deals with the experiment conducted during the period 1988-1990 to test the synergism between fluoride and aluminum on the absorption and elimination of these ions from the body of the American kestrel. There is a brief review of the background literature upon which the rationale of the experiment was developed, the methodology, results and discussions, and a conclusion based on the findings.

The two chapters of this thesis are intended to be submitted to appropriate journals, the first as a review paper and the second as an original research paper. Literature search, experimental planning, data collection, chemical and statistical analyses were conducted independently by the author under the supervision of Dr. David M. Bird. The tables and references follow the sections in which they

are cited. A few tables with detailed statistical data are put in the Appendix for reference purposes.

I hope the results from this study can add to the vast pool of knowledge on fluoride and aluminum metabolism to further the understanding on this subject, and lead to more research on the phenomenon of synergism.

REGULATION ON THESIS PRESENTATION

The following is included in accordance with the regulations of the Faculty of Graduate Studies and Research of McGill University:

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While the inclusion of manuscripts co-authored by the Candidate and others is not prohibited for a test period, the Candidate is warned to make an explicit statement on who contributed to such work and to what extent, and supervisors and others will have to bear witness to the accuracy of such claims before the Oral Committee. It should also be noted that the task of the External Examiner is much more difficult in such cases."

CHAPTER 1

PHYSIOLOGICAL EFFECTS OF FLUORIDE AND ALUMINUM: A REVIEW

I. Introduction

Fluoride and aluminum are among the earth's most common substances. Great controversy has arisen from their roles and impacts on living organisms. Dentists, together with municipalities, have constantly been pushing for fluoridation of drinking water as a means to combat caries; aluminum has been linked to the occurrence of Alzheimer's disease, and so on. Therefore, there is a need for greater understanding of these two substances before we can decide how harmful or beneficial they are to living organisms. Some of the most recent findings concerning these two substances are reported below.

II. Prevalence of Fluoride (F^-) in the Environment and Its Effects on Living Organisms

1. *Major Forms and Sources of Fluoride in the Environment*

Since the fluorine atom is very reactive, with only 7 electrons in its outermost electron shell and a relatively small radius, it is almost always ionized to form the fluoride ion which carries a single negative charge (F^-). The fluoride ion can also form a covalent bond with hydrogen to become hydrogen fluoride (HF) which is highly volatile and corrosive.

Industrial origins of gaseous fluoride in Canada include primary aluminum production, phosphate fertilizer and elemental phosphorus plants, primary iron and steel production, power generation, solid waste incineration, and various other commercial and industrial activities (Environment Canada, 1976). The gaseous emissions may later be dissolved in the water bodies near the points of atmospheric discharge and become aqueous fluoride ions.

There have not been as many data on the volumes and concentrations of fluoride-containing wastes released into lakes, rivers and oceans, as gaseous emission is the predominant mode of release of fluoride into the environment (Rose and Marier, 1977). Effluents and overflows from limed settling ponds may have contributed to aqueous fluoride levels. Nevertheless, the following industries have been noted for their release of large quantities of fluoride into waterways: aluminum industry, phosphate fertilizer, steel production, and uranium tetra- and hexa-fluoride synthesis, to name a few (Rose and Marier, 1977).

Fluoride-containing solid wastes from the above industries may also be used as landfill or buried. The fluoride therein will probably enter the surface and groundwater by means of leaching.

Although fluoride has been considered ubiquitous and present in almost all natural substances, an atmospheric level of soluble fluoride above 0.05 $\mu g/m^3$ can be attributed to anthropogenic sources (Rose and Marier, 1977). Since fluoride can readily circulate among the three states of matter, it is often difficult to pin-point the exact origin of a particularly high fluoride level.

With the fluoridation of municipal drinking water and the increasingly popular consumption of mineral water which may sometimes be rich in fluoride, even humans who live away from major fluoride-emitting facilities are increasingly subjected to chronic exposure to this ion in the daily diet.

Numerous incidents have been reported where fluoride pollution resulted in ill health of factory workers and people living in the vicinity of those facilities (e.g. Yang et al., 1987). Observations in both human subjects (Yang et al., 1987) and laboratory animals such as guinea pigs (Bourbon et al., 1984) have shown correlations between the degree of fluoride exposure and the levels of fluoride in blood plasma and urine even on a short-term basis. Investigators hope to be able to make use of such correlations to detect fluoride accumulation at an early stage in order to prevent massive deposition of fluoride in the hard tissue.

Fluoride has been repeatedly reported to accumulate in wildlife living in the vicinity of fluoride-emitting facilities (Dabkowska and Machoy, 1989), most notably at high levels in the bones of predatory birds (Seel and Thomson, 1984), and perhaps in carnivorous mammals as well, which have the chance of consuming prey items highly contaminated with this substance (Thomson, 1987). The effect of fluoride intoxication can therefore be magnified by bioaccumulation along the food chain. Skeletal fluorosis may result in the loss of mobility and even a mild condition of such can greatly increase the chance of the afflicted animal falling prey to a predator in the wild.

2. *Acute Toxicity of Fluoride*

Whether in humans or other animals, an overdose of fluoride can be fatal. The lethal dose of sodium fluoride in humans is about 70 - 140 mg/kg body weight (Mitchell, 1983). This substance was widely used in the first half of this century in rodenticides and

insecticides. Numerous cases of fatal poisoning as a result of the ingestion of such chemicals have been reported (Poklis and Mackell, 1989).

The chemical sodium fluoride, a common form for the anion, is rapidly and completely absorbed from the stomach, the bioavailability being almost 100% (Mitchell, 1983). While in the stomach, fluoride reacts with hydrochloric acid to form the highly corrosive hydrofluoric acid (or hydrogen fluoride). It damages the gastric mucosa, causing gastroenteritis. After being absorbed into the bloodstream, fluoride enters the bone by replacing ions associated with hydroxyapatite crystals to form fluorohydroxyapatite (Gosselin et al., 1976). This accounts for 95% of the total body load of fluoride. The renal clearance is 48 - 147 ml/min (Gosselin et al., 1976). The body can remove over 20% of an ingested dose through the kidneys within 4 hours.

The symptoms of acute fluoride poisoning include vomiting, abdominal pain, diarrhea, convulsions, generalized and muscular weakness, collapse, dyspnea, paresis, difficulty in articulation, thirst, weakness of pulse, disturbed color vision, and loss of consciousness (Poklis and Mackell, 1989).

Human volunteers given a single dose of 20 ml sodium fluoride solution containing 20 mg fluoride (53 mmol/L) displayed petechiae erosion in the body of the stomach and changes in the antrum (Spak et al., 1989). The surface epithelium, gastric pits and superficial stroma of the gastric mucosa were affected. Such medical doses are often given to osteoporotic patients. Among the subjects in the above

study, only 4 out of 12 developed nausea while all subjects showed symptoms of gastric mucosal damage. Thus nausea is not a suitable indicator for the first sign of fluoride toxicity, which is, in practice, often used as such by physicians (Spak et al., 1989).

3. *Chronic Effects of Fluoride in Hard Tissues*

Skeletal fluorosis and bone pain have resulted from long-term consumption of mineral water containing 8.5 mg/L fluoride. The intake of 20 - 30 mg fluoride daily can also result in increased bone mineral density and serum osteocalcin, the latter being a sensitive and specific marker of osteoblastic bone formation (Meunier et al., 1989). Thus fluoride is often employed in treating patients suffering from osteoporosis to stimulate bone formation.

The above observations were supported by another report from Dandona et al. (1989), who, however, pointed out that whether the bone produced in response to fluoride supplement was architecturally and mechanically functional remained to be tested. This point was also put forward by Brown (1989), who stated that the bone formed during the administration of fluoride was poorly mineralized unless calcium and vitamin D were administered concurrently. Furthermore, the fluoride treatment often has to be discontinued before the end of a trial period owing to negative side effects such as nausea, gastrointestinal hemorrhage, tendonitis and stress fractures (Hodsman and Drost, 1989).

On the other hand, positive evidence supporting the use of fluoride therapy against osteoporosis and bone weakness and fractures

was provided by Farley et al. (1989) and Mackie et al.(1989). Long-term fluoride therapy was recommended as a measure for increasing bone mass and reducing the risk of spinal fractures.

Fluoride was demonstrated to facilitate osteoblast proliferation and collagen formation at the concentration of 10^{-6} - 10^{-5} M both *in vitro* and *in vivo*, but the same concentration of fluoride suppressed marrow stromal cell proliferation (Kidder et al., 1989). Thus the osteogenic effect of fluoride is a consequence of its action on the differentiated osteoblasts rather than on the precursor cell population.

In an *in vivo* study on osteoclasts resorbing bone above the erupting first molar tooth in Sprague-Dawley rats, a single high dose of fluoride (60 mg NaF/kg) was given. The results showed a significant reduction in the number of actively resorbing osteoclasts in the above tissue at 2 and 6 hours after injection, and an increase in the number of inactive osteoclasts detached from bone surfaces, where they normally belonged (Lindskog et al., 1989).

Although low levels of fluoride have previously been shown to induce bone growth, calves born to fluoride-loaded cows were found to display symptoms such as atrophy of osteoblasts, osteopenia, atrophy of bone marrow cells and bone marrow fat, severely stunted growth, brown mottling of bone and enamel of teeth, and severe retardation of cartilage cell differentiation (Maylin et al., 1987). Such observations were attributed to chronic intoxication of the cows by feeding on fluoride-contaminated feed, which also caused dental fluorosis and decrease in milk yield to those cattle. The fluoride

contained in the cows' bodies was transferred to the fetuses *in utero*, therefore aggravating the toxic effects of fluoride at the early stage of cellular differentiation in the fetuses.

While detrimental effects of fluoride on bone are frequently reported for mammals, avian species seem to be more tolerant of fluoride in their bodies (Rose and Marier, 1977). They may even obtain beneficial results from drinking fluoridated water (Merkley, 1981), notably the strengthening of long bones in caged hens, without affecting their ability to produce eggs.

By supplementing the diet of Japanese quail (*Coturnix japonica*) with 0.075% fluoride, growth and calcium retention were enhanced in formerly calcium-deficient individuals (Chan et al., 1973). The birds' bone integrity and strength were little affected by the additional fluoride.

It is possible for female birds to alleviate their body load of fluoride through deposition in the egg shell (Carrière et al., 1987). This may partly explain the apparent resistance to fluoride intoxication, as most of the birds used in such experiments were females active in egg-laying.

The presence of fluoride in rabbit collagen disrupts the normal amino acid composition, especially by reducing the proportion of hydroxylated proline and lysine residues, which are responsible for forming cross-linkages between collagen fibers (Susheela and Sharma, 1982). The animals were treated with 10 mg/kg NaF daily for their study. When various osseous and nonosseous tissues from rabbits given 50 mg NaF/kg daily were examined, the uptake of ^{14}C-proline, an

indicator of collagen synthesis, was found to be significantly reduced in all types of tissues. The cross-linking agents in collagen were also diminished in quantity at the same dosage of fluoride. The rate of collagen catabolic activity was also increased after fluoride ingestion. All of the above suggest that excessive fluoride ingestion affects the normal rate of formation and structure of collagen. Similar effects were also observed in female rats given daily dosages of fluoride (Bély et al., 1988).

Studies on the effects of dietary fluoride on teeth have varying conclusions. The ingestion of 3.5 mg F^-/kg body weight by Thone and Marthod ewes for 3 months at various stages of their growth resulted in damage to the molars, a characteristic of dental fluorosis in sheep (Milhaud et al., 1987). On the other hand, Sprague-Dawley rats given up to 1 ppm fluoride in their drinking water showed better resistance to induced caries by the inoculation of Streptococcus mutans 6715 (Beiraghi et al., 1989).

The difference in the pattern of wearing of the teeth between these two kinds of animals, the dosage of fluoride given, and the duration of experiment can contribute to the apparent discrepancies. It is possible that a low level of dietary fluoride (1 ppm or less) can be effective in preventing caries by interrupting the enzymatic pathways in micro-organisms (at a concentration non-toxic to higher animals), or by enhancing remineralization of the teeth. Such arguments have often been put forth by parties in favour of fluoridation of drinking water. However, the low level of fluoride at which caries reduction is achieved is often exceeded by the total

daily intake. Fluoride, which occurs commonly in most foods, may be present in sufficient quantities to make fluoridation redundant or even harmful to human health.

