

Effect of Level of Fluoride Ingestion on Fluoride Uptake

In all hard tissues at all stages of development, the level of fluoride is related to ingestion.

Bones

Zipkin et al. (1958) showed that the relationship between the fluoride concentrations in different bones from the same individual is maintained at varying levels of fluoride ingestion. Reports from many animal experiments also agree that there is a fairly consistent pattern of fluoride distribution between the different types and regions of bone at various levels of fluoride administration (see Table 10).

Such consistent relationships are maintained so long as the intake of fluoride is not so high, or of such duration, as to cause the skeletal changes associated with skeletal fluorosis. The most obvious symptom of this condition is the pathological growth of exostoses, the sites and forms of which are many and various. In man, these may occur after long periods of fluoride ingestion at levels of 4-8 ppm or above in water. Wherever such exostoses arise, the uptake of fluoride increases. Analyses of exostotic bone and adjacent normal bone from fluorotic animals clearly demonstrated the enhanced fluoride uptake in the rapidly forming exostotic bone (Weidmann & Weatherell, 1959).

Teeth

In teeth, as in bone, the concentration of fluoride relates closely to the quantity ingested. Fluoride levels of both dentine and enamel correlate well with the concentration of fluoride in the drinking water (Table 11). Although the absolute levels may rise or fall, depending on fluoride intake, the differential between dentine and enamel is maintained throughout the life of the tooth (Jackson & Weidmann, 1959).

The figures given in Table 11 suggest a linear relationship between the level of fluoride in the drinking water and the concentration of the element in the enamel surface. Such remarkable correlation, however, is to some extent fortuitous, because the variations between different individual age-groups will be appreciable and the thickness of the surface layer analysed by the different workers extremely variable.

During the period of tooth formation, the ingestion of amounts of water-borne fluoride as low as 1 ppm may produce slight white spots in the enamel surfaces in a few cases. These may be due to the effect of fluoride upon the ameloblast during tooth formation. They are not aesthetically displeasing and resemble other imperfections in enamel commonly seen in teeth from districts where the fluoride content of the drinking water seems low

TABLE 11
FLUORIDE CONCENTRATION (EXPRESSED AS PPM IN ASH) IN DENTINE AND ENAMEL AT
DIFFERENT LEVELS OF FLUORIDE INGESTION

Reference	Species	Age	Dose (ppm F in water)	Duration	Enamel			Dentine whole
					surface	interior	whole	
McClure & Likins (1951)	Man	Adult	0.1 7.6	Life-long Life-long	— —	— —	86 658	332 1 968
Jackson & Weidmann (1959)	Man	20-49 years	<0.5	Life-long	—	—	108	508
		20-35 years	1.2	Life-long	—	—	180	922
		20-35 years	1.9	Life-long	—	—	320	1 290
Jenkins & Speirs (1953)	Man	Adult	<0.25	Life-long	590	80	—	—
			1.4	Life-long	960	110	—	—
			2.0	Life-long	1 310	270	—	—
Brudevold, Steadman & Smith (1960)	Man	20-29 years	0.1	Life-long	571	48	—	—
			1.0	Life-long	889	129	—	—
			3.0	Life-long	1 930	152	—	—
			5.0	Life-long	3 370	570	—	—

enough to preclude the possibility of fluorosis. Higher levels of fluoride ingested during amelogenesis increase both the incidence and the severity of the fluorotic lesions.

Influence of Fluoride Saturation

Fluoride is taken up at crystallite surfaces and it has been debated whether after long periods of exposure, or at relatively high levels of incorporation, saturation of the available sites might occur and subsequent uptake be reduced.

Bones

Several workers have noticed that the rate of uptake by bones of experimental animals gradually decreased as the periods of fluoride exposure were prolonged. This observation could imply saturation, but might also be partly due to a gradual fall in bone activity or growth rate as the animal aged during the period of fluoride administration. Suttie & Phillips (1959) found, however, that both young and old rats tended to take up fluoride less rapidly when they had been subjected to prior doses of the element. A diet containing 500 ppm fluoride had been supplied to the rats in question, and it seems feasible that at such high dosage some saturation of available bone surfaces might occur. At much lower levels of ingestion, such as those usually encountered by man, it is extremely unlikely that any saturation could arise. The normal processes of bone activity, i.e., exchange,

recrystallization and remodelling, would ensure a constant renewal of bone mineral, either by removing fluoride from the crystal surfaces by back-exchange and recrystallization, or by replacement of fluorotic bone with new bone during the normal process of remodelling.

Teeth

In dentine and cementum the situation appears somewhat analogous to bone; at low rates of ingestion the continued formation of tissue will tend to offset fluoride saturation.

