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Fluoride Metabolism

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Abstract

Knowledge of all aspects of fluoride metabolism is essential for comprehending the biological effects of this ion in humans as well as to drive the prevention (and treatment) of fluoride toxicity. Several aspects of fluoride metabolism – including gastric absorption, distribution and renal excretion – are pH-dependent because the coefficient of permeability of lipid bilayer membranes to hydrogen fluoride (HF) is 1 million times higher than that of F^-. This means that fluoride readily crosses cell membranes as HF, in response to a pH gradient between adjacent body fluid compartments. After ingestion, plasma fluoride levels increase rapidly due to the rapid absorption from the stomach, an event that is pH-dependent and distinguishes fluoride from other halogens and most other substances. The majority of fluoride not absorbed from the stomach will be absorbed from the small intestine. In this case, absorption is not pH-dependent. Fluoride not absorbed will be excreted in feces. Peak plasma fluoride concentrations are reached within 20–60 min following ingestion. The levels start declining thereafter due to two main reasons: uptake in calcified tissues and excretion in urine. Plasma fluoride levels are not homeostatically regulated and vary according to the levels of intake, deposition in hard tissues and excretion of fluoride. Many factors can modify the metabolism and effects of fluoride in the organism, such as chronic and acute acid-base disturbances, hematocrit, altitude, physical activity, circadian rhythm and hormones, nutritional status, diet, and genetic predisposition. These will be discussed in detail in this review.

Fluorine is a natural component of the biosphere. It is the thirteenth most abundant element in the earth's crust, constituting in the combined state around 0.065% by weight of the crust. Due to the small radius of the fluorine atom, its effective surface charge is the highest among all elements. As a consequence, fluorine is the most electronegative and reactive of all elements and hardly occurs in nature in its elemental form. Instead, it is found most frequently as inorganic fluoride that is widely distributed [1]. Besides its ubiquitous natural occurrence, widespread acceptance of the cariostatic properties of fluoride has led to its addition to systemic (such as water, salt, sugar, milk and supplements) and topical vehicles (such as toothpastes, gels, foams, mouth rinses and varnishes) which are widely employed for caries control [Buzalaf et al., this vol., pp. 97–114; Pessan et al., this vol., pp. 115–132; Sampaio and Levy, this vol., pp. 133–145]. It can be inferred therefore that the human organism is broadly exposed to fluoride. The main

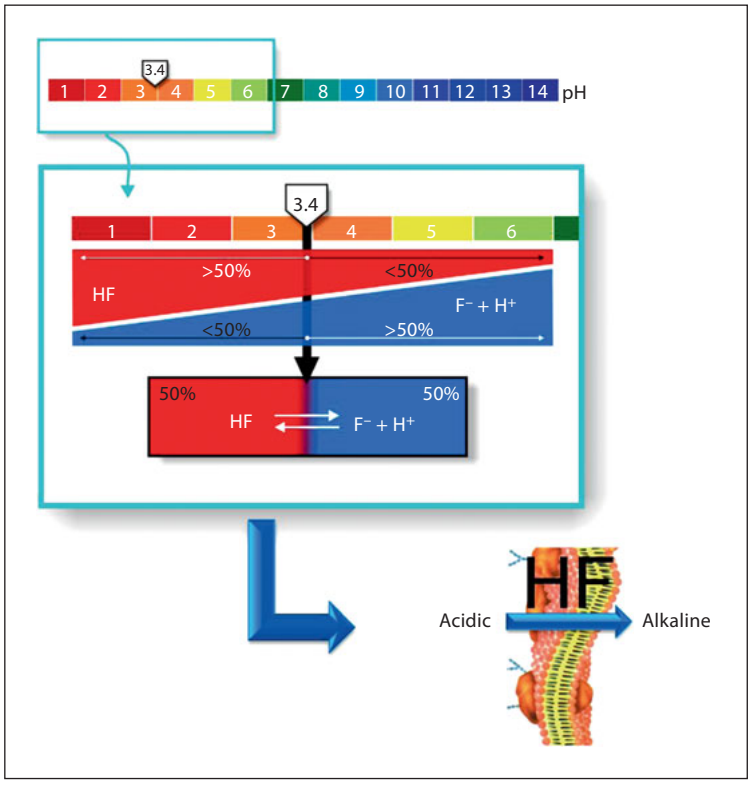

Fig. 1. pH-dependency of fluoride metabolism. HF is a weak acid with a pK_a of 3.4. Thus, at pH 3.4, 50% of fluoride is in the undissociated form (HF) while the remaining 50% is in the dissociated or ionic form (F^-). As pH decreases from 3.4, the concentration of HF increases, and as pH increases, the concentration of F^- increases. The coefficient of permeability of lipid bilayer membranes to HF is 1 million times higher than that of F^-. Therefore, fluoride crosses cell membranes as HF, in response to a pH gradient (goes from the more acidic compartment to the more alkaline compartment).

sources of fluoride intake were described in the chapter by Buzalaf and Levy [this vol., pp. 1–19].

Despite its proven benefits for caries control [2], there is a benefit/risk ratio that needs to be taken into account. The acute ingestion of a large dose can provoke gastric and kidney disturbances or even death in extreme cases [Whitford, this vol., pp. 66–80]. Lower levels of excessive intake on a chronic basis can affect the quality of the developing mineralized tissues, resulting in dental or skeletal fluorosis, depending on the amount and duration of intake [DenBesten and Li, this vol., pp. 81–96]. Thus, knowledge of all aspects of fluoride metabolism is essential for not only understanding the biological effects of this ion in humans, but also to optimize opportunities to prevent or treat cases of excess fluoride ingestion.