In summary, skeletal fluorosis, resulting from chronic exposure to fluoride, occurs more often in older animals (Wix and Mohamedally, 1980), in individuals which are undergoing active bone and/or tooth formation at the time of exposure, and in animals with poor nutritional status (Rose and Marier, 1977). The chemical form and solubility of fluoride and the presence of other components in the diet can also influence its bioavailability. The health of the kidney determines the rate at which excess fluoride is excreted and thus the amount of fluoride available for affecting body cells (Franke, 1989). Furthermore, several dose-dependent sex differences were found in the response pattern of long bones to excessive fluoride (Mittal et al., 1985).

4. *Chronic Effects of Fluoride in Soft Tissues*

The results of recent studies on the effects of long-term exposure to fluoride are summarized in Table 1 (p. 50). For earlier references, see the review by Monsour and Kruger (1985).

Generally speaking, fluoride exerts its physiological effects on soft tissues through several mechanisms: (1) by altering (in most cases, inhibiting) the activities of various enzymes, especially those responsible for hydrolysis, which control a lot of the reactions involved in the metabolism of proteins, carbohydrates and lipids (Shashi et al., 1989); (2) by disturbing calcium and phosphate

balance, and indirectly affecting the production of parathyroid hormone (Monsour and Kruger, 1985), and by upsetting the balance of trace elements such as zinc and manganese (Singh, 1984); (3) in the case of the stomach, by the liberation of protons to form the extremely corrosive hydrofluoric acid (Shayiq et al., 1984).

Energy deficiency may result from the inhibition of enzymes involved in cellular respiration, loss of the ability to synthesize ATP, and disintegration of mitochondrial membranes as a result of inhibition of the synthesis of lipid and protein by fluoride. As a consequence, mitochondria are enlarged to compensate for the lost function and the smooth endoplasmic reticulum (SER) is enlarged to speed up the detoxification process (Zhan and Huo, 1988).

Overall, inhibition of the activities of a wide range of enzymes by fluoride has broad implications, as it affects the normal functioning of virtually every organ over the long term.

5. *Effects of Fluoride at the Molecular Level*

To date, very few studies have examined the possible mutagenic effects of fluoride, particularly under chronic exposure. Such genotoxicity studies involving chronic treatment are mostly inconclusive (Kram et al., 1978; Martin et al., 1979), using only one single dosage of fluoride. In a more recent and comprehensive study on the mutagenicity of fluoride (Li et al., 1989), there was no apparent increase in the frequency of sister-chromatid exchange, an indicator of mutagenic activities, in cells isolated from male Chinese hamsters given 0, 1, 10, 50 and 75 ppm fluoridated drinking water for

24 weeks. Neither in vivo nor in vitro studies revealed any genotoxic effect of fluoride in the above animal (Li et al., 1987).

6. *Effects of Other Substances on Fluoride Toxicity*

Since it is impossible to find fluoride existing by itself in the natural world, it is worthwhile to examine what kind of influence other ions have on the physiological effects of fluoride in living organisms.

Among such elements, one that attracts most attention is aluminum (Shore et al., 1985; Spencer et al., 1985; Hahn and Guenter, 1986; Kessabi et al., 1988; Kawase et al., 1989; Walton, 1989). By far, aluminum is the strongest complexing agent for fluoride (Brudevold et al., 1972). In fact, aluminum was shown to be an effective treatment for fluorosis in sheep and cattle because of its ability to inhibit fluoride absorption (Underwood, 1971). By the same principle, fluoride has been tested for its ability to lower the level of aluminum in the body of patients suffering from Alzheimer's disease, for which aluminum is suspected to be a cause (Shore et al., 1985). Other practical uses of aluminum include the co-administration of the antacid aluminum hydroxide during fluoride therapy for osteoporosis in order to counteract gastric discomfort caused by large doses of fluoride (Spencer et al., 1985).

That aluminum is capable of decreasing the digestive absorption of fluoride was demonstrated in male Sardy sheep (Kessabi et al., 1988), among other farm and laboratory animals. The levels of fluoride in serum, bone, teeth and urine were significantly reduced by

the concurrent administration of aluminum in the diet.

High levels of fluoride (1300 ppm) in drinking water can suppress feed intake and egg production of laying Single Comb White Leghorn hens (Gallus domesticus) (Hahn and Guenter, 1986). Such effects were alleviated by the use of dietary aluminum at a ratio of 1 part fluoride to 0.8 part aluminum. The level of fluoride accumulation in tissues such as bone and serum, and organs like liver and kidney were also reduced by the presence of aluminum.

Besides aluminum, chloride can also influence the uptake of fluoride. It was shown, using laboratory rats, that fluoride uptake and retention was greatest at low levels of dietary chloride (Cerklewski, 1986; Cerklewski et al., 1986). However, high chloride intake did not inhibit fluoride absorption.

Despite the effects fluoride has on the normal balance of zinc and iron in the body (Singh, 1984), these two elements did not exhibit any effect on skeletal uptake of fluoride (Cerklewski and Ridlington, 1985), implying that fluoride is not in direct competition with zinc or iron in its absorption into the body.

Another ion the metabolism of which is closely related to fluoride is calcium, mostly owing to the fact that both are heavily involved in mineralization of osseous tissues. Forsyth et al. (1972) revealed an antagonistic effect between the uptake of fluoride and calcium in the diet of Yorkshire pigs. When calcium level was low in the feed, more fluoride accumulated in the bone, its prime target.

Similar results were obtained in a study on male Sprague-Dawley rats, in which femoral fluoride concentration was significantly lower

in rats fed both fluoride and calcium than in those fed fluoride alone (Ericsson et al., 1986).

Such antagonistic effects between calcium and fluoride were also shown in the process of amelogenesis in the hamster tooth germ *in vitro* (Bronckers et al., 1989). The inhibition of mineralization of enamel matrix by the presence of fluoride in the culture medium was aggravated by lowering the level of calcium in the medium, and was counteracted by high calcium levels.

III. The General Chemistry and Toxicity of Aluminum

1. *Aquatic Chemistry*

Aluminum (Al) is the most prevalent metal and the third most abundant element in the earth's crust. It is a normal constituent of vegetable and animal tissues, and is present in raw untreated water (Wills and Savory, 1989). As a result of increasing acidification of lakes and inland waters, the elevated mobilization of aluminum from the edaphic to the aquatic environment has become a serious problem for biological communities, particularly those inhabiting aquatic systems (Driscoll et al., 1980).

The chemistry of aluminum in water is essentially the chemistry of aluminum hydroxide, which possesses the following three important properties:

a) It is readily amphoteric.

b) It forms complex ions with other substances in the water.

c) It tends to polymerize.

Therefore, the form and concentration of aluminum in water depends on the pH and the nature of substances dissolved in the receiving waters, and, to a lesser extent, on the temperature and the duration of exposure to the water (Burrows, 1977).

In the dilute solution commonly found in natural waters, aluminum can exist in the following forms:

a) Monomeric aluminum hydrates -- These are formed by dissolving an aluminum salt of a noncomplexing acid, e.g. aluminum perchlorate, in pure water. The resulting solution is acidic. By successive hydrolysis of the aluminum ion, it can become univalent, colloidal

aluminum hydroxide, and finally the aluminate anion.

$$Al^{3+} + H_2O \rightleftharpoons AlOH^{2+} + H^+$$

$$AlOH^{2+} + H_2O \rightleftharpoons Al(OH)_2^+ + H^+$$

$$Al(OH)_2^+ + H_2O \rightleftharpoons Al(OH)_3 + H^+$$

$$Al(OH)_3 + H_2O \rightleftharpoons Al(OH)_4^- + H^+$$

b) Polymeric aluminum hydrates -- They are formed by the addition of hydroxide to an aluminum solution. The strong tendency for dissolved aluminum to form dimeric, oligomeric, and polymeric species is enhanced as the ratio of aluminum-bound hydroxide to aluminum increases from 0 to 3. The process of polymerization occurs gradually over time. Thus, an organism in contact with a freshly prepared solution of aluminum may be exposed to a spectrum of hydrated aluminum species with different toxic potentials. An aged solution may exert a different order of toxicity due to lower concentrations of monomers and absence of intermediate polymers.

c) Complex ions -- These occur mainly in the presence of fluoride and/or sulphate ions, and in various forms. The presence of complexing ligands such as fluoride and sulphate in water increases the amount of dissolved aluminum in equilibrium with solid aluminum hydroxide. In other words, their presence enhances the solubility of aluminum hydroxide. Other substances such as dissolved silica, phosphoric acid, ethylenediamine tetraacetic acid (EDTA), nitrilotriacetic acid (NTA), and sodium tripolyphosphate (STPP) can also form complex ions with aluminum.

d) Natural organic complexes -- These are produced when aluminum combines with humic and fulvic acids from decaying leaf litter and a

mixture of polyphenols, reducing sugars, and organic acids present in forest canopy drip (Burrows, 1977).

2. *Environmental Prevalence*

Aluminum in the metallic form is used extensively for both industrial and domestic purposes, and a variety of aluminum salts are widely used in food, fluid, and medication.

Aluminum is normally found in all foods (Sorensen et al., 1974). The natural aluminum content of food varies mainly according to the soil on which that food has been grown. It is also possible for aluminum to be leached from cooking utensils or containers when preparing or storing acidic food.

Aluminum salts are widely employed as food additives where they fulfill a number of functions (Lione, 1983). Sodium aluminum phosphates are the most commonly used of 6 aluminum salts that have been approved as food additives. The acidic forms of this salt are used as leavening agents in reaction with sodium bicarbonate in cake mixes and self-rising flour. The alkaline forms are used as emulsifying agents in processed cheeses, and for providing soft texture, storage capability, and easy melting characteristics. Aluminum silicates are used as anticaking agents to facilitate the flow characteristics of dry powdered products.

Aluminum is normally present in raw water, with usually low concentrations in ground waters and higher levels in surface waters (Miller et al., 1984). This is probably due to the occurrence of acid rain in these areas. Aluminum is among the metals that are mobilized

from soil and rock in acid rain-sensitive areas with a consequent high concentration in the surface run-off (Cronan and Schofield, 1979). Areas most susceptible to acid rain are those with little or no soil overlying granite or gneiss bedrock. It is from these areas with poorly buffered aquatic ecosystems that the lethal effects of acid rain have been reported, involving the disappearance of fish and a variety of other forms of animal and plant life.

In domestic tap water, aluminum has been added as a flocculant during the purification process to remove organic materials present in surface water that might affect the color or taste of the finished product. The usual flocculant is aluminum sulphate.

As medication, aluminum in the form of the hydroxide, phosphate and potassium sulphate salts is used in minute amounts in the preparation of toxoid vaccines and allergens (Lione, 1985). Aluminum salts, particularly aluminum hydroxide, are commonly used as antacid drugs. They are also used for reducing the gastric irritation caused by aspirin, by means of the buffering capacity of aluminum hydroxide.

Aluminum absorption from aluminum-containing phosphate-binding antacids is also a major source of aluminum loading in patients on dialysis, besides the uptake of aluminum from untreated municipal water in the dialysate. One example of such phosphate-binding agents is sucralfate, containing 21% aluminum by weight. It is widely used for treating peptic ulcer disease and gastritis (Robertson et al., 1989). It does not alter plasma aluminum levels in normal subjects, but aluminum can dissociate from sucralfate at pH 6 or lower and become bioavailable. This can cause aluminum toxicity in patients

with renal failure, who cannot excrete the excess aluminum through the kidney.

In aquatic systems, soluble aluminum occurs at much higher levels in acidic, highly saline, hot, or moving waters. Thus, other than acid rain, acid mine drainage as practised in coal mining has also been identified as a major cause of high aluminum levels in water bodies (Burrows, 1977).

3. *Toxicity in Aquatic Organisms*

Aluminum occurs in aquatic ecosystems in a wide array of forms, depending upon the environmental conditions of the water body such as pH, salinity, ionic strength, temperature, flow rate, turbidity, amount of organic matter, and so on. Therefore each toxicity test of aluminum on aquatic life in the natural environment can be considered unique in itself. However, efforts have been made to summarize and compare most of the acute toxicity studies of aluminum in aquatic systems (Burrows, 1977), with the experimental conditions specified.