Enamel presents a somewhat different picture. Once enamel has been formed, cellular activity ceases and the incorporation of fluoride depends solely upon ion-exchange mechanisms. Penetration of fluoride into the interior regions of enamel is obstructed by the chemical trapping of ions in the outer regions and, presumably, by the low permeability of this highly calcified structure. This results in the precipitous fall of fluoride concentration from external to internal regions shown in Fig. 2.

The concentration of fluoride at any point within the enamel appears to fall exponentially from surface to interior. Saturation might occur in the surfaces of the outermost crystallites, but this remains a matter of conjecture. Perhaps the slight wear to which tooth surfaces are subjected is sufficient to prevent it. Whether saturation does occur in the outermost layer is difficult to assess. Chemical analysis gives only an average value and can never reveal variations in the fluoride content of crystal surfaces. In shark teeth, and probably in those of some other fish continually exposed to the high fluoride content of a marine environment, almost complete deposition of tooth mineral as fluorapatite (i.e., 3.8% F) has been demonstrated (Büttner, 1966). The surface layers of enamel from human teeth rarely contain even a third of this amount.

Removal of Fluoride from Hard Tissues

The discussion so far has not taken into account the circumstances by which fluoride might be removed from skeletal and dental systems. Several authors have noticed that, once present, fluoride is lost with difficulty from hard tissues. Savchuck & Armstrong (1951) observed that after withdrawal of fluoride approximately 10-15% was eliminated from the rat skeleton in 40 days; the remainder appeared to be more firmly fixed and after 40 days the level changed little. McCann & Bullock (1957) also found that after long periods of fluoride exposure *in vivo* or *in vitro* skeletal fluoride was removed with difficulty. They suggested that, although fluoride incorporated by surface exchange may be removable, that which is more deeply incorporated into the crystal is removed by an extremely slow reaction.

Bones

Fluoride is certainly not irrevocably deposited in skeletal tissues. Experiments with rats of various ages have shown that there is an initial rapid decrease in the skeletally bound fluoride, followed by a more gradual removal (Savchuck & Armstrong, 1951). Hodge (1952) pointed to a similar phenomenon in man. He offered the explanation that the escape of fluoride from mineral to tissue fluids by back-exchange with ions in the hydration shell could account for the relatively rapid loss of fluoride in the period immediately following incorporation.

Whereas there are good reasons to believe that the element will be removed relatively quickly as long as it is situated at the surfaces of crystallites, there is no direct experimental evidence in support of Hodge's hypothesis. It is not possible to say, therefore, to what extent exchange or osteoclastic activity is responsible for the initial removal of fluoride from the skeleton. According to Hodge, the remodelling of bone by osteoblastic and osteoclastic activity was responsible for the more gradual, later phase of fluoride removal, accounting for a slower but considerable loss of the element. There is no doubt that some of the incorporated fluoride will eventually be buried deeply by crystal growth and subsequent apposition of new tissue. In this state, although fluoride could not escape from the mineral crystallites by exchange, it could still be released by osteoclastic resorption.

Measurements of fluoride levels in bone or urine give no direct indication of the extent of fluoride mobilization, for some of the fluoride released may be reincorporated. Likins et al. (1959) found that, although some of the fluoride present in the proximal metaphysis of the growing rat tibia had been lost during bone growth, there was considerable uptake in the adjacent developing bone segment. They came to the conclusion that fluoride, released during remodelling, did not necessarily enter the general circulation but redeposited in nearby sites of growth. It does indeed seem likely that some fluoride released into tissue fluid by resorption or exchange will redeposit rapidly in adjacent areas of active formation.

The evidence of fluoride removal from the human skeleton rests entirely upon measurements of urinary excretion rates. There is no way of directly detecting skeletal fluoride loss in man. Largent (1961) examined the urinary excretion of stored fluoride in persons who had ingested large amounts for long periods. For some time after discontinuation of fluoride administration, urinary excretion remained in excess of ingestion. The levels of excretion fell according to an exponential equation and were still in excess of intake in the 96th week after discontinuation of high-level absorption. Largent estimated that the decline of excess urinary fluoride reached its midpoint in 75 to 80 weeks and that a state of balance was reached in 200 to 225 weeks. He assumed that eventually the urinary fluoride concentration would reach a level similar to that in the drinking water. This

assumption is supported by the evidence of McClure & Kinser (1944), who found that the concentration of fluoride in human urine bore a linear relationship to the fluoride content of the domestic drinking-water supply.