General Features of Fluoride Metabolism

Several aspects of fluoride metabolism – including gastric absorption, distribution and renal excretion – are pH-dependent. Hydrogen fluoride (HF) is a weak acid with a pK_a of 3.4. Thus, at pH 3.4, 50% of fluoride is in the undissociated form (HF) while the remaining 50% is in the dissociated or ionic form (F^-). As pH decreases from 3.4, the concentration of HF increases, and as pH increases, the concentration of F^- increases [3]. The coefficient of permeability of lipid bilayer membranes to HF is 1 million times higher than that of F^- [4]. This means that fluoride crosses cell membranes as HF, in response to a pH gradient between adjacent body fluid compartments, i.e. HF goes from the more acidic compartment to the more alkaline compartment (fig. 1).

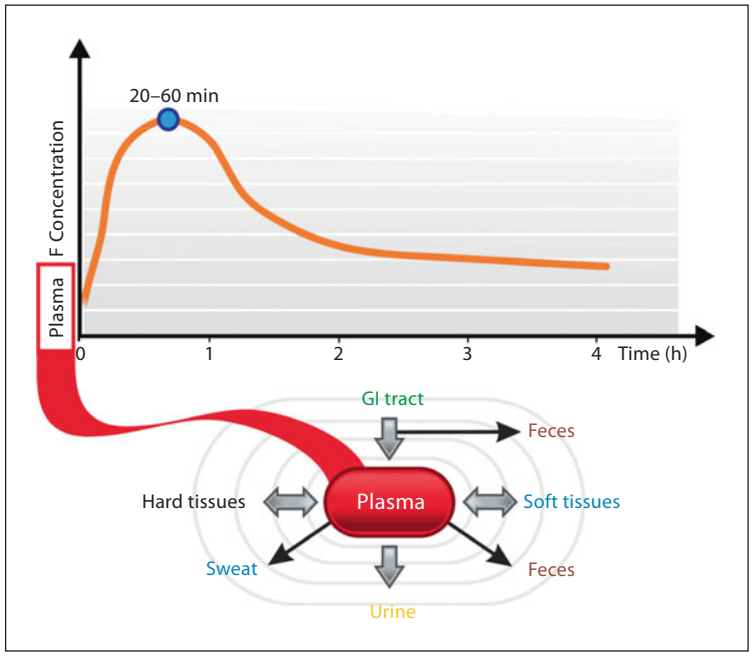

Fig. 2. Typical plasma fluoride concentration curve after ingestion of a small amount of fluoride and general features of fluoride metabolism. After ingestion, plasma fluoride levels increase rapidly, reaching a peak within 20–60 min due to absorption of fluoride in the GI tract and lung (to a lesser extent). Fluoride not absorbed will be excreted in feces. Plasma is the central compartment from which and into which fluoride must transit for its later distribution to hard and soft tissues and excretion. In adults, approximately 50% of an absorbed amount of fluoride will become associated with calcified tissues (mainly bone), where 99% of fluoride in the body is found. However, fluoride is not irreversibly bound to bone and can be released back into plasma when plasma fluoride levels fall (bidirectional arrows). A small amount of fluoride is found in soft tissues, where a steady-state distribution between extracellular and intracellular fluids is established. Most of the fluoride absorbed and not taken up by mineralized tissues is excreted in urine, while only a small amount of absorbed fluoride is excreted in sweat and feces.

General features of fluoride metabolism are described in figures 2 and 3. Figure 2 also illustrates a typical plasma fluoride concentration curve after ingestion of a small amount of fluoride. After ingestion, plasma fluoride levels increase rapidly (fig. 2) due to the ready absorption from the stomach, an event that is pH-dependent and distinguishes fluoride from other halogens and most other substances [3]. The majority of fluoride not absorbed from the stomach will be absorbed from the small intestine, but in this case absorption is not pH-dependent (fig. 3) [5, 6]. Fluoride not absorbed will be excreted in the feces [3].

Peak plasma fluoride concentrations are reached within 20–60 min following ingestion (fig. 2) and the levels start declining thereafter due to two main reasons: uptake in calcified

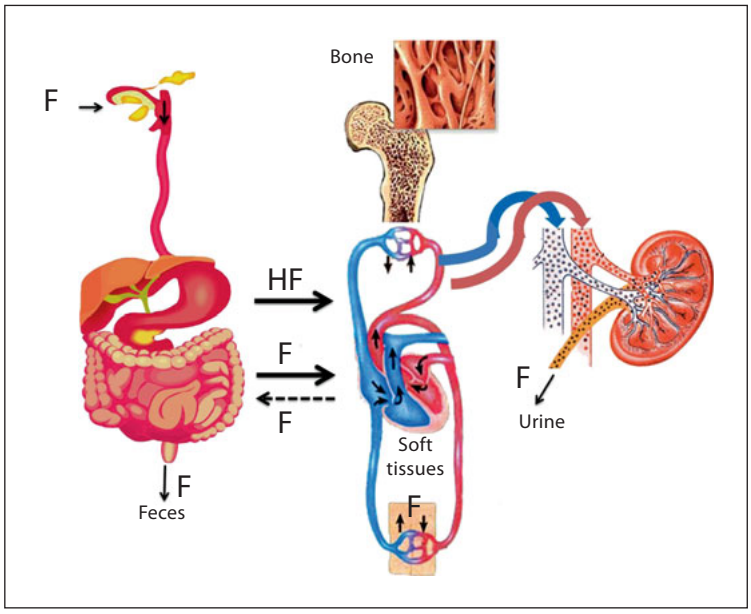

Fig. 3. General features of fluoride metabolism.

tissues and excretion in urine (fig. 2, 3). Plasma is the central compartment from which and into which fluoride must transit for its later distribution to hard and soft tissues and excretion. In adults, approximately 50% of an absorbed amount of fluoride will become associated with calcified tissues (mainly bone), where 99% of fluoride in the body is found [7]. However, fluoride is not irreversibly bound to bone and can be released back into plasma when plasma fluoride levels fall (bidirectional arrows in fig. 2, 3). A small amount of fluoride absorbed is found in soft tissues, where a steady-state distribution between extracellular and intracellular fluids is established. Most of the absorbed fluoride not taken up by mineralized tissues is excreted in urine while only a small amount of absorbed fluoride is excreted in sweat and feces. If the amount of fluoride ingested is small, the plasma fluoride concentrations return to baseline levels within 3–6 h (fig. 2) [3].