More recent aluminum toxicity tests done on brook trout fry (Salvelinus fontinalis, Mitchill) and white sucker fry (Catostomus commersoni, Lacepede) (Driscoll et al., 1980), Atlantic salmon fry (Salmo salar) (Birchall et al., 1989), and rainbow trout (S. gairdneri) (Playle et al., 1989) also indicated that pH, presence of calcium, fluoride, and silicon play a major role in influencing aluminum toxicity in these animals. The presence of aluminum in the waters to which the above organisms were exposed led to deaths, gill damage, upset of plasma sodium and chloride levels, red cell swelling,

hemoconcentration, reduced blood oxygen tension, elevated blood carbon dioxide tension and increased blood lactate in these fishes.

Other aquatic life, including amphibians, crustaceans, other invertebrates and even protozoans and bacteria have also been tested for their resistance to aluminum toxicity. The results are summarized by Burrows (1977). Toxic effects of aluminum to these organisms range from activity inhibition to death.

4. *Toxicity in Mammals, Including Humans*

Absorption of aluminum in mammals usually takes place through gastrointestinal absorption following oral intake of food and fluids. This is of critical importance in organisms with chronic renal failure. Other ways of aluminum uptake are through nasal mucosa and lungs, but they have not been as thoroughly studied. Such uptake pathways appear to depend on both the particle size and the chemical state of aluminum present in the substance inhaled.

The gastrointestinal tract is normally relatively impermeable to aluminum, which therefore has a very low fractional absorption rate in organisms with normal renal functions, since most of the aluminum absorbed after oral intake would be rapidly excreted through urine production.

In a gut perfusion experiment conducted on rats in vivo, the absorption of aluminum was found to take place passively in the duodenum, proportional to the aluminum concentration in the perfusate (Rumbelow et al., 1989). The absorbed aluminum was subsequently found to be sequestered in the duodenal mucosa, and it is possible that

aluminum is held in those cells until they are shed, thereby excreting the aluminum at the same time through the colon.

Several toxicokinetic studies of aluminum were done on Wistar rats (Burnatowska-Hledin et al., 1985; Olaizola et al., 1989) and New Zealand white rabbits (Bertholf et al., 1989; Yokel and McNamara, 1989) with respect to age, degree of aluminum overload, either orally or intraperitoneally, and chronic renal failure. Younger rats showed a higher rate for both aluminum absorption and excretion. Chronic renal failure caused rats to show a higher serum and urinary aluminum level increase than normal rats (Olaizola et al., 1989). Aluminum was found to accumulate after 4 hours of intravenous injection, in the bile, kidney, liver, lung, serum, and spleen of rabbits (Yokel and McNamara, 1989). The persistence of aluminum in these tissues (with half-lives from 2 to 3 days in kidney to 113 days in spleen of rabbit) may serve as a reservoir for continuous aluminum exposure for sensitive organs such as the central nervous system.

A significant correlation was found between bone aluminum and serum and dialysate aluminum levels in human subjects at age ranging from 30 to 83 years old, undergoing continuous ambulatory peritoneal dialysis for 1 to 87 months due to end-stage renal failure (Joffe et al., 1989).

The species of aluminum in the gastrointestinal tract seems to be a critical factor in the absorption process, since aluminum is capable of forming complexes with a series of organic and inorganic substances, which in turn affect the bioavailability of aluminum in the intestines.

Evidence suggests that the intestinal absorption of aluminum is linked with the 2-stage calcium transport mechanism (Provan and Yokel, 1988), with a non-saturable component and a vitamin D-dependent saturable component (Adler and Berlyne, 1985).

In humans, 80% of aluminum in serum is nondiffusible and protein-bound, while the remaining 20% is diffusible (Wills and Savory, 1989). Citrate is found to be the dominant, small-molecular binder of aluminum in plasma (Martin, 1986), whereas the protein-bound portion of aluminum in plasma is associated with albumin or transferrin (King et al., 1982; Trapp, 1983).

The inhalation of dust heavily contaminated with aluminum has been recognized as an industrial cause of pulmonary fibrosis although the mechanism is not clear (McLaughlin et al., 1962). Less commonly, pulmonary granulomatosis and desquamative interstitial pneumonia have also been linked with industrial exposure to aluminum dust (Herbert et al., 1982; DeVuyst et al., 1987).

Nonindustrial exposure to aluminum has been implicated in 2 specific neurological disorders, amyotrophic lateral sclerosis and Parkinsonism, in 3 geographical locations in the western Pacific (Perl et al., 1982) in humans with normal renal function. The soil and drinking water from areas with a high incidence of these neurological disorders contained a high concentration of aluminum and low concentrations of calcium and magnesium. It was suggested that chronic deficiency of the latter two elements might cause secondary hyperparathyroidism, resulting in an increased intestinal absorption of aluminum and its deposition in the central nervous system.

It was found that the presence of aluminum could cause premature onset of deterioration in fully differentiated neuroblastoma clone cells, but it did not affect the normal pattern of differentiation of developing neuroblastoma cells. Deterioration of these neurons included depolarization of cell surface potentials, loss of excitable properties, and structural deformation (Roll et al., 1989).

In a study using another cell line of neuroblastoma clone cells, Shea et al. (1989) reported the appearance of whorls of intermediate filaments in neuronal perikarya in both differentiated and undifferentiated cells at the ultrastructural level, as a result of treatment with aluminum salts, when those filaments should normally only occur within axonal neurites. This is one of the tell-tale symptoms in a variety of degenerative disease states such as Alzheimer's disease (Cork et al., 1986), amyotrophic lateral sclerosis (Munoz et al., 1988), and Parkinson's disease (Forno et al., 1986).

The mechanism of action of aluminum in neurotoxicity may be through direct binding to critical sites, such as phosphate groups in deoxyribonucleic acids (DNA), proteins and lipids, or through an intracellular mediator, probably cyclic AMP and cyclic GMP, among others (Jope, 1986).

The accumulation of aluminum in the serum and tissues of patients with chronic renal failure and its subsequent toxic effects have long been established (Wills and Savory, 1989). Often these people absorb large quantities of aluminum through long-term hemodialysis using untreated water with traces of aluminum, coupled with the inability of their kidneys to excrete the excess aluminum. The consequent

phenomena of increased body burden of aluminum include dialysis encephalopathy, osteomalacic dialysis osteodystrophy, and microcytic hypochromic anemia.

The symptoms of patients with dialysis encephalopathy are speech disorders, followed by the development of dementia, convulsions, and myoclonus (Alfrey et al., 1972). The mechanism by which aluminum acts in the pathogenesis of the dialysis encephalopathy syndrome has not been clearly defined, but a detailed discussion of the various possibilities is provided by Wills and Savory (1989).

Dialysis osteodystrophy is a term used for the progressive metabolic bone diseases seen in uremic patients on long-term treatment with intermittent hemodialysis. The bone diseases include osteitis fibrosa, osteomalacia, osteosclerosis, and osteopenia, consequences of disturbances in calcium and phosphorus homeostasis, and metastic and soft-tissue calcification. Osteomalacia is often associated with bone fractures which may be unresponsive to treatment with vitamin D, and is caused by phosphate deficiency. Aluminum can bind dietary phosphorus and block its absorption by the intestines (Wills and Savory, 1989).

The anemia caused by aluminum in patients with chronic renal failure is non-iron-deficient, microcytic, hypochromic, and responds to the use of a low-aluminum dialysate (O'Hare and Murnaghan, 1982; Kaiser and Schwartz, 1985). It is considered a specific symptom of aluminum toxicity, probably a result of the inhibitory effect of aluminum on hemoglobin synthesis (Altmann et al., 1988), among other possible mechanisms. The binding of aluminum with transferrin as a

means of plasma transport of the ion may also be a factor in the pathogenesis of aluminum-induced anemia despite the presence of adequate iron.

Even in patients on hemodialysis without any apparent symptoms, certain abnormalities were found in the myocardium owing to aluminum accumulation (London et al., 1989). Of 50 such patients, 36 were found to have stainable cortical bone aluminum (SCBA). The other 14 patients without SCBA had lower left ventricular mass, increased rate of circumferential fiber shortening and higher mitral E-F slope (an index of ventricular filling) than people not on dialysis.

The motor activity of Swiss-Webster mice was tested as a measurement of aluminum toxicity (Golub et al., 1989). Despite the absence of any neurotoxic sign after 5 weeks of dietary aluminum treatment, the overall activity level in the high-dose group (1000 ug/g diet) was significantly lower than control group, with the vertical movement more seriously affected than horizontal movement, and both being less frequent than in the control group.

The effect of orally administered aluminum on the neuromotor abilities of newborn Wistar rats was tested with respect to grasping reflex, negative geotaxis, suspension and locomotor coordination. With the exception of the grasping reflex, all the other tests revealed aluminum-induced poorer response, especially in the high-dose group (300 mg Al/kg body weight/day) (Bernuzzi et al., 1989). Another effect of ingesting aluminum included delayed growth in a dose-dependent manner.

In New Zealand white rabbits, the acquisition and retention of a conditioned reflex, nictitating membrane extension, was significantly affected after 20 subcutaneous injections of aluminum lactate at 200 μmole/kg body weight over a period of 4 weeks (Yokel, 1989). A difference was also observed between young rabbits (1-2 months old) and aged rabbits (2-3 years old) in that the behavioral responses of aged rabbits were more seriously affected by the aluminum injections than those of young ones. Renal function, as measured by creatinine clearance, was also more impaired in aged rabbits.

As for hypercalcemic dialysis patients, two-thirds of those studied had low-turnover osteodystrophy (LTO), predominantly osteomalacia, a disorder significantly more frequent than among non-hypercalcemic dialysis patients (Piraino et al., 1989). Those hypercalcemic patients with LTO had higher levels of surface bone aluminum than nonhypercalcemic patients with LTO. The aluminum-related bone disease also increased the mortality and disability of hypercalcemic patients.

Aluminum overload, as a consequence of aluminum contamination in albumin solutions used as replacement fluid in plasma exchange, can cause osteoporosis, aluminum deposits in bone, and low bone formation in patients with normal renal function (Mousson et al., 1989).

Aluminum was demonstrated to be able to deactivate the enzymes glucose-6-phosphate dehydrogenase (G6PD) isozymes I and II, isolated from pig and human brains (Cho and Joshi, 1989). The inhibition could be prevented by preincubation of the aluminum salt with citrate, $NADP^+$, EDTA, sodium fluoride, adenosine triphosphate (ATP), and

apotransferrin, and reversed by citrate, sodium fluoride, and apotransferrin only. The inhibition of G6PD activity by low levels of aluminum shown in that study suggested a role for aluminum in energy metabolism of the brain, thus constituting part of its neurotoxicity.

5. *Toxicity in Birds*

In principle, most of the toxic effects of aluminum occurring in mammals can also be found in birds. There are many similarities in basic modes of metabolism, especially with regard to the absorption of food in the digestive tract between the two groups of vertebrates. Discrepancies, however, may exist during periods of special physiological needs, e.g. during egg-laying. Therefore, a lot of the toxicological studies of the effects of aluminum on birds have focused on the mineral balance in egg-laying birds (e.g. Lipstein and Hurwitz, 1981; Miles and Rossi, 1985; Hussein et al., 1988; 1989).

Aluminum of up to 0.15% in the form of aluminum sulphate in the diet was found to reduce food intake, egg production, body weight, tibia breaking strength and plasma inorganic phosphorus in laying Hy-Line W-36 hens after 28 days (Hussein et al., 1989). Similar results were also obtained from Japanese quail hens which were fed 0.30% aluminum. Feed intake and egg production by those birds were reduced as a result.

The reduction in plasma phosphorus of laying hens owing to dietary aluminum also corresponds to findings in humans fed dietary aluminum (Spencer et al., 1982). The phosphorus metabolism of human subjects was negatively affected after small doses of aluminum-

containing antacids, indicating a partial resemblance in response to aluminum toxicity between avian and mammalian species.