According to Largent (1961), the total amount of fluoride deposited in bones relates to the level of fluoride ingestion. So long as the level of ingestion remains unchanged, any further storage will eventually be offset by the mobilization of some previously stored fluoride. A balance appeared to be maintained between absorption and storage, on the one hand, and mobilization and excretion on the other. In the case of ten human subjects, he found that intake and output of fluoride were nearly equal. Two residents from each of five cities were examined, the fluoride of the water supplies in these cities ranging from 2 to 20 ppm. The findings suggested that in each of the five cities the residents stored enough fluoride to reach a state of equilibrium. This appears to support the view expressed by several workers that the fluoride concentration of bones increases with age for a time but that eventually a steady state is reached, after which there is no further rise in skeletal fluoride concentration. More will be said about this later.

Teeth

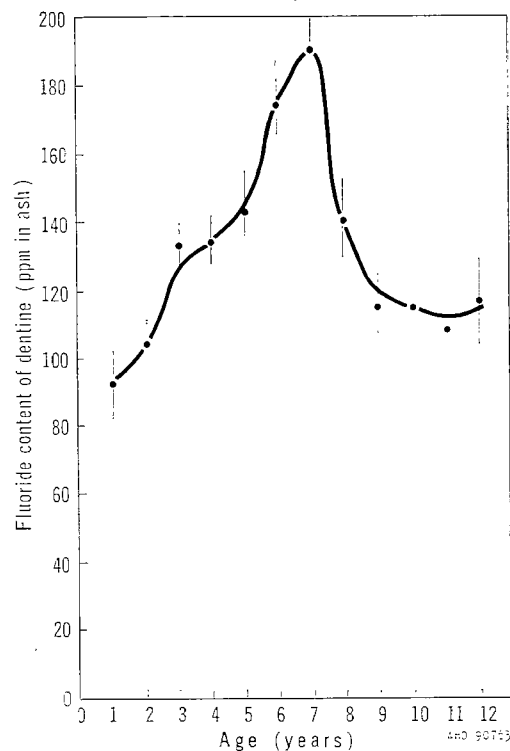
Direct evidence of fluoride removal from dentine emerged from a study of deciduous teeth. Hargreaves & Weatherell (1965) showed that the fluoride concentration of dentine from the crowns of deciduous teeth rose with age to a maximum and then dropped steeply when the process of resorption, which precedes the exfoliation of deciduous teeth, began (Fig. 3). The fall in fluoride level appeared to be due entirely to the osteoclastic removal of high-fluoride dentine from the pulpal surface.

There is little information about the lability of enamel-bound fluoride. No doubt the incorporated fluoride of the interior regions is firmly fixed, but that present in the tooth surface may be less permanently bound. Some surface fluoride may escape by exchange with the ions present in saliva and some may be lost by wear due to attrition or abrasion during mastication (Hallsworth & Weatherell, 1969). In the continuously growing teeth of rodents, fluoride is lost as teeth grow and are continually worn away. Weidmann (1962) demonstrated this type of fluoride loss in the case of rat incisors; in the permanent dentition of the cat such loss was not observed. It seems likely that the wear sustained by enamel during the long life-span of human teeth might result in the removal of a few microns of tissue from the enamel surface, together with its associated fluoride. This could explain the low levels of fluoride found in the labial surfaces of some incisors from persons over the age of 50 (Weatherell & Hargreaves, 1966).

In conclusion, three mechanisms have been considered for the removal of fluoride from hard tissues: back-exchange with ions contained in tissue

FIG. 3

VARIATIONS WITH AGE IN THE FLUORIDE CONTENT OF DECIDUOUS DENTINE IN SECOND MOLAR TEETH FROM CHILDREN LIVING IN AREAS SUPPLIED WITH DRINKING WATER CONTAINING < 0.5 ppm F (STANDARD DEVIATION SHOWN FOR EACH AGE-GROUP)^a



^a After Hargreaves & Weatherell (1965).

fluids; resorption of tissue; and mechanical abrasion. A major loss of fluoride by back-exchange is difficult to visualize since it would act against surface accumulation, which is a well-established fact. In contrast, resorption has been proved to remove demonstrable amounts of fluoride. Whether it is the sole agent or not will have to await further information about bone activity.