It is important to highlight that these general characteristics of fluoride metabolism are subject to variation due to dietary, environmental, genetic, physiological and pathological variables that will be discussed later in this chapter.

Fluoride Absorption

In the absence of high amounts of bi- and trivalent cations such as calcium, aluminum and magnesium that may complex fluoride and form insoluble compounds, approximately 80–90% of an amount of ingested fluoride is absorbed from the gastrointestinal tract [3]. Fluoride absorption occurs by passive diffusion (not against a concentration gradient), and is not affected by temperature changes or metabolic inhibitors. Fluoride absorption occurs rapidly, with a half time of approximately 30 min. Unlike most substances, roughly 20–25% of the total fluoride ingested is absorbed from the stomach, while the remainder is absorbed from the proximal small intestine [3, 6, 8, 9]. Although fluoride absorption from the stomach occurs rapidly, the rate

of absorption is determined by gastric acidity [10, 11] and velocity of gastric emptying [6, 12]. Other factors that influence fluoride absorption are fluoride intake with other foods [13–15] and the specific salt of fluoride ingested [13, 15, 16].

Gastric fluoride absorption is inversely related to the pH of the stomach content because, in the stomach, fluoride is absorbed predominantly as HF [10]. When ionic fluoride enters the acidic gastric lumen environment, it is converted into HF which is an uncharged molecule that readily crosses cell membranes, including the gastric mucosa [4]. Thus, the higher the acidity of the gastric content, the faster the fluoride absorption from the stomach. As a consequence, peak plasma concentrations will be reached more quickly and sooner from an acidic environment than from a more neutral environment. The pH of the solution in which fluoride is administered, under conditions of normal gastric acid secretion, has little or no effect on fluoride absorption. However, animal studies have suggested that the pH of the solution exerts a profound short-term effect on fluoride absorption when drugs that inhibit gastric acid secretion are used. Solutions with lower pH would lead to a greater rate of fluoride absorption in the short term [11]. The extent of fluoride absorption from the stomach as a function of pH has important implications both for the treatment of acute fluoride toxicity [Whitford, this vol., pp. 66–80] and the therapeutic use of fluoride.

Another factor that interferes with gastric fluoride absorption is the rate of gastric emptying. Animal studies have shown that even at early time periods, while most of the fluoride dose still remained in the stomach, the majority of fluoride absorption occurred from the proximal small intestine. Thus, delayed gastric emptying might result in slower and smaller increases in plasma fluoride levels [6, 12].

Most of fluoride that is not absorbed from the stomach will be absorbed from the proximal small intestine (around 70–75% of absorbed fluoride) [5, 6]. The small intestine has a huge capacity for fluoride absorption and fluoride is rapidly absorbed following emptying from the stomach. Fluoride absorption from the small intestine, differently from what happens in the stomach, is unaffected by pH and occurs predominantly as the ionic fluoride (fig. 3) crosses the leaky epithelia through the tight junctions between the cells or paracellular channels [5]. The massive fluoride absorption from the small intestine compensates for the low gastric absorption at high pH, so that overall fluoride absorption is relatively unaffected by gastric acidity [11].

Fluoride absorption is affected by the composition of the diet and intake with foods. For a soluble fluoride compound, such as sodium fluoride (NaF) added to water, almost 100% of the fluoride is absorbed. If fluoride is ingested with milk (or baby formula) or with foods, especially those containing high amounts of divalent or trivalent cations that can complex fluoride and form insoluble compounds, the degree of absorption is reduced [13–15, 17]. This is the basis for using calcium-containing solutions to lavage the stomach in cases of acute fluoride toxicity [Whitford, this vol., pp. 66–80].

Regarding the type of fluoride ingested, most of the published studies are in agreement that the total amount of fluoride absorbed from disodium monofluorophosphate (SMFP) is similar to that absorbed from NaF [14, 16]. However, since absorption of fluoride from SMFP requires enzymatic hydrolysis of the moiety by phosphatases, fluoride absorption from SMFP occurs more slowly than from NaF. This leads to lower and delayed peak plasma fluoride levels compared to those seen after ingestion of NaF [13, 15, 16]. Similarly, the bioavailability of fluoride when ingested from naturally or artificially fluoridated water, which usually have different fluoride compounds, does not differ [18, 19].

Fluoride Distribution

After absorption, fluoride is rapidly distributed throughout the organism. Plasma fluoride levels start to increase within 10 min following fluoride intake and peak concentrations are reached within 20–60 min. Baseline plasma fluoride levels are usually reached within 3–11 h after ingestion, depending on the amount ingested [3].

From a pharmacokinetic point of view, plasma is regarded as the central compartment for fluoride distribution, since it is the fluid from which and into which fluoride must pass to be distributed to hard and soft tissues and excreted. A small part (<1%) of absorbed fluoride is found in soft tissues, where a steady-state distribution between extracellular and intracellular fluids is established [3]. This means that when there is an increase or decrease in plasma fluoride levels, a proportional change occurs in the fluoride concentrations of the extracellular and intracellular fluids. Most fluoride absorbed (around 35% for healthy adults) is taken up by calcified tissues where fluoride is reversibly bound and can be released back into plasma when plasma fluoride levels fall (fig. 2) [7].

The quantitative and qualitative aspects of fluoride distribution to each of these compartments will be detailed below.