6. *Effects of Aluminum at the Molecular Level*

Recently the studies on aluminum toxicity have also investigated its effects on the genetic material. Aluminum was found to be an effective precipitant of chromatin isolated from rat liver and brain by compacting the chromatin through the high charge density and low ionic radius of aluminum (Walker et al., 1989). In general, the more compact the chromatin fiber is, the less accessible it is to exogenous nucleases. Thus in the study above, aluminum was also found to inhibit the digestion of both brain and liver chromatin by deoxyribonuclease (DNase) I even at the lowest concentration of aluminum tested (100 μM). The inhibitory effect was more prominent at higher concentrations of up to 500 μM. Moreover, the chromatin prepared from the cortical area of the brain was more sensitive to aluminum-induced alterations in chromatin structure than that from the liver. This effect was demonstrated to be the result of altering the conformation of chromatin rather than that of inhibiting the enzyme DNase per se. The fact that nuclei from the neuron-rich area of the brain (the cortex) were more sensitive to aluminum than those from the liver implies a strong relationship between aluminum action in the brain and the neurological symptoms of Alzheimer's disease, which exhibits a localized concentration of aluminum in the brain tissue of such patients.

7. Influence of Other Substances on Aluminum Toxicity

As mentioned in the earlier sections, I-6 and II-1, the most potent inorganic complexing agents of aluminum in the environment include fluoride and sulphate ions, among others. Nonetheless, there are also a number of organic agents capable of interacting with aluminum inside the body of living organisms.

Citrate has been repeatedly identified to play a part in augmenting aluminum absorption in humans (e.g. Froment et al., 1989b; Mischel et al., 1989; van der Voet et al., 1989). It is often given to uremic patients as a phosphate-binder, and co-administered with aluminum gel to those patients who are refractory to either agent alone. It was found that standard dosages of citrate can enhance the absorption of aluminum by 5- to 10-fold, which may cause the patients to suffer from severe aluminum toxicity (Mischel et al., 1989). It was deduced from an <u>in vitro</u> experiment that citrate enhances aluminum absorption by opening the tight junctions on the duodenal epithelium, in addition to the passive uptake of aluminum through the paracellular pathway (Froment et al., 1989b).

A similar suggestion has also been put forward after a study on the gastrointestinal absorption of aluminum in the Sprague-Dawley rat by using different kinds of aluminum solutions with or without additional citrate (Froment et al., 1989a). Percentage absorption of aluminum as determined from changes in plasma aluminum levels was much higher whenever citrate was present in the solution given, irrespective of the other anions present.

As previously mentioned in section II-4, the uptake of aluminum

in the gastrointestinal tract is probably through the calcium transport mechanism, with a saturable and a non-saturable component. Thus, it is possible that vitamin D, which is essential in calcium mobilization, exerts a similar action in aluminum transport. Through an *in vivo* experiment carried out using vitamin D-replete and vitamin D-deficient Sprague-Dawley rats, it was found that both groups of rats exhibited a similar rate of uptake of aluminum in the duodenum by the nonsaturable mechanism, which is vitamin D-independent. However, there was significantly lower aluminum absorption by the saturable mechanism in the vitamin D-deficient rats than in the vitamin D-replete ones (Adler and Berlyne, 1985). Aluminum also reduced the amount of calcium absorbed through the duodenum in vitamin D-replete rats by 33% but not in the other group. This implies a competition between aluminum and calcium uptake, a possible result of utilizing the same channels for absorption.

In another study, parathyroid hormone (PTH) was found to increase aluminum concentration in tibia of Wistar rats after just 5 days of administration (Ballanti et al., 1989). This agrees with the findings of Mayor et al. (1977). It has been proposed that PTH may contribute to the hyperaluminemia and increased tissue content of aluminum in patients with chronic renal failure by facilitating the intestinal absorption of the metal ion (Mayor et al., 1980; Mayor and Burnatowska-Hledin, 1983). Interestingly, aluminum also exhibits an inhibitory action on the secretion of PTH as well, demonstrated *in vivo* using Wistar rats, both in individuals with normal renal functions and those with chronic renal failure (Virgos et al., 1989).

IV. Summary

Fluoride has been demonstrated to have major influence on bone metabolism in mammals and birds, particularly in individuals undergoing rapid bone and tooth formation, older individuals, and those with poor nutritional status. It is readily absorbed through the digestive tract. On the other hand, aluminum has very low bioavailability in healthy individuals with normal kidney functions, despite the fact that it is the most abundant metal and third most abundant element in the world. Yet, neither ion exists in solitude in the natural world. Both are highly interactive with other substances and with each other. Therefore, more research into their interaction is warranted in order to give a more realistic picture of how these 2 ions affect humans as well as other living organisms.

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TABLE 1. Summary of chronic effects of fluoride on the soft tissues of various organisms

ORGAN/TISSUE	ANIMAL SP.	F-DOSAGE	EFFECTS	REFERENCE
mammary gland	commercially raised silver fox	97.6-136.8 ppm in commercial fox feed	agalactia of vixens, causing starvation of fox pups	Eckerlin et al., 1986
liver	albino rabbit	10, 50 mg NaF/kg/day for 7 months	enlarged mitochondria, broken mitochondrial cristae; reduced no. of RNA granules and RER, enlarged cisterns of SER; scant lipid droplets	Zhan and Huo, 1988
	male albino rabbit	s.c. injection of 10 mg NaF/kg for 3.5 months	decrease in cholesterol and total triglyceride	Singh et al., 1985
	albino mice	50, 100, 200 ppm F in drinking water for 16 weeks	decrease in activity of acid and alkaline phosphatases, succinic and lactic dehydrogenases, glutamic oxaloacetic and glutamic pyruvic transaminases; increase in ATPase activity; decrease in zinc, copper and manganese; increase in iron content	Singh, 1984
kidney	albino rabbit	10, 50 mg NaF/kg/day for 7 months	scant and swollen mitochondria in podocytes; swollen mitochondria with disintegrated cristae, thickened basal membrane and reduced RER in cells of proximal convoluted tubules	Zhan and Huo, 1988

TABLE 1 (Cont'd)

ORGAN/TISSUE	ANIMAL SP.	F-DOSAGE	EFFECTS	REFERENCE
kidney	albino mice	50, 100, 200 ppm F in drinking water 16 weeks	increase in the activity of ATPase and alkaline phosphatase; decrease in activity of acid phosphatase, succinic and lactic dehydrogenases, glutamic oxaloacetic and glutamic pyruvic transaminases; drop in level of zinc, copper, manganese; rise in iron	Singh, 1984
	1-day-old domestic chicken	150 ppm F in diet supplement for 4 weeks	rise in ascorbic acid level	Yu and Driver, 1978
stomach	male albino rat	infiltration with 7-9 ml of 24, 48, 96 mM NaF solution *in situ*	24, 48 mM: increase in activity of v-glutamyl transpeptidase, ATPase, 5'-nucleotidase, sucrase, alkaline phosphatase; 96 mM: more sucrase activity but less activity of glutamyl transpeptidase, ATPase, nucleotidase and alkaline phosphatase	Rastogi et al., 1987
		25 mg NaF/kg/day for 60 days	increase in gastric volume, free acidity and peptic activity	Shayiq et al., 1984
	albino rabbit (both sexes)	s.c. injection of 5, 10, 20, 50 mg NaF/kg/day for 100 days	fall in both acidic and basic protein contents	Shashi et al., 1987

TABLE 1. (Cont'd)

ORGAN/TISSUE	ANIMAL SP.	F-DOSAGE	EFFECTS	REFERENCE
thyroid gland	albino rabbit	10, 50 mg NaF/kg/day for 7 months	swollen mitochondria with disintegrated cristae, enlarged cisterns in SER, increased lysosomes in follicular epithelial cells	Zhan and Huo, 1988
duodenum and ileum	albino rabbit	s.c. injection of 5, 10, 20, 50 mg NaF/kg/day for 100 days	decrease in both acidic and basic proteins	Shashi et al., 1987
skeletal muscle	ditto	ditto	hypertrophy of muscle fibers, retraction from perimysial sheath, disintegration and necrosis of sarcoplasm, nuclear hypoplasia in endomysial connective tissue	Shashi, 1989
pectoralis muscle	1-day-old domestic chicken	150 ppm F in diet supplement for 4 weeks	increased weight gain of the muscle	Yu and Driver, 1978
pectoralis muscle, brain, gizzard, heart, spleen, pancreas	ditto	ditto	decrease in ascorbic acid levels	ditto
lung	ditto	ditto	increase in ascorbic acid level	ditto

TABLE 1. (Cont'd)

ORGAN/TISSUE	ANIMAL SP.	F-DOSAGE	EFFECTS	REFERENCE
lung	albino rabbit (both sexes)	s.c. injection of 5, 10, 20 mg NaF/kg/day for 100 days	decrease in total lipids, neutral lipids, triglyceride and cholesterol; phospholipid dropped in 5mg/kg group but rose in 10 and 20 mg/kg groups in females; neutral lipids rose in 5 mg/kg group but dropped in 10 and 20 mg/kg groups in males	Shashi et al., 1989
trachea	ditto	ditto	fall in total lipids, neutral lipids, triglyceride, free fatty acids and phospholipids; cholesterol dropped in 5 mg/kg group but rose in 10, 20 mg/kg groups in males, it dropped in 5, 10 mg/kg groups and rose in 20 mg/kg group in females	ditto
seminal vesicle	male albino mice (Swiss strain)	10, 20 mg NaF/kg/day orally for 30 days	increase in weight, rise in fructose level	Chinoy and Sequeira, 1989
prostate gland	ditto	ditto	increase in weight, increase in protein content and acid phosphatase activity	ditto
testis	ditto	ditto	decrease in succinate dehydrogenase activity	ditto
caput and cauda epididymus	ditto	ditto	decrease in ATPase activity and sialic acid level	ditto

TABLE 1. (Cont'd)

ORGAN/TISSUE	ANIMAL SP.	F-DOSAGE	EFFECTS	REFERENCE
vas deferens	male albino mice (Swiss strain)	10, 20 mg NaF/ kg/day orally for 30 days	increase in glycogen content	Chinoy and Sequeira, 1989
blood	ditto	ditto	decrease in serum testerone level	ditto

CONNECTING STATEMENT

Chapter 1 summarized the major findings in the fields of fluoride and aluminum research. It was obvious that little emphasis has been put on studying interactive effects of 2 or more substances together, as this is a more realistic picture inside any organism. Thus in Chapter 2, an experiment performed to further understand synergistic actions is reported, using fluoride and aluminum as the target substances and the American kestrel as the organism of study.

CHAPTER 2

EFFECTS OF ORALLY ADMINISTERED FLUORIDE AND ALUMINUM ON THE RATES OF ELIMINATION AND DISTRIBUTION PATTERNS OF THESE TWO IONS IN THE AMERICAN KESTREL

INTRODUCTION

Fluoride is a common constituent in industrial effluents, but it is especially prevalent in discharges from aluminum plants. Moreover, aluminum fluoride is used extensively as a raw material for these plants (Patel et al., 1986). Aluminum is also the world's most abundant metal, becoming more widespread as an aquatic pollutant through leaching from the soil by acid precipitation (Driscoll et al., 1980).

Fluoride and aluminum tend to form complexes when they are present together in a given solution. Such a chemical property is often made use of in treating patients suffering from Alzheimer's and other aluminum-related diseases. There have been attempts to remove excess aluminum from the body by fluoride administrations (Shore et al., 1985).

By the same principle, aluminum sulphate has been used for alleviating the symptoms of experimental fluorosis in sheep (Kessabi et al., 1988). Aluminum was found to reduce the digestive absorption of fluoride by 33 to 45%, hence lowering the fluoride in serum, urine, bones and teeth.

In a similar study on human subjects by Spencer et al. (1985), the intake of aluminum hydroxide was associated with a significant increase in fecal fluoride and a significant decrease of the net absorption of fluoride by an average of 57%.

Dietary aluminum administered to laying hens on a short term basis was effective in protecting the hen from fluorine toxicosis as a result of high dietary fluoride intake (Hahn and Guenter, 1986).