Effect of Age on Accumulation of Fluoride

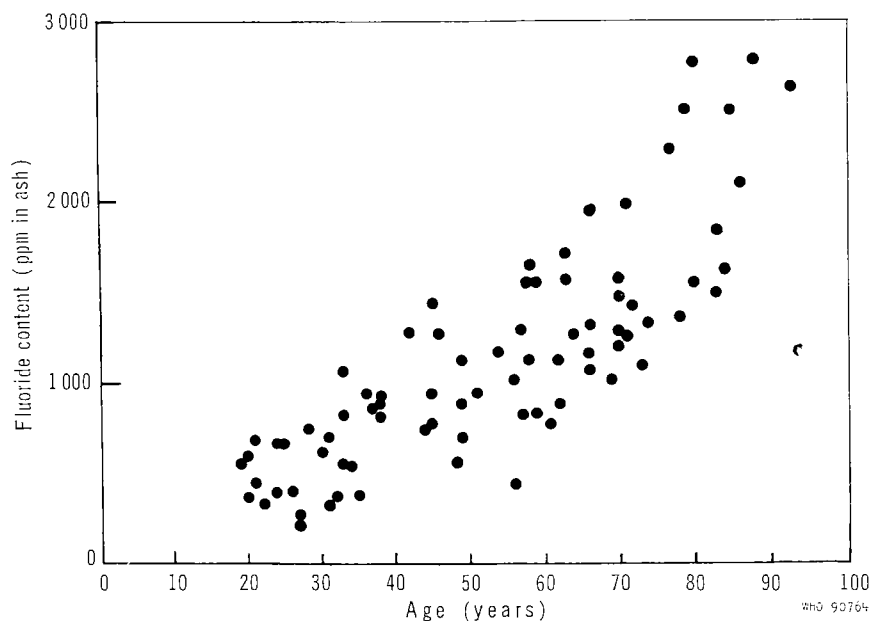
Estimations of fluoride levels show that both bones and teeth tend to accumulate fluoride with age. Provided that the level of ingestion does not decrease, the balance between fluoride uptake and removal is clearly in favour of uptake.

Bones

The concentration of fluoride in bone undoubtedly increases with time and a number of surveys have been carried out to establish the extent to which the element accumulates throughout life. The first investigation into human bone of different ages was that of Glock, Lowater & Murray (1941), who estimated the fluoride content of ground samples from 14 whole ribs taken *post mortem* from persons in two London hospitals. A rise in fluoride content with age was clearly demonstrated, but the rate of accumulation could not be established because of the large scatter of values. Such scatter is inevitable when samples are taken indiscriminately from a bone with so variable a structure as rib. The wide disparity between the fluoride levels of tissue surfaces compared to interior regions and between compact and cancellous bone has been stressed earlier. Reliable sampling techniques are, therefore, crucial, since comparisons between the fluoride content of specimens from different individuals can only be made when they are carefully chosen from similar anatomical sites and structures. To avoid errors arising from injudicious faulty sampling, Smith, Gardner & Hodge (1953) compared specimens of rib cortex and Jackson & Weidmann (1958) restricted their analyses to rib cancellum. Both groups of workers demonstrated that the fluoride content of bone rose with age. But whereas the results of Smith and co-workers (*op. cit.*) seemed to indicate a linear increase in fluoride concentration with age, Jackson & Weidmann (1958) found that fluoride tended to accumulate less readily in older specimens and that a plateau level of fluoride concentration was reached at the age of about 55. Blayney, Bowers & Zimmerman (1962) made a survey of iliac crest specimens and came to conclusions similar to those of Jackson & Weidmann. It is doubtful, however, whether either of these two studies presented conclusive evidence that a plateau was reached, because the scatter of results was very large and the number of observations relatively small. Zipkin et al. (1958) presented data from analyses of iliac crest, rib and vertebrae from persons who lived in areas with 0.1-4 ppm fluoride in the drinking water. No levelling with age was observed in these predominantly cancellous structures in either low (0.1 ppm) or high (2.6 ppm) fluoride groups, although the years of residence varied between 10 and 90 years. Recent analyses (Fig. 4) of compact bone from the femora of persons living in an area containing 0.1 ppm fluoride in the drinking water do not suggest that the rate of fluoride accumulation in this tissue declines with age (Weatherell, 1966). More work is required before any categorical statement about reaching a plateau level can be made.

It is difficult to predict from the knowledge of bone activity what the true situation might be. Assuming that the rate of bone formation decreases as the skeleton ages, it would seem reasonable to expect that less fluoride is taken up by older bone. There is, on the other hand, less remodelling in the older skeleton as well as less growth and, therefore, a larger fraction of

FIG. 4
 FLUORIDE CONTENT OF FEMORAL COMPACTA FROM HUMANS OF DIFFERENT
 AGES LIVING IN DISTRICTS SUPPLIED WITH DRINKING WATER CONTAINING
 $< 0.5 \text{ ppm F}^a$