Fluoride in Blood Plasma

There are two general forms of fluoride in human plasma. One fraction is ionic fluoride (also called inorganic or free fluoride) that can be detected by the fluoride ion-specific electrode. Ionic fluoride is not bound to other plasma constituents and is the form of significance in dentistry, medicine and public health. In the blood, ionic fluoride is not equally distributed between plasma and blood cells (its concentration in plasma is twice as high as that found in the cells). The other fraction is the non-ionic fluoride whose biological function has not been established yet, although its concentration is usually higher than that of ionic fluoride. This fraction: (1) seems to be composed chiefly of different types of lipid-like molecules that bind to plasma proteins; (2) can only be detected in plasma by the electrode after ashing; (3) is not expected to increase with increasing levels of chronic fluoride intake, suggesting little or no exchange between the two pools. Together, the non-ionic and ionic fractions constitute the so-called 'total' plasma fluoride [3, 20].

It is important to highlight that plasma ionic fluoride concentrations, unlike most other biologically relevant ions, are not homeostatically regulated. Instead they increase or decrease according to the amount of fluoride intake, deposition and removal in soft and hard tissues and urinary excretion [3]. As a consequence, plasma fluoride levels have been used as contemporary biomarkers of exposure to fluoride (indicate present exposure), although many physiological factors can influence plasma concentrations, regardless of fluoride intake [Rugg-Gunn et al., this vol., pp. 37–51].

Distribution to Soft Tissues

Fluoride in plasma is rapidly distributed to all tissues and organs. The velocity of distribution is determined by the rate of blood flow to the different tissues [20]. When considering fluoride distribution to soft tissues, it is useful to keep in mind that fluoride accumulates in the more alkaline compartment in response to a pH gradient (diffusion equilibrium of HF across cell membranes). In other words, fluoride goes from the more acidic to the more alkaline environment (fig. 1). Considering that the cytosol of mammalian cells is usually more acidic than extracellular fluid, intracellular fluoride levels are typically 10–50% lower than those found in plasma and extracellular fluid (fig. 4, 5), as shown by short-term experiments with radioactive fluoride in laboratory animals. However, intracellular fluoride concentrations change simultaneously and in proportion to changes in plasma fluoride levels [21]. Considering that the pH gradient across the membranes of most cells can be changed by

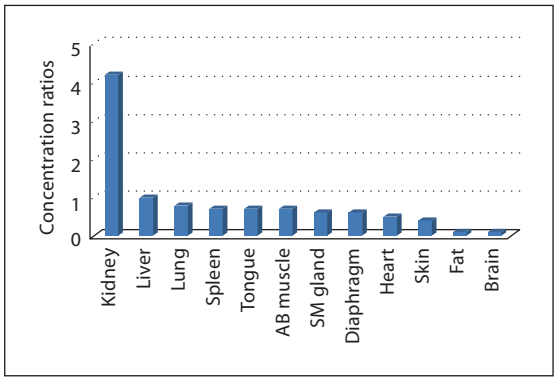

Fig. 4. Tissue/plasma fluoride (^{18}F) concentration ratios of soft tissues from the rat. AB = Abdominal; SM = submandibular [21].

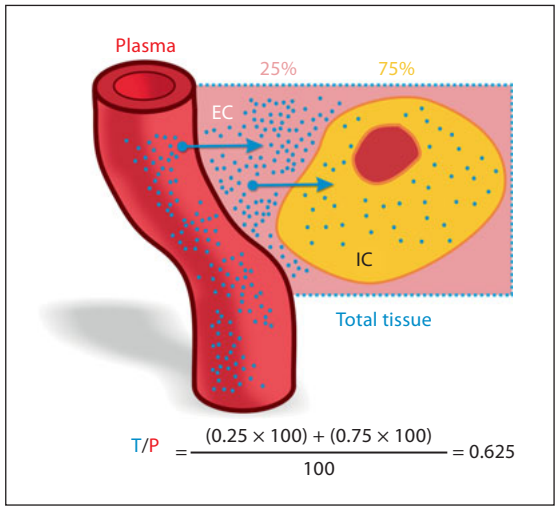

Fig. 5. Distribution of fluoride in the water spaces of soft tissues. The concentrations of fluoride in plasma and interstitial fluid are assumed to be the same. The intracellular (IC) fluoride concentration is lower than that of the extracellular (EC) fluid. T/P = Tisue/plasma. Modified from Whitford [3].

altering extracellular pH, it is possible to promote the net flux of fluoride into or out of cells. For this reason, the recommended treatment in cases of acute and potentially toxic levels of fluoride ingestion includes alkalinization of the body fluids as a means to promote a net flux of fluoride out of cells, favoring fluoride elimination in the urine [22; Whitford, this vol., pp. 66–80].

Figure 4 shows tissue/plasma fluoride (^{18}F) concentration ratios of different soft tissues from published animal studies. The ratios are typically between 0.4 and 0.9 [21]. Exceptions are the brain (<0.1), because the blood-brain barrier is relatively impermeable to fluoride, and the kidney (>4.0), due to the high fluoride concentrations within the tubular and interstitial fluids.

Distribution to Specialized Body Fluids

Fluoride concentrations in some specialized body fluids are different from those found in plasma, but the concentrations change simultaneously and in proportion to those found in plasma. This is the case for cerebrospinal fluid and milk, which have fluoride concentrations 50% or less than that of plasma [3]. Gingival crevicular fluid fluoride levels are slightly higher than those in plasma, whereas the concentrations in parotid and submandibular ductal saliva are slightly lower. Ductal salivary-to-plasma fluoride concentration ratios have been reported to be around 0.9 and 0.8 for submandibular and parotid secretions, respectively [7]. Ductal saliva has been employed as a contemporary biomarker of fluoride exposure rather than plasma to estimate the bioavailability of fluoride from fluoridated products or fluoridated water [23–25]. Whole saliva usually has fluoride concentrations more variable and higher than those seen in ductal saliva due to exogenous contamination and is not recommended to estimate plasma fluoride levels [26]. For more details, see the chapter by Rugg-Gunn et al. [this vol., pp. 37–51].