Few reports have documented the degree to which wild animals are affected by the existence of aluminum and fluoride, or their complexes, in the natural environment. The subjects of study include brook trout fry (Salvelinus fontinalis, Mitchill) (Driscoll et al., 1980), various predatory birds, including great crested grebe (Podiceps cristatus), grey heron (Ardea cinerea), buzzard (Buteo buteo), sparrowhawk (Accipiter nisus), merlin (Falco columbarius), kestrel (F. tinnunculus), barn owl (Tyto alba), little owl (Athene noctua), tawny owl (Strix aluco), long-eared owl (Asio otus), short-eared owl (A. flammeus), and kingfisher (Alcedo atthis) (Seel and Thomson, 1984), commercially farmed silver foxes (Eckerlin et al., 1986), barn owls (Tyto alba) (Thomson, 1987), red deer (Cervus elaphus) (Dabkowska and Machoy, 1989), and rainbow trout (Salmo gairdneri) (Playle et al., 1989).

The American kestrel (Falco sparverius) has been the subject of fluoride studies (Carrière et al., 1987). It reproduces easily in captivity and its widespread nature in the wild facilitates comparisons of laboratory and field results. Captive propagation and handling procedures for the kestrel have been well established at the Macdonald Raptor Research Centre of McGill University (Bird, 1982) where the present study was conducted.

The overall aim of the experiment was to simulate the natural intake of the two mineral ions, fluoride and aluminum, by feeding contaminated mice to captive kestrels for a short duration in order to detect any synergistic or antagonistic effects. The consumption of fluoride- and aluminum-contaminated food by kestrels can occur readily

in the wild through taking small rodents which have been drinking from polluted water sources, and which may have varying degrees of accumulation of the two substances under investigation.

Specifically, two hypotheses were tested: (1) dietary aluminum can decrease the digestive absorption of fluoride by the American kestrel; and (2) dietary aluminum can alter the pattern of distribution of fluoride among the various internal organs/tissues of the American kestrel.

MATERIALS AND METHODS

1. Dosing Procedure

Thirty-six American kestrels, 28 males and 8 females (3 $\pm$ 1 years of age) were chosen for the experiment. They were evenly divided into 6 treatment groups, with independent random allocation of males and females to each group so as to avoid having one particular treatment group dominated by one sex. All kestrels were adults with a maximum age difference of 2 years since the uptake of fluoride into the body can be influenced by age and stage of growth (Rose and Marier, 1977).

Each of the birds was kept separately in a holding cage (46.3 cm x 49.8 cm x 67.0 cm, l x w x h) constructed with rigid opaque plastic (Coroplast Inc., Granby, Quebec) on a wooden frame. Each cage was equipped with a rope perch, and had a dowelled open window on the front trap door. All cages were elevated 0.6 m above ground.

The birds were allowed to acclimate in their cages for a week and then sham-dosed with deionized water for another week prior to actual dosing to accustom them to handling procedures. Afterwards, oral dosing was carried out each day for 30 days according to the following scheme.

Group 1 (C_O): deionized water

Group 2 (C_{F30}): 30 mg/kg NaF

Group 3 ($F_{30}AL_{24}$): 30 mg/kg NaF and 24 mg/kg $Al_2(SO_4)_3$

Group 4 (C_{F24}): 24 mg/kg NaF

Group 5 ($F_{24}AL_{24}$): 24 mg/kg NaF and 24 mg/kg $Al_2(SO_4)_3$

Group 6 (C_{AL24}): 24 mg/kg $Al_2(SO_4)_3$

The upper limit of the dosage for fluoride (F) was set at 30 mg/kg body weight. In other experiments involving kestrels at the Macdonald Raptor Centre, this dose was found to bring about a detectable level of accumulation in the long bones within a relatively short period of time (i.e. a month) without causing severe crippling and other bone disorders (Shutt, personal communication). Since there had never been any experiment performed on the American kestrel using aluminum (Al), no known safe dosage was available for reference. The dosage of 24 mg/kg was used because of the F:Al ratio of 1:0.8 to be tested. In a preliminary experiment to assess the feasibility of these dosages, birds which received 24 mg/kg of Al did not display any noticeable health problems such as drastic weight loss or loss of appetite (Chu, unpublished data). During the course of the experiment, the health of all birds was closely monitored.

The total volume of each dose was made up to about 0.8 ml with deionized water in proportion to the body weight of the bird which was recorded daily prior to dosing. This volume allowed sufficient dilution of the presumably caustic chemicals and yet was small enough to avoid congestion and interference with normal food consumption (Chu, unpublished data). This also served the purpose of administering a uniform amount of fluid to each bird. A dose period of 30 days allowed time for significant accumulation of the chemicals in the kestrel's body.

Food in the form of skinned mice homogenized in a food processor was provided ad libitum immediately after each dose. At the end of each day, the daily feed intake was calculated by subtracting the

weight of left-over food from the initial ration. Food consumption, together with body weight, was used informally as an index of the health of the birds during the course of the experiment.

To test the first hypothesis, excreta were collected every 24 hours between 2 consecutive doses and analyzed for fluoride (F), aluminum (Al), calcium (Ca) and total phosphate (P). The latter 2 ions were monitored because they have been reported to be closely affected by elevated F and Al levels in the body (Forsyth et al., 1972; Spencer et al., 1985; Bronckers et al., 1989). Under the conditions of the experiment, it was not possible to separate feces (indigestible substances removed from the digestive tract) from excretory products (metabolic wastes removed through the kidneys) since they are both ejected from the cloaca. Hence, the rate of removal of F and Al was calculated as a combination of the rates of egestion and excretion.

A piece of rigid plastic sheeting was placed at the bottom of the cage and removed daily to have the excreta scraped off for chemical analysis. The plastic sheets were cleaned every day to avoid contamination from previous days.

The basal intake of F and Al was calculated from the analysis of food given to the birds.

To test the second hypothesis, the kestrels were euthanized at the end of the dose period by means of cervical dislocation. Femurae and soft tissues, including alimentary canal, liver, kidneys, and pectoral muscle, were collected and analyzed for F, Al, Ca and P. Femurae were collected because the fluoride content in long bones is a good indicator of the degree of fluoride accumulation (Mittal et al.,

1985). The alimentary canal, especially the stomach, was the first part of the body in contact with the chemicals given. Liver and kidneys were responsible for removing these substances from the body. Muscular tissue was reported to be adversely affected by the presence of fluoride and aluminum (London et al., 1989; Shashi, 1989).

2. Chemical Analysis

All the organic constituents in the samples were first broken down into inorganic components. Since F has been reported to interact with glass (McCann, 1968; Capar and Gould, 1979), only plastic apparatus was used.

The excreta samples were air-dried for 24 hours and then oven-dried at 70°C for 4 hours. Portions roughly 0.3 g in weight were obtained from the dried, powdery excreta for subsequent acid digestion. The total weight of excreta from each bird on each sampling day was also noted to determine the absolute amount of removal of each ion per unit body weight.

The excreta sub-samples were digested with 0.6 ml of 60% perchloric acid and 4.5 ml of 70% nitric acid over a hot water bath for an hour, or until the mixture turned clear. The resulting solution was filtered through Whatman filter paper no. 541 to remove any residue or gelatinous precipitate. The pH of the filtrate was adjusted to approximately 5.5 using sodium hydroxide solution (NaOH) before further analysis.

The soft tissues collected, i.e. kidney, liver, alimentary canal (sub-divided into 1-oesophagus, 2-stomach and gizzard, and 3-small and

large intestines), and pectoral muscle, were oven-dried at 70°C overnight. They were then homogenized by mortar and pestle or with a food processor. In the case of kidney, oesophagus, and stomach, the whole organ was used in chemical analysis because of the small amount available. All organs were weighed and digested in the same manner as the excreta.

After all the attached muscles and tendons were cleared from its surface, the left femur of each bird was subjected to dry ashing at 600°C in a muffle furnace for 4 hours. Weights were determined before and after ashing. The bone ash was ground to powder with a mortar and pestle. A solution was formed by dissolving the powder in hydrochloric acid following the method of Singer and Armstrong (1968).

F in the above solutions was measured using the fluoride ion-selective electrode (Orion Research Cat. no. 960900, Orion Research Inc., Cambridge, MA) in the presence of Total Ionic Strength Adjusting Buffer (TISAB) II (Appendix). Al was determined using an atomic absorption spectrophotometer (Perkin-Elmer 2380, Perkin-Elmer Corp., Norwalk, CT) with either the flame or graphite furnace, depending on concentration of the sample after appropriate dilutions. Ca was also analyzed with the atomic absorption spectrophotometer. P was measured with a UV/visible spectrometer (LKB Biochrom Ultrospec II, LKB Biochrom Ltd., Cambridge, England) following the procedure by Murphy and Riley (1962).

3. Statistical Analysis

Any differences due to sex were tested using the general linear

models (GLM) procedure (SAS, 1982) on all organs and excreta samples collected each day. If sex was found to be significant in any of these tests, then analysis of covariance would be used in subsequent analysis for those particular data sets. In this case, no significant sex differences were detected using the above procedure.

The daily variations in the levels of the 4 ions in excreta were also tested for significance at 0.05 and 0.01 levels using the GLM procedure. Where no statistically significant variation was found, the levels were pooled across days.

In testing the hypotheses, the effect of different dose levels of F (0, 24, and 30 mg/kg) and of Al (0 and 24 mg/kg) were tested by the GLM procedure with a 3x2 factorial design. Where interactions between F and Al were found to be statistically significant, the effects of F-Al combination were examined and used for comparisons of means. In other words, the effect of different treatment groups was tested.

Lotus 1-2-3 releases 1A and 2 (Lotus Development Corp., 1984, 1985), Statgraphics (Statistical Graphics Corp., 1984), and Statistical Analysis Systems (SAS Institute Inc., 1982) were employed in the above analyses as appropriate.

RESULTS

1. Analysis of excreta

All the levels of ions measured in excreta samples are expressed in μg/g dry weight/kg body weight.

1-1. Effect of sex difference

Using the analysis of covariance procedure of SAS (1982), the effect of sex was analyzed as a covariable, and found to be not significant ($p>0.05$, $n=467$) for all 4 ions.

1-2. Fluoride

a. Difference between day 0 and days 1-29

Samples from days 0, 1, 2, 4, 6, 8, 10, 12, 15, 17, 19, 22, 27, and 29 were used in chemical analysis for all ions measured. In order to determine if there was any change in the rate of excretion or elimination of F from birds receiving different treatments before and after the experiment started, the observed values of F removed from their bodies through the excreta on day 0 (i.e. before experiment started) and those on subsequent days were compared within each type of treatment (i.e. chemical constituents of the oral dose) using the GLM procedure. The probability values from the test are summarized in Table A1 in the Appendix (p.101).

Except for groups C_o and C_{AL24}, which did not receive any F in the oral dose, all other groups showed significantly higher levels of F excreted following the oral administration of either 24 or 30 mg/kg of F solution. This indicates that there was minimal cross-contamination between groups during both the dose administration and

the excreta collection.

b. Effect of treatments

The effect of different dose levels of F and Al on F excreted was tested using the GLM procedure. The effect of different dose levels of F (0, 24, and 30 mg/kg), Al (0 and 24 mg/kg) and F-Al interaction are all significant ($p<0.01$). The daily variation of F in excreta is significant ($p<0.05$), but no obvious pattern or trend can be detected.

In order to locate differences in the F content of excreta among the treatment groups, contrasts and Duncan's multiple range test were done using the GLM procedure. The results are shown in Table 1 (p.85). In general, the addition of Al to the oral dose has a significantly positive influence ($p<0.001$, Table A2, Appendix) on the amount of F excreted. The amount of F is much higher in the excreta from group $F_{30}AL_{24}$ than the corresponding amount from group C_{F30}. However, no such difference is seen between groups $F_{24}AL_{24}$ and C_{F24}. The level of F excreted from group $F_{30}Al_{24}$ was also significantly higher ($p<0.01$) than that of group $F_{24}AL_{24}$, but no such difference was observed between groups C_{F30} and C_{F24}.