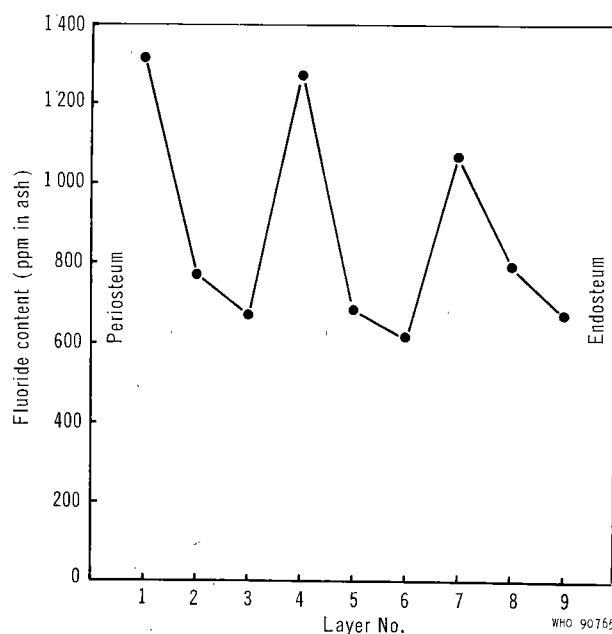
^a After Weatherell (1966).

“static” bone in which fluoride might gradually accumulate by ionic exchange. It should also be considered what differences could exist in these respects between cancellous and cortical structures. Only further data can resolve these questions.

It can be visualized that less fluoride leaves the bones than enters them by exchange. This would imply that the fluoride ion present in tissue fluid exchanges more readily with groups of the apatite crystallite than *vice versa*. Such a tendency is quite feasible in view of the greater stability and insolubility of fluorapatite in comparison with hydroxy- or carbonato-apatites. This view is empirically supported by the fact that hard tissues, e.g., the surface of enamel, or archaeological specimens of bones and teeth buried for long periods, absorb and accumulate fluoride by exchange even when the fluoride concentration in the surrounding environment is low. Thus, fluoride might accumulate by exchange not only in new bone, when the apatite crystallites are small and well hydrated, but also in the mature, less available crystal surfaces of biologically static regions, uninvolved in the processes of remodelling. In older persons small areas of bone very likely persist for many years in such a static state, during which time they might

well accumulate comparatively high levels of fluoride by exchange. If the level of fluoride ingestion remains low, areas of newly formed bone might therefore have considerably lower levels of the element than older inactive regions. The distribution of fluoride across the cortex of an adult human femur can occasionally reveal a very complex situation in which extreme differences in fluoride concentration are found in contiguous layers of bone (Fig. 5). The most likely explanation of this phenomenon seems to be that the regions of bone containing relatively low levels of fluoride were of more recent origin than those corresponding to the peak values.

FIG. 5
FLUORIDE DISTRIBUTION ACROSS THE FEMORAL CORTEX OF AN ADULT HUMAN



The over-all accumulation of fluoride with age, however, might not only result from an increase of fluoride in static regions, but also be due to a progressive rise in the fluoride content of the more labile areas. If, as Likins et al. (1959) suggested, some of the fluoride mobilized by bone resorption is re-incorporated into adjacent areas of formation, the tendency in labile regions would be towards a fluoride increase. In adult individuals the amount of bone resorbed is balanced by the amount formed. Should the newly laid down bone always contain some of the fluoride mobilized from nearby areas of resorption, however, there will be a small but continuous gain of fluoride.

The process of bone removal itself could lead to an enhancement of the bone fluoride levels. While it is debatable whether the osteoclast can discriminate between fluoridated and non-fluoridated bone, resorption usually affects anatomically specific areas of tissue, e.g., the endosteal region of long bones from which in old persons a considerable proportion of bone may be removed. Such resorption would increase the fluoride concentration of the tissue if the region removed had relatively low levels of fluoride, leaving behind areas of bone which fortuitously contained higher concentrations of the element. An alteration in fluoride concentration by this means was described earlier (see page 121); in the case cited, the preferential removal of high-fluoride dentine from the pulpal surfaces of deciduous teeth led to a lowered fluoride concentration. In the human femur, the rise in fluoride concentration after the age of 50 (Fig. 4) may partly be due to the preferential removal of low-fluoride endosteal bone as the cortex thins with age.

Finally, the purely speculative point might be mentioned as to whether a preferential dissolution of bone mineral might occur, leaving behind an increased concentration of fluorapatite. Such a possibility has been mooted (Neuman & Neuman, 1958; Jenkins, 1962) but there is no experimental evidence that osteoclasts are influenced by fluoride concentration.

The question of skeletal fluoride accumulation is clearly a complicated one. It is still a matter of conjecture whether predominance of exchange over back-exchange