Distribution to Mineralized Tissues

Fluoride is an avid mineralized tissue seeker. Approximately 99% of all fluoride retained in the human body is found in mineralized tissues, mainly in bone but also in enamel and dentin [7]. Fluoride

concentration in bone is not uniform. In long bone, the concentrations are higher in the periosteal and endosteal regions. Cancellous bone has higher fluoride concentrations than compact bone due to its greater surface area in contact with the surrounding extracellular fluid [27]. Bone fluoride concentrations tend to increase with age due to continuous fluoride uptake throughout life [27–29].

It is estimated that approximately 36% of the fluoride absorbed each day by healthy adults (18–75 years) becomes associated with the skeleton, while the remainder is excreted in urine. In children (<7 years), the degree of retention is much higher (around 55%) [30] due to the richer blood supply and larger surface area of bone crystallites, which are smaller, more loosely organized, and more numerous than those of mature bone [7].

Fluoride uptake by bone occurs in different stages [31]. The initial uptake occurs by iso- and heteroionic exchange on the hydration shells of bone crystallites. These ion-rich shells are continuous with the extracellular fluids. In fact, it is believed that a steady-state relationship exists between the fluoride concentrations in the extracellular fluids and the hydration shells of bone crystallites. According to this concept, there is a net transfer of fluoride from plasma to the hydration shells when the plasma concentration is rising and in the opposite direction when the plasma concentration is falling [7]. For this reason, bone surface has been suggested as a terminal biomarker of acute fluoride exposure [32–34; Rugg-Gunn et al., this vol., pp. 37–51]. Later stages involve fluoride association with or incorporation into precursors of hydroxyfluorapatite and finally into the apatitic lattice itself [31].

A physiologically based pharmacokinetic model considers that bone has two compartments: a small, flow-limited, rapidly exchangeable surface bone compartment and a bulk, virtually non-exchangeable, inner bone compartment. Fluoride associated with the inner bone compartment is not irreversibly bound. Over time, it may be mobilized through the continuous process of bone remodeling in the young, bone resorption and bone remodeling in the adult [35].

Dentin fluoride concentrations are similar to bone fluoride concentrations and both tend to increase with age, i.e. they are proportional to the long-term level of fluoride intake. Dentin fluoride levels are higher close to the pulp and reduce progressively towards the dentin-enamel junction [36]. Enamel fluoride concentrations are usually lower than the levels found in dentin; no correlation has been found between the fluoride concentrations in these two dental tissues [37, 38]. Enamel fluoride concentrations tend to decrease with age in areas subjected to tooth wear, but increase in areas that accumulate dental biofilm [39]. The fluoride concentrations of tooth enamel generally reflect the level of fluoride exposure during its formation [36]. However, a significant correlation between the severity of dental fluorosis and tooth fluoride concentrations has been found for dentin, but not for enamel [37, 38, 40].

Renal handling of Fluoride

Kidneys represent the major route of fluoride removal from the body. Under normal conditions, roughly 60% of fluoride absorbed each day by healthy adults (18–75 years) is excreted in urine. The corresponding percentage for children is 45% [30]. As a consequence, plasma and urinary excretion reflect a physiologic balance determined by previous fluoride intake, rate of fluoride uptake and removal from bone and the efficiency with which the kidneys excrete fluoride.

Since ionic fluoride is not bound to plasma proteins, its concentration in the glomerular filtrate is the same found in plasma. After entering the renal tubules, a variable amount of the ion is reabsorbed (from 10 to 90%) and returns to the systemic circulation, while the remainder in excreted in urine [20]. This process, together with glomerular filtration rate, is the main determinant of the amount of fluoride excreted in urine. The reduction in

glomerular filtration rate that occurs in chronic renal dysfunction as well as in the last decades of life, when the number of functional nephrons is declining, will result in lower excretion and increased plasma fluoride levels [20, 41].

The renal clearance of fluoride (around 35 ml/min in healthy adults) is unusually high when compared with the clearance of the other halogens (usually less than 1 or 2 ml/min). There is, however, a high variation among individuals [7] that is attributed to alterations in glomerular filtration rate [42], urinary pH [43–45] and flow rate [45, 46].

The mechanism of renal tubular reabsorption of fluoride, as happens for gastric absorption and transmembrane migration of fluoride, is also pH-dependent and occurs by diffusion of HF [44]. Thus, when the pH of the tubular fluid is relatively high, the proportion of fluoride as HF is lower while there is a higher proportion of fluoride as F^-. As a consequence, only a small amount of HF crosses the epithelium of the renal tubule to be reabsorbed and a high amount of fluoride is excreted in urine as F^-. On the other hand, when the pH of the tubular fluid is lower, high amounts of HF cross the tubular epithelium into the interstitial fluid where the pH is relatively high (around 7) which promotes the dissociation of HF. F^- is then released and diffuses into the peritubular capillaries returning to the systemic circulation. The renal clearance rate, in this case, is low (fig. 6). Thus, all conditions that alter urinary pH can affect the metabolic balance and tissue concentrations of fluoride. These include diet composition, certain drugs (such as ascorbic acid, ammonium chloride, chlorothiazide diuretics and methenamine mandelate), metabolic and respiratory disorders, and altitude of residence. These will be discussed later.

Fecal Fluoride

Most of the fluoride found in feces corresponds to the fraction that was not absorbed. Fecal fluoride usually accounts for less than 10% of the amount of ingested fluoride. Thus, more than 90% of ingested fluoride is usually absorbed [47, 48].

Fluoride present in feces, however, does not correspond solely to fluoride that was not absorbed. In two other situations, increased fecal fluoride excretion has been reported in rats: when plasma fluoride levels are high and when the diet contains high amounts of calcium (1% or higher). High plasma fluoride levels would cause net migration of fluoride from the systemic circulation into the intestinal tract. On the other hand, when diets containing high amounts of calcium are consumed, it is believed that unabsorbed calcium in the chyme binds fluoride entering the intestinal tract, thus reducing the concentration of diffusible fluoride and allowing the migration of more fluoride into the tract [49].