1-3. Aluminum

a. Difference between day 0 and days 1-29

There is no significant difference between the level of Al excreted on day 0 and subsequent days in group C_0 (Table A1, Appendix). The level of Al is significantly higher on days 1-29 than on day 0 for groups C_{F30} and $F_{30}AL_{24}$ ($p<0.05$), and for groups C_{F24}, C_{AL24}, and $F_{24}AL_{24}$ ($p<0.01$).

b. Effect of treatments

The effect of different dose levels of Al (0 and 24 mg/kg) on the amount of Al in excreta was significant ($p<0.01$) whereas that of different dose levels of F and F-Al interaction was not ($p>0.05$). The amount of Al in excreta varied little between days, except on day 29, on which there was an abnormally high Al level.

By means of contrasts and Duncan's multiple range test, levels of Al in excreta were significantly higher in group $F_{30}AL_{24}$ than in group C_{AL24} ($p<0.05$), lower in group C_{F30} than in group $F_{30}AL_{24}$ ($p<0.05$), lower in group C_0 than in group C_{AL24} ($p<0.05$), and lower in group C_{F24} than in group $F_{24}AL_{24}$ ($p<0.05$, Table 2).

1-4. Calcium

a. Difference between day 0 and days 1-29

There was significant difference between the levels of Ca in excreta before and after starting the experiment ($p>0.05$, Table A1, Appendix), despite wide fluctuations throughout the period of the experiment.

b. Effect of treatments

The effects of individual dose levels of F and Al were not significant in affecting Ca excretion ($p>0.05$, analysis of variance), although F-Al interaction is significant ($p<0.0001$).

By using Duncan's multiple range test to detect which F-Al combinations were effective in affecting Ca excretion, the level of Ca in group C_0 was significantly higher than in group C_{AL24} ($p<0.05$), and lower in group C_{F30} than in group $F_{30}AL_{24}$ ($p<0.05$, Table 3). By means of contrasts, Ca content from group C_0 was found to be significantly

higher than that from groups C_{F30} and C_{F24} ($p<0.01$), and lower in group C_{AL24} than in groups $F_{30}AL_{24}$ and $F_{24}AL_{24}$ ($p<0.01$). Group C_{F30} also has significantly less Ca in the excreta than group C_{F24} ($p<0.05$, Table A4, Appendix).

1-5. Phosphate

a. Difference between day 0 and days 1-29

The only difference in P excretion between day 0 and subsequent days was found in group $F_{30}AL_{24}$ ($p<0.05$, Table A1, Appendix). The daily variations were wide without a consistent trend, therefore not statistically significant ($p>0.05$).

b. Effect of treatments

The effects of different dose levels of F and Al were not significant in influencing the amount of P in excreta ($p>0.05$), but F-Al interaction was significant ($p<0.01$). The daily variations in P excreted was significant ($p<0.01$), although the variations did not follow a discernible trend.

By comparing different treatments with Duncan's multiple range test, P level in excreta of group C_0 was significantly higher than both group C_{AL24} and group C_{F30} ($p<0.05$, Table 4), as was group $F_{30}AL_{24}$ higher than groups C_{F30} and group C_{AL24} ($p<0.05$).

2. Analysis of organs

2-1. Effect of sex difference

The only significant difference between the 2 sexes was found in the P content of the intestines ($p<0.05$). No other significant

sex difference was observed for the rest of the ion-tissue combinations ($p>0.05$).

2-2. Bone

a. Fluoride

The F content in femurae was influenced significantly by the dose levels of both F ($p<0.01$) and Al ($p<0.01$) as well as F-Al interaction ($p<0.05$).

Using Duncan's multiple range test, the concentrations of F in groups C_{F30} and C_{F24} were found to be significantly higher than in groups $F_{30}AL_{24}$ and $F_{24}AL_{24}$, which in turn had more F in their bones than groups C_0 and C_{AL24} ($p<0.05$, Table 5). The difference in F content of femurae within each pair of groups above was not significant ($p>0.05$) although the dosage of F was different.

b. Aluminum

The effects of different dose levels of F and Al amd F-Al interaction on the amount of Al in femurae were not significant ($p>0.05$).

Using Duncan's multiple range test, a significantly lower Al level was found in femurae from groups C_{F24} than those from group $F_{24}AL_{24}$ ($p<0.05$, Table 6).

c. Calcium

The effect of different dose levels of F or Al or F-Al interaction on Ca content in the femur was not significant ($p>0.05$).

The Ca content in femur was found to be significantly different between groups C_{F30} and $F_{24}AL_{24}$ ($p<0.05$), and between C_{F30} and C_{AL24} ($p<0.05$, Table 7). However, it is hard to make any logical comparison

for these two pairs of treatments since they differed from each other in more than one way.

d. Phosphate

No significance could be detected in the effects of different dose levels of F, Al, and F-Al interaction on P content in femurae ($p>0.05$).

Nevertheless, difference in P content was found between femurae from groups C_{F30} and C_{F24} ($p<0.05$), between groups C_{F30} and $F_{24}AL_{24}$ ($p<0.05$), and between groups C_{F30} and C_{AL24} ($p<0.05$, Table 8). Among them, only one reasonable comparison can be made, i.e. between groups C_{F30} and C_{F24}, where the treatments only differed by the dose level of F.

2-3. Kidney

a. Fluoride

Dose levels of F and Al and their interaction had no significant effect on the amount of F recovered from kidneys ($p>0.05$, Table 9).

b. Aluminum

The effects of different dose levels of F and Al on Al content in the kidney were both significant ($p<0.01$), as was F-Al interaction ($p<0.05$).

Using Duncan's multiple range test, the Al content of kidneys from group $F_{30}AL_{24}$ (the highest mean Al content) was found to be significantly different from all the other groups ($p<0.05$, Table 10). A significant difference was also found between groups C_{F30} and $F_{24}AL_{24}$ ($p<0.05$).

c. Calcium

The concentrations of Ca in kidneys from different treatments did not differ significantly from one another ($p>0.05$, Table 11).

d. Phosphate

The effects of different dose levels of F and Al on P concentration in kidneys were not significant ($p>0.05$). Only the F-Al interaction was significant ($p<0.05$). Using Duncan's multiple range test, group C_0 was found to have significantly lower P in the kidney than group C_{F24} ($p<0.05$). Group C_{F24} had significantly higher P content in the kidney than group $F_{24}AL_{24}$ ($p<0.05$, Table 12).

2-4. Oesophagus, stomach, intestine, muscle, and liver

No significant difference was detected in the content of any of the 4 ions in each of these 5 organs among all treatment groups ($p>0.05$).

3. Others

3-1. Body weight

The body weights of the kestrels in the experiment ranged from 95g to 120g, males and females included. This is considered within the normal range. Analyses of variance were done on the body weights of the birds with respect to treatment groups on a daily basis. No significant difference was found among treatment groups on any day of the experiment ($p>0.05$).

The mean body weights of each treatment group were plotted against days of the experiment in Figure A1 (Appendix, p. 107). No significant variation was detected by analysis of variance in the body

weight of any treatment group throughout the duration of the experiment ($p>0.05$).

3-2. Organ weight

The range of weight of each organ analyzed is presented in Table 13. The result from the analysis of variance did not show any significant difference in weight among different groups in any of the organs ($p>0.05$).

3-3. Feed consumption

The unit feed consumption for each bird on each day was obtained by dividing the intake of food by the body weight on a given day, which varied slightly on a daily basis. Analyses of variance were done to test the effect of treatment on the unit feed consumption. No significant result was obtained ($p>0.05$).

Simple linear regression was done on the feed consumption within each group over the whole period of the experiment, and the results are shown in Table A6 (Appendix). Groups C_{F30} and C_{F24} showed a significant trend of increasing unit feed consumption over the period ($p<0.05$)

Since there was no significant difference in feed consumption among different treatment groups over the period of the experiment, and the feed itself was homogenized with a food processor before being fed to the birds, it is assumed that the variations in feed intake would not have any significant influence over the amount of uptake of the 4 ions. Nonetheless, samples of food given to the birds were analyzed for F, Al, Ca, and P as a reference (Table 14).

DISCUSSION

1. Analysis of excreta

A significant difference in F level in excreta was detected between groups C_{F30} and $F_{30}AL_{24}$ ($p<0.05$, Table 1). This likely resulted from the additional Al given to group $F_{30}AL_{24}$, demonstrating an antagonistic effect of Al on F uptake. The same effect has been demonstrated in sheep (Kessabi et al., 1988) in that animals given an oral dose of Al and F eliminated significantly more F in their feces than those that were given F alone.

However, in this study there was no detectable difference in the amount of F removed in excreta between groups C_{F24} and $F_{24}AL_{24}$, although birds in group $F_{24}AL_{24}$ were dosed with Al while those in group C_{F24} were not. The discrepancy may lie in the variation in the F:Al ratio of the oral dose, being 1:0.8 in group $F_{30}AL_{24}$ and 1:1 in group $F_{24}AL_{24}$. The former ratio was effective in facilitating the removal of F from the bodies of laying hens (Hahn and Guenter, 1986). In their study, the level of Al was varied while that of F was kept constant in dosages used, and it was found that a F:Al ratio of 1:0.4 was less effective than 1:0.8. Setting the F:Al ratio at 1:1 for group $F_{24}AL_{24}$ in the present experiment tested whether an even larger proportion of Al would increase the synergistic effect. It now appears that there is an optimal ratio between the two ions as the above ratio of 1:1 did not facilitate the removal of F in the present experiment. Kessabi et al. (1988) suggested that the synergistic effect was F dose-related. In their study, the amount of reduction of F in the

serum of sheep as a result of the addition of Al in the diet was related to the amount of F given.

Significantly more Al was removed from the birds in groups C_{F30} and C_{F24} after the experiment began, even though no extra Al was ever given to them (Table A1, Appendix). Cross-contamination between groups was not likely, as the level of Al excreted in group C_o was very low throughout the whole period of the experiment, and all groups were subjected to the same experimental conditions. However, it is possible that the mere application of F resulted in removing indigenous Al from the body. In fact, clinical trials using F to remove excessive Al in patients suffering from Al-related illnesses have been done. Shore et al. (1985) tested the possibilities of using F as a complexing agent to prevent Al from binding to nuclear chromatin in the brain. Their study focused on the ability of F to complex the Al which had already been absorbed rather than trying to prevent further absorption of Al, since most of the patients obtained excessive Al through hemodialysis using unpurified municipal water.

In the present study, since the excretory products could hardly be separated from feces, it was not possible to determine whether the Al contained in excreta came from the intestines (i.e. it was never absorbed) or from the kidneys (i.e. it was the result of cellular metabolism). Therefore, a more precise experiment in which the feces and urates can be separated is needed to substantiate the claim that excess F can help eliminate Al from the body.

On the other hand, the addition of Al in the diet did not have any effect on F elimination from the body. This was demonstrated by

the minimal amount of F found in the excreta of group C_{AL24} (in which birds were given only Al), which was not significantly different from the level of F found in the excreta of group C_o (in which birds were given deionized water only) (Table 2). Hahn and Guenter (1986) also found in their study on laying hens that no detrimental effect from Al alone was observed on the production parameters of the hens over a 16-week period. Moreover, Al was actually important in maintaining feed intake at near normal levels during their experiment with F.

The various types of complexes formed between Al and other ions in aquatic systems have been detailed by Burrows (1977). In most cases, a complex between Al and F probably exists in the form of AlF_3, but the form in which the complex is displayed inside the body is not known, in view of the great spectrum of both organic and inorganic compounds within the body. The stability of the complex can also be influenced by the ambient pH, which varies from organ to organ. It would be worthwhile to investigate the structure of such a complex inside the body systems in order to further comprehend its possible physiological effects.

The effects of either F or Al or both on the elimination of Ca and P were rather dose-specific (Tables 3 and 4). Besides, Ca and P metabolism are influenced by a wide range of factors and substances and vary greatly among individuals. Since there was no previous record of Ca and P in excreta for birds from the same breeding colony as the ones used in this experiment, it was impossible to determine whether the variations in the levels are characteristic of these birds. In this study, the measurement of Ca and P in excreta served

more as indicators of the general health conditions (e.g. whether the birds were starved or suffering from mineral imbalance) than for determining the precise influences of F and Al on these 2 ions.