Factors That Modify the Metabolism or Effects of Fluoride

By analyzing the general features of fluoride metabolism, it becomes clear that any condition – systemic, metabolic or genetic – which interferes with the absorption or excretion of fluoride, will influence its fate in the body, and ultimately may alter the relationship between fluoride intake and the risk of dental or skeletal fluorosis. Variables that have been reported to modify the general features of fluoride metabolism in the organism include chronic and acute acid-base disturbances, hematocrit, high altitude, physical activity, circadian rhythm and hormones [3]. Other predisposing factors suggested are impaired kidney function, genetic predisposition and nutritional status. These will be discussed in more detail below.

Acid-Base Disturbances

Due to the effects of urinary pH on the efficiency of kidneys to remove fluoride from the body, chronic acid-base disturbances play an important role on the balance and tissue concentrations of fluoride. Factors that chronically alter the acid-

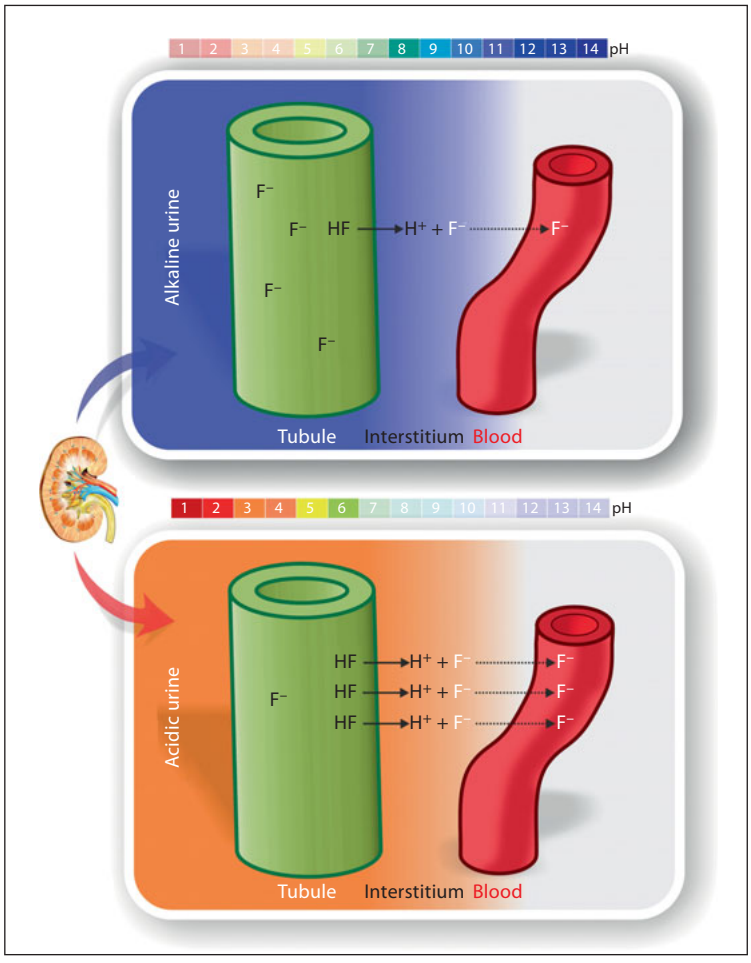

Fig. 6. Mechanism of fluoride reabsorption from the renal tubule. When urine is alkaline, there is a low concentration of HF and most of fluoride remains in the tubule to be excreted. When urine is acidic, there is a high concentration of HF that crosses the tubule membrane towards the interstitium where it dissociates originating F^- that diffuses into the peritubular capillaries and returns to the systemic circulation. Modified from Whitford [3].

base equilibrium include diet composition (vegetarian diet tends to increase urinary pH, while a diet with a high composition of meat tends to decrease urinary pH), certain drugs, a variety of metabolic and respiratory disorders, the level of physical activity and the altitude of residence [3]. Acute respiratory acid-base disorders affect renal excretion of fluoride in the same manner as the metabolic disorders [3].

Renal Impairment

Renal impairment in children has been associated with tooth defects that include enamel pitting and hypoplasia. The effects of uremia (increased concentrations of urea in blood) on tooth formation were evaluated in nephrectomized rats exposed to 0 or 50 ppm NaF in drinking water [50]. Intake of fluoride by nephrectomized rats increased plasma F levels twofold. It was also shown that uremia affected the formation of dentin and enamel, and was more extensive than the effect of fluoride alone, demonstrating that intake of fluoride by rats with reduced renal function impairs fluoride clearance from the plasma and aggravates the already negative effects of uremia on incisor tooth development. In humans, several studies have

shown a direct relationship between renal impairment and enamel defects, which include hypoplasia [51–53]. In a study comparing the frequency of dental fluorosis in children with renal disease and healthy children, although no significant difference was observed in the frequency of dental fluorosis between the 2 groups, patients with renal disease presented more severe dental fluorosis than children without renal disease [54].

Altitude of Residence

Researchers have noted that enamel disturbances are exacerbated in rats raised in hypobaric chambers which simulated high altitudes, regardless of the levels of ingested fluoride [3]. Alterations in acid-base balance, caused by hypobaric hypoxia during residence at high altitude, were cited as the cause of decreased urinary excretion of fluoride and therefore greater retention of fluoride [55]. In humans, a significantly higher prevalence of fluorosis has been observed in Tanzanian communities at a high altitude (1,463 m), in contrast with a low altitude area (100 m), but with similar food habits and low levels of fluoride in the drinking water [56]. The authors concluded that the severity of enamel disturbances at the high altitude area was not consistent with the low fluoride concentration in drinking water, suggesting that altitude, along with other factors, is a variable which may be contributing to the severity of dental enamel disturbances occurring in that area. Studies conducted in other countries confirmed this finding [57–60], suggesting that physiological changes associated with residence at high altitude are able to exacerbate the effects of fluoride in mineralized tissues. Such disturbances may be due to hypoxia in high altitude areas. This ultimately leads to a decrease in urinary pH, reducing fluoride renal excretion and, therefore, increasing fluoride concentrations in the body.

Physical Activity

In prolonged physical activity, there is a reduction in the pH gradient across cell membranes, especially skeletal muscle cells, which promotes the diffusion of fluoride (as HF) from the extracellular to the intracellular fluid. In addition, renal vasoconstriction can occur due to increased secretion of catecholamines and muscular blood flow during exercise. Depending on the balance of several factors, exercise could be associated with either decreased or increased circulating fluoride levels [3]. It must be considered, however, that although physical activity may alter the pattern of fluoride excretion, the impact of such findings on the development of dental fluorosis seem to be negligible, as prolonged physical activity in children at the age risk for fluorosis is uncommon.

Circadian Rhythm and Hormones

The possibility of existence of a biological rhythm in plasma fluoride levels was raised based on reports of circadian rhythms for calcium and phosphate [61, 62]. The daily variations of these ions are partially attributed to the balance between bone accretion and resorption, which are influenced by bone-active hormones. As the bulk of fluoride is contained in the skeleton, it was hypothesized that plasma fluoride levels would exhibit a circadian rhythm similar to, and in phase with, that of calcium and phosphate. Such rhythmicity was verified in dogs, with a mean peak fluoride concentration around 9 a.m., followed by a decrease around 9 p.m. [3].

The administration of parathormone or salmon thyrocalcitonin to humans demonstrated for the first time that alterations in hormone-mediated bone accretion and resorption are reflected in plasma and urinary fluoride levels. However, as reported in published animal studies [61, 62], the rhythmic pattern for calcium and phosphate occurred in the opposite way of that verified for fluoride, suggesting that a physiological system, other than bone, would be the responsible for the characteristics of the biological rhythmicity of fluoride in plasma. A recent study suggested that the renal system is involved with such rhythmicity in humans. Cardoso et al. [63] demonstrated

a rhythmicity for fluoride concentrations in plasma, with mean peak (0.55 μmol/l) at 11 a.m. and the lowest concentrations (mean of 0.50 μmol/l) occurring between 5 and 8 p.m. Plasma fluoride concentrations were positively correlated with urinary fluoride excretion rates and with serum parathormone levels, suggesting that both the renal system and hormones might be involved in the rhythmicity for plasma fluoride concentrations in humans. It was also recently demonstrated that the diurnal average fractional urinary fluoride excretion is significantly lower than the average nocturnal one [64], which is in line with the findings of Cardoso et al. [63] and the suggested rhythmicity for plasma fluoride concentrations. The existence of this rhythmicity may alter the relationship between fluoride intake and the risk of dental or skeletal fluorosis.

Nutritional Status

Although an association between malnutrition and dental fluorosis prevalence and severity has been suggested for decades, the evidence for such a relationship is controversial and difficult to interpret. If a fasting child may absorb fluoride from water or other sources more quickly than a well-fed child (due to the inexistence of complexes of fluoride in an empty stomach), a malnourished child, on the other hand, may have low fluoride deposition over a long-term period of time (due to slower bone growth).

A statistically significant relationship between water fluoride concentration, socioeconomic status, nutritional status and the prevalence of diffuse enamel lesions (DDE index) in boys from Saudi Arabia has been demonstrated by Rugg-Gunn et al. [65]. Although the diffuse enamel defects of the DDE index are considered as an indicator of dental fluorosis, direct comparisons between the DDE index and specific dental fluorosis indices have been discouraged [66]. In a study with Tanzanian children, Yoder et al. [56] suggested a direct relationship between malnutrition and dental fluorosis. Such assumptions, however, must be considered with caution to avoid misinterpretation. The authors correlated their findings (high prevalence of dental fluorosis) with previous information on nutrition in 2 of the 3 areas evaluated, but no direct comparison between children with or without malnutrition regarding the prevalence of dental fluorosis was carried out.

Correia Sampaio et al. [67] demonstrated that dental fluorosis is independent of nutritional status. Nutritional status was assessed by the height-for-age (chronic malnutrition) and weight-for-age (general malnutrition) indexes, recommended by the WHO. A significant relationship between dental fluorosis and water fluoride concentration was found, but not with regard to nutritional status or sex. Dental fluorosis may be related to other factors, like infant dietary habits or increased consumption of fluoridated water. Future studies on this subject should consider a longitudinal study design where nutritional status, infant dietary habits and fluoride intake are assessed during the tooth formation period. This is particularly important for developing countries, where malnutrition and dental fluorosis are prevalent and fluoride-containing products are introduced in order to control dental caries.

Diet Composition

The acidification and subsequent alkalinization of urine by ingestion of NH_4Cl and $NaHCO_3$, respectively, led to significant differences in urinary fluoride clearance and plasma half-lives of 5 adult volunteers [68]. Similar findings were obtained by acidifying and alkalinizing urine by following a protein-rich (meat/dairy products) and a vegetarian diet, respectively. These results strongly suggested that long-term diet-induced changes in urinary pH could decrease (alkaline urine) or increase (acidic urine) the risk of dental fluorosis [55]. The prevalence and severity of dental fluorosis were compared among vegetarian and non-vegetarian children and adolescents living in an area with endemic dental fluorosis in India [69]. Vegetarianism was inversely associated with the

prevalence of dental fluorosis. The prevalence and severity (Thylstrup and Fejersko index ≥4) of dental fluorosis were 67 and 21%, respectively, in the vegetarian group, and 95 and 35%, respectively, in the nonvegetarian group ($p < 0.05$). In addition, multiple logistic regression analysis showed that the risk of developing dental fluorosis was 7 times higher among nonvegetarians than among vegetarians. Tamarind has also been shown to increase urinary fluoride excretion by increasing urinary pH in schoolchildren [70]. In a study conducted in a fluoride endemic area in South India, a significant decrease in urinary fluoride excretion was seen after volunteers were supplied with defluoridated water for 2 weeks. Then half of the subjects were supplemented with tamarind for 3 weeks, while the control group received defluoridated water for the same period. A significant increase in fluoride excretion and urinary pH was observed in the experimental group [71]. Tartaric acid is a major component of the tamarind paste (8.4–12.4%), which does not get metabolized and is excreted as such through the urine.