2. Analysis of organs/tissues

2-1. Bone

The F content of femurae from group C_{F30} was significantly higher than that found in group $F_{30}AL_{24}$ ($p<0.05$, Table 5) with a corresponding significant increase in F in the excreta from the latter group. It has been suggested that the amount of F accumulated in the long bone is proportional to the amount absorbed into the body (Carrière et al., 1987). Therefore, if the amount of F absorbed was reduced in the intestines by the presence of Al, the amount accumulating in the bone would certainly have been smaller. Increased excretion of F by the co-existence of Al is less plausible as an explanation to the above finding since F has a high affinity for the bone matrix once it enters the body.

Peculiarly enough, there was no significant difference in the F content of femurae from groups C_{F30} and C_{F24} despite a difference in dosage. A previous study by Bird et al. (1992) showed a F level as high as 10,000 ppm in the femurae of kestrels (from the same colony) fed dietary F for 21 days without suffering from growth depression. Therefore, it was unlikely that the bones of these kestrels could have been saturated with F (maximum F content being 1092.00 µg/g in this study).

Although there was no significant difference in F of excreta from

groups C_{F24} and $F_{24}AL_{24}$, F of femurae from the former group was significantly higher than that from the latter. One possible explanation is that the F level in excreta was measured on a daily basis whereas that in the femur was a result of accumulation over 30 days of dosing. A slight difference on each day might have translated into a significant difference at the end of the 30-day period.

In general, Al content of femurae was not significantly different among treatment groups (Table 6). This is expected since Al has never been reported to congregate significantly in the bone within such a short time. Al overload causing structural abnormalities in bone was reported for human patients receiving long-term (3 to 5 years) plasma exchange treatment with albumin replacement solutions. The resultant bone Al concentration was about twice normal. The dose period employed in this study, however, is considered too short to have caused any significant changes in the bone as a result of dietary Al. This mineral ion is not readily absorbed into the body through the digestive system, and the amount that can be retained even after intravenous infusion is very small in people with normal renal function (Mousson et al., 1989).

2-2. Kidney

No significant difference could be found in F content of kidneys among groups (Table 9). This is expected since deposition of excess F will only occur after the full capacity for the storage of F is reached in the long bones during prolonged exposure to F. The dosing period in this experiment was relatively short, and the F content of

femurae from all treatment groups was below saturation level.

Furthermore, most of the studies on the effects of F on the kidney have focused on the histological or the biochemical aspects (Yu and Driver, 1978; Singh, 1984; and Zhan and Huo, 1988), making it hard to compare the results obtained from this study with the others.

Interestingly, not only Al, but also F and F-Al interaction, had significant influences on Al content of the kidney ($p<0.01$ for effects of F and Al, $p<0.05$ for effect of F-Al interaction). This agrees with the interactions found between F and Al on the deposition of F in the femur, as well as on the removal of F from the body, as discussed earlier.

Since synergism affects both sides of the relationship, it is possible that the phenomenon could hamper the absorption of F and facilitate the absorption of Al at the same time. In fact, more Al was found in the kidneys from group $F_{30}AL_{24}$ than the other two groups which were given the same dosage of Al but less F (Table 10). The content of Al in the excreta from group $F_{30}AL_{24}$ was also higher than in group C_{AL24}, which was not given any F. The greater amount of Al in both the kidney and the excreta of group $F_{30}AL_{24}$ coincided with a higher dosage of F given to this group. As a result, it can be inferred that at least part of the extra Al in the excreta of group $F_{30}AL_{24}$ has come from excretion, i.e. after cellular metabolism, as the patterns of the relative abundance of Al in the excreta and in the kidney corresponded to each other. That in turn implies that more Al has been absorbed through the alimentary canal in this group than others. The extra amount of Al in the kidney of group $F_{30}AL_{24}$ could

not have solely been a result of leaching of indigenous Al by F, since the level is still higher than any of the groups which were not given Al, i.e. groups C_O, C_{F30}, and C_{F24}.

On the other hand, since it was previously assumed that Al coupled with F thus aiding in the removal of exogenous F from the intestines, a major portion of the Al in the excreta had to have come from digestive removal as well. Therefore, there appeared to be more Al being eliminated in total from the kestrels' bodies in group $F_{30}AL_{24}$ than in groups $F_{24}AL_{24}$ or C_{AL24}. A possible explanation for the extra amount of Al removed is the reported ability of F to leach indigenous Al from the body, as discussed previously.

Obviously, much of the above discussion is hypothetical, and needs to be substantiated by experimental evidence. Unfortunately, there is very limited literature on the interactions between F and Al on the ionic balance of the various internal organs or soft tissues. Moreover, a much longer study period is required in order to demonstrate any effect of the accumulation of F on the soft tissues. Further research into the mechanism by which the 'F-Al complex' is taken up by the digestive system would also assist in greater understanding of the chemistry occurring at the cellular level.

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TABLE 1. Comparison of means using Duncan's multiple range test on the fluoride levels in excreta of American kestrels dosed with fluoride and/or aluminum. Groups with the same Duncan grouping are not significantly different at 0.05 level.

TREATMENT GROUP	MEAN F LEVEL ± S.D. (µg/g dry wt./kg b.wt.)	NO. OF SAMPLES	DUNCAN GROUPING
C_0	0.65 + 1.06	78	d
C_{F10}	3.20 ± 1.75	78	c
$F_{10}AL_{24}$	5.20 ± 3.31	78	a
C_{F24}	3.84 + 2.15	78	b,c
$F_{24}AL_{24}$	3.95 ± 2.11	78	b
C_{AL24}	0.56 + 1.15	77	d

TABLE 2. Comparison of means using Duncan's multiple range test on the aluminum levels in excreta of American kestrels dosed with fluoride and/or aluminum. Groups with the same Duncan grouping are not significantly different at 0.05 level.

TREATMENT GROUP	MEAN AL LEVEL ± S.D. (µg/g dry wt./kg b.wt.)	NO. OF SAMPLES	DUNCAN GROUPING
C_0	1.01 ± 1.17	78	c
C_{F30}	1.23 ± 0.86	78	c
$F_{30}AL_{24}$	8.07 ± 7.32	78	a
C_{F24}	1.42 ± 1.04	78	c
$F_{24}AL_{24}$	6.83 ± 5.19	78	a,b
C_{AL24}	6.35 ± 5.00	77	b

TABLE 3. Comparison of means using Duncan's multiple range test on the calcium levels in excreta of American kestrels dosed with fluoride and/or aluminum. Groups with the same Duncan grouping are not significantly different at 0.05 level.

TREATMENT GROUP	MEAN CA LEVEL ± S.D. (µg/g dry wt./kg b. wt.)	NO. OF SAMPLES	DUNCAN GROUPING
C_O	936.02 ± 420.59	78	a
C_{F30}	692.60 ± 403.83	78	b
$F_{30}AL_{24}$	932.30 ± 452.07	78	a
C_{F24}	831.41 ± 430.37	78	a,b
$F_{24}AL_{24}$	851.13 ± 436.85	78	a
C_{AL24}	689.66 ± 397.23	77	b

TABLE 4. Comparison of means using Duncan's multiple range test on the phosphate levels in excreta of American kestrels dosed with fluoride and/or aluminum. Groups with the same Duncan grouping are not significantly different at 0.05 level.

TREATMENT GROUP	MEAN P LEVEL ± S.D. (µg/g dry wt./kg b. wt.)	NO. OF SAMPLES	DUNCAN GROUPING
C_O	874.44 ± 402.34	78	a
C_{F30}	703.39 ± 415.56	78	b
$F_{30}AL_{24}$	872.96 ± 400.64	78	a
C_{F24}	790.18 ± 433.31	78	a,b
$F_{24}AL_{24}$	792.34 ± 410.02	78	a,b
C_{AL24}	657.66 ± 392.57	77	b

TABLE 5. Comparison of mean fluoride concentrations in long bones of American kestrels dosed with fluoride and/or aluminum by Duncan's multiple range test. Groups with the same Duncan grouping are not significantly different at 0.05 level.

GROUP	MEAN F IN BONE ± S.D. (µg/g ash weight)	NO. OF INDIVIDUALS	DUNCAN GROUPING
C_0	137.46 ± 23.96	6	a
C_{F30}	823.47 ± 153.67	6	c
$F_{30}AL_{24}$	511.70 ± 137.14	6	b
C_{F24}	833.43 ± 216.13	6	c
$F_{24}AL_{24}$	493.51 ± 95.85	6	b
$C_{Al\,24}$	173.10 ± 70.72	6	a

TABLE 6. Comparison of mean aluminum concentrations in long bones of American kestrels dosed with fluoride and/or aluminum by Duncan's multiple range test. Groups with the same Duncan grouping are not significantly different at 0.05 level.

GROUP	MEAN AL IN BONE ± S.D. (µg/g ash weight)	NO. OF INDIVIDUALS	DUNCAN GROUPING
C_0	79.72 ± 26.55	6	a,b
C_{F30}	76.57 ± 8.36	6	a,b
$F_{30}AL_{24}$	79.06 ± 16.07	6	a,b
C_{F24}	59.45 ± 14.19	6	b
$F_{24}AL_{24}$	98.94 ± 37.03	6	a
C_{AL24}	84.84 ± 14.53	6	a,b

TABLE 7. Comparison of mean calcium concentrations in long bones of American kestrels dosed with fluoride and/or aluminum by Duncan's multiple range test. Groups with the same Duncan grouping are not significantly different at 0.05 level.

GROUP	MEAN CA IN BONE ± S.D. (mg/g ash weight)	NO. OF INDIVIDUALS	DUNCAN GROUPING
C_0	343.6 ± 37.5	6	a,b
C_{F30}	310.7 ± 64.2	6	b
$F_{30}AL_{24}$	332.0 ± 58.1	6	a,b
C_{F24}	355.9 ± 24.6	6	a,b
$F_{24}AL_{24}$	379.7 ± 14.2	6	a
C_{AL24}	373.8 ± 9.6	6	a

TABLE 8. Comparison of mean phosphate concentrations in long bones of American kestrels dosed with fluoride and/or aluminum by Duncan's multiple range test. Groups with the same Duncan grouping are not significantly different at 0.05 level.

GROUP	MEAN P IN BONE ± S.D. (mg/g ash weight)	NO. OF INDIVIDUALS	DUNCAN GROUPING
C_O	165.5 ± 17.1	6	a,b
C_{F30}	148.8 ± 30.1	6	b
$F_{30}AL_{24}$	156.6 ± 27.7	6	a,b
C_{F24}	174.9 ± 9.4	6	a
$F_{24}AL_{24}$	181.0 ± 7.6	6	a
C_{AL24}	177.7 ± 7.9	6	a

TABLE 9. Comparison of mean fluoride concentrations in kidneys of American kestrels dosed with fluoride and/or aluminum by Duncan's multiple range test. Groups with the same Duncan grouping are not significantly different at 0.05 level.

GROUP	MEAN F IN KIDNEY ± S.D. (µg/g dry weight)	NO. OF INDIVIDUALS	DUNCAN GROUPING
C_0	1.28 ± 0.88	6	a
C_{F30}	1.14 ± 0.68	6	a
$F_{30}AL_{24}$	1.34 ± 0.79	6	a
C_{F24}	1.29 ± 0.65	6	a
$F_{24}AL_{24}$	1.52 ± 0.71	6	a
C_{AL24}	1.05 ± 0.36	6	a

TABLE 10. Comparison of mean aluminum concentrations in kidneys of American kestrels dosed with fluoride and/or aluminum by Duncan's multiple range test. Groups with the same Duncan grouping are not significantly different at 0.05 level.

GROUP	MEAN AL IN KIDNEY ± S.D. (µg/g dry weight)	NO. OF INDIVIDUALS	DUNCAN GROUPING
C_0	5.39 ± 4.59	6	b,c
C_{F30}	2.72 ± 2.78	6	c
$F_{30}AL_{24}$	28.65 ± 15.33	6	a
C_{F24}	4.24 ± 2.55	6	b,c
$F_{24}AL_{24}$	13.69 ± 9.37	6	b
C_{AL24}	6.41 ± 3.00	6	b,c

TABLE 11. Comparison of mean calcium concentrations in kidneys of American kestrels dosed with fluoride and/or aluminum by Duncan's multiple range test. Groups with the same Duncan grouping are not significantly different at 0.05 level.