Other dietary constituents also seem to play an important role in the balance between fluoride and fluorosis. High dietary concentrations of certain cations, especially calcium, can reduce the extent of fluoride absorption [49]. In a study conducted in the province of Jiangxi, China, where the prevalence of dental fluorosis is reported to be above 50%, the incidence rates of dental fluorosis were found to differ markedly, depending on whether or not the children consumed milk. The rate of dental fluorosis of the milk-drinking group was 7.2%, whereas that of the non-milk-drinking group was 37.5% [72]. In India, where approximately 62 million people (including 6 million children) have dental fluorosis (mainly endemic), some studies have been conducted in order to identify components other than fluoride associated with an increased risk of dental fluorosis. Low calcium concentrations in the drinking water were demonstrated to be inversely related to the prevalence of dental fluorosis [73, 74]. It was suggested that calcium supplementation should be implemented in areas with endemic fluorosis in order to minimize the effects of fluoride on mineralized tissues [74]. However, there is not enough evidence to support this, since none of the above-mentioned studies were able to determine the effect of calcium alone in communities with similar background exposure to fluoride from water.

The usual diet also appears to be important. Fluoride retention and resulting toxicity were found to be higher with sorghum (also called jowar) or sorghum-based diets than with rice- or wheat-based diets when the fluoride intakes were similar. Fluoride excretion in urine was significantly high on rice-based diets as compared with the sorghum-based diet [75].

Genetic Factors

Epidemiological observations of marked variation in dental fluorosis prevalence in subjects from areas with comparable levels of fluoride intake [56], or even in studies showing different degrees of susceptibility to fluorosis between certain ethnic groups [76–78] have led to the assumption that the predisposition to dental fluorosis is genetically determined [79, 80].

In a study conducted with Tanzanian children from three distinct areas, which differed regarding water fluoride concentrations and altitude, it was observed that even in the two sites with more severe fluorosis, several children had very little evidence of enamel disturbances [56]. These 'resistant' children were lifelong residents of the same area of the 'susceptible' children. Urinary fluoride values and meal fluoride values from children were also similar between the two groups. The question of possible genetic influence became more evident due to the tribal homogeneity in the area were fluorosis prevalence was unexpectedly high (no fluoridated drinking water).

The possibility of genetic predisposition to dental fluorosis was demonstrated using a

mouse model system where genotype, age, gender, food, housing and drinking water fluoride levels were under control [81]. Examination of 12 inbred strains of mice showed differences in susceptibilities to dental fluorosis. The A/J mouse strain was highly susceptible, with a rapid onset and severe development of dental fluorosis compared to the other strains tested, whereas the 129P3/J mouse strain was less affected, with minimal dental fluorosis. It was latter demonstrated that these 2 strains also have different bone responses to fluoride exposure [82]. It was hypothesized that the different susceptibility to dental fluorosis between these two 2 strains was due to differences in fluoride metabolism, i.e. it was expected that the resistant strain would excrete more fluoride which in turn would lead to decreased susceptibility to dental fluorosis. Thus, a metabolic study was conducted to test this hypothesis. Surprisingly, the resistant strain (129P3/J) excreted a significantly lower amount of fluoride in urine than the susceptible strain (A/J) and, as a result, had significantly higher plasma and bone fluoride concentrations. Despite this, the amelogenesis in the 129P3/J strain was remarkably unaffected by fluoride [83]. Dental fluorosis-associated quantitative trait loci were detected on mouse chromosomes 2 and 11. Histological examination of maturing enamel showed that fluoride treatment resulted in accumulation of amelogenins in the maturing enamel of A/J mice, but not of 129P3/J mice [84]. The physiological, biochemical and/or molecular mechanisms underlying this resistance remain to be determined.

In humans, the possibility of gene-environment interaction was assessed by determining differential susceptibility to fluorosis at a given level of fluoride exposure based upon genetic background. A case-control study was conducted among children between 8 and 12 years of age with (n = 75) and without (n = 165) dental fluorosis in two counties in Henan Province, China. The PvuII and RsaI polymorphisms in the COL1A2 gene were genotyped. Calcitonin and osteocalcin levels in the serum were measured. Children carrying the homozygous genotype PP of COL1A2 PvuII had a significantly increased risk of dental fluorosis (OR = 4.85, 95% CI: 1.22–19.32) compared to children carrying the homozygous genotype pp in an endemic fluorosis village. However, the risk was not elevated when the control population was recruited from a non-endemic fluorosis village. Additionally, fluoride levels in urine and osteocalcin levels in serum were found to be significantly lower in controls from non-endemic villages compared to cases. However, the differences in fluoride and osteocalcin levels were not observed when cases were compared to a control population from endemic fluorosis villages. This study provided the first evidence of an association between polymorphisms in the COL1A2 gene with dental fluorosis in high-fluoride-exposed populations [85].

Conclusion

In view of the diverse effects that fluoride can produce in biological systems, it is not surprising that it has been the subject of thousands of scientific reports. It is clear that the beneficial as well as the adverse effects of fluoride can be attributed to the magnitude and duration of the concentration of the ion at specific tissue or cellular sites. In addition to the level of prior fluoride exposure, these concentrations are determined by the characteristics of the general metabolism of fluoride within the individual. As has been made clear in this chapter, these characteristics are not constant within or among individuals or populations. Instead they are subject to the effects of diverse environmental, biochemical, physiological and pathological factors. While much has been learned during the last few decades, much remains to be done – particularly in clearly defining the mechanisms underlying the metabolism and biological effects of fluoride. With the continuing

development of advanced analytical, diagnostic, molecular and genetic techniques, we can expect our knowledge to grow and, with that growth, the beneficial effects of fluoride will be enhanced and the unwanted effects minimized.

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