GROUP	MEAN CA IN KIDNEY ± S.D. (μg/g dry weight)	NO. OF INDIVIDUALS	DUNCAN GROUPING
C_0	299.56 ± 41.42	6	a
C_{F30}	341.00 ± 79.59	6	a
$F_{30}AL_{24}$	337.52 ± 87.60	6	a
C_{F24}	288.21 ± 39.45	6	a
$F_{24}AL_{24}$	296.45 ± 86.00	6	a
C_{AL24}	326.88 ± 127.38	6	a

TABLE 12. Comparison of mean phosphate concentrations in kidneys of American kestrels dosed with fluoride and/or aluminum by Duncan's multiple range test. Groups with the same Duncan grouping are not significantly different at 0.05 level.

GROUP	MEAN P IN KIDNEY ± S.D. (mg/g dry weight)	NO. OF INDIVIDUALS	DUNCAN GROUPING
C_0	6.03 ± 0.23	6	b
C_{F30}	6.33 ± 0.58	6	a,b
$F_{30}AL_{24}$	6.32 ± 0.42	6	a,b
C_{F24}	6.71 ± 0.24	6	a
$F_{24}AL_{24}$	5.95 ± 0.37	6	b
C_{AL24}	6.30 ± 0.60	6	a,b

Table 13. Summary of the range of fresh weight of each organ/tissue of American kestrels dosed with fluoride and/or aluminum and the probabilities of a greater F value from the analyses of variance on the effect of treatment on the weights of different tissues/organs. No significant effect was found.

ORGAN/TISSUE	NO. OF SAMPLES	RANGE (g)	PROB (>F)
femur	36	0.2 - 0.4	0.6830
kidney	36	0.7 - 1.3	0.1159
heart	36	1.0 - 1.9	0.5974
oesophagus	36	0.2 - 0.7	0.2447
stomach	36	1.4 - 3.6	0.2444
intestine	36	2.1 - 5.0	0.8183
liver	36	1.9 - 4.6	0.5676
muscle	36	5.8 - 10.9	0.7752

Table 14. Ionic composition of mashed skinned mice fed to the kestrels during the experiment.

ION	CONCENTRATION (μg/g dry wt)
Ca	8.56×10^3
P	2.24×10^4
F	15.65
Al	22.06

CONCLUSION

The interaction between fluoride and aluminum was most pronounced when the ratio of F:Al was 1:0.8, at which the removal of both fluoride and aluminum from the body was enhanced, deposition of fluoride in the bone was reduced, and aluminum content of the kidney was increased. The F:Al ratio of 1:1 was also effective in increasing the aluminum content of kidney and reducing the fluoride deposit in bone. The effect of aluminum on fluoride uptake and elimination appeared to be related to the dosage of fluoride, or the ratio between fluoride and aluminum.

APPENDIX

Procedure for preparing Total Ionic Strength Adjusting Buffer (TISAB) II: (p.64)

To prepare 1L of the buffer solution, dissolve 58g of sodium chloride (NaCl) and 1g of CDTA in a mixture of 57ml of anhydrous acetic acid (NaOAc) and approximately 500ml of deionized water. Titrate the resulting mixture to a pH of 5.8 with 5M sodium hydroxide solution (NaOH). Allow the solution to cool to room temperature and make it up to 1L in a volumetric flask.

TABLE A1. Probabilities of a greater F value for testing the null hypothesis Ho: there is no significant difference between the level of an ion measured in the excreta obtained on day 0 and those obtained on days 1 - 29 in a treatment group. The null hypothesis is rejected for a value of p smaller than 0.05.

IONS	C_O	C_{F30}	$F_{30}AL_{24}$	C_{F24}	$F_{24}AL_{24}$	C_{AL24}
FLUORIDE	0.5482	0.0006***	0.0007***	0.0019**	0.0001****	0.6403
ALUMINUM	0.1344	0.0335*	0.0104*	0.0056**	0.0033**	0.0036**
CALCIUM	0.0550	0.5292	0.0519	0.8304	0.5874	0.9909
PHOSPHATE	0.2612	0.1551	0.0260*	0.7024	0.9364	0.6748

* significant at 0.05 level

** significant at 0.01 level

*** significant at 0.001 level

**** significant at 0.0001 level

TABLE A2. Contrasts using GLM procedure in SAS on the fluoride levels in excreta of American kestrels dosed with fluoride and/or aluminum.

CONTRAST	df	p(>F)
C_0, C_{F30}, C_{F24} vs $F_{30}AL_{24}, F_{24}AL_{24}, C_{AL24}$	1	0.0005***
C_0 vs C_{F30}, C_{F24}	1	0.0001****
C_{AL24} vs $F_{30}AL_{24}, F_{24}AL_{24}$	1	0.0001****
C_{F30} vs C_{F24}	1	0.0537
$F_{30}AL_{24}$ vs $F_{24}AL_{24}$	1	0.0002***

*** significant at 0.001 level

**** significant at 0.0001 level

TABLE A3. Contrasts using GLM procedure in SAS on the aluminum levels in excreta of American kestrels dosed with fluoride and/or aluminum.

CONTRAST	df	p(>F)
C_O, C_{F30}, C_{F24} vs $F_{30}AL_{24}, F_{24}AL_{24}, C_{AL24}$	1	0.0001****
C_O vs C_{F30}, C_{F24}	1	0.6005
C_{AL24} vs $F_{30}AL_{24}, F_{24}AL_{24}$	1	0.1022
C_{F30} vs C_{F24}	1	0.7815
$F_{30}AL_{24}$ vs $F_{24}AL_{24}$	1	0.0720

**** significant at 0.0001 level

TABLE A4. Contrasts using GLM procedure in SAS on the calcium levels in excreta of American kestrels dosed with fluoride and/or aluminum.

CONTRAST	df	p(>F)
C_O, C_{F30}, C_{F24} vs $F_{30}AL_{24}, F_{24}AL_{24}, C_{AL24}$	1	0.9213
C_O vs C_{F30}, C_{F24}	1	0.0034**
C_{AL24} vs $F_{30}AL_{24}, F_{24}AL_{24}$	1	0.0007***
C_{F30} vs C_{F24}	1	0.0434*
$F_{30}AL_{24}$ vs $F_{24}AL_{24}$	1	0.2341

* significant at 0.05 level

** significant at 0.01 level

*** significant at 0.001 level

TABLE A5. Contrasts using GLM procedure in SAS on the phosphate levels in excreta of American kestrels dosed with fluoride and/or aluminum.

CONTRAST	df	p(>F)
C_O, C_{F30}, C_{F24} vs $F_{30}AL_{24}, F_{24}AL_{24}, C_{AL24}$	1	0.6921
C_O vs C_{F30}, C_{F24}	1	0.0250*
C_{AL24} vs $F_{30}AL_{24}, F_{24}AL_{24}$	1	0.0023***
C_{F30} vs C_{F24}	1	0.1861
$F_{30}AL_{24}$ vs $F_{24}AL_{24}$	1	0.2193

* significant at 0.05 level

*** significant at 0.001 level

Table A6. Summary of the results from simple linear regression of the unit feed consumption on day of the experiment for each treatment group. A positive slope indicates an increasing trend for feed consumption over the course of the experiment, and vice versa.

GROUP	PARAMETER	ESTIMATE (g/g b.wt.)	STANDARD ERROR	T-VALUE	PROB (H_0: slope=0)
C_O	intercept	0.2670	0.0089	30.12	
	slope	0.0004	0.0005	0.77	0.4419
C_{F30}	intercept	0.2498	0.0087	28.82	
	slope	0.0013	0.0005	2.45	0.0155*
$F_{30}AL_{24}$	intercept	0.2801	0.0098	28.47	
	slope	0.0007	0.0006	1.21	0.2287
C_{F24}	intercept	0.2459	0.0101	24.24	
	slope	0.0013	0.0006	2.24	0.0267*
$F_{24}AL_{24}$	intercept	0.2574	0.0078	32.85	
	slope	0.0007	0.0005	1.41	0.1593
C_{AL24}	intercept	0.2581	0.0092	28.17	
	slope	0.0007	0.0005	1.21	0.2277

* significant at 0.05 level

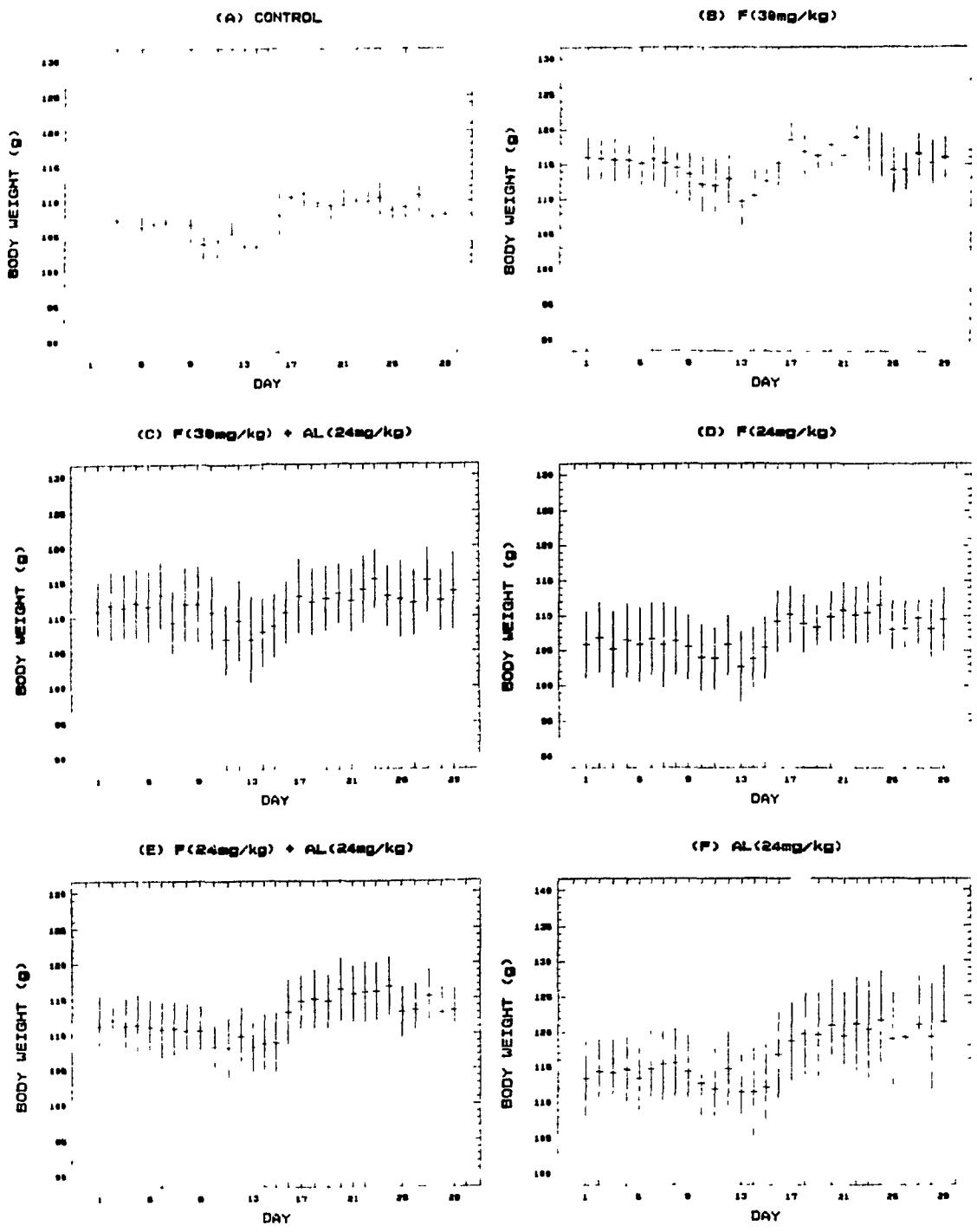

FIGURE A1. Plots of mean daily body weights ± standard error of mean (S.E.M.) of each treatment group versus day of the experiment. The effect of day on mean body weights was not significant for any treatments ($p>0.05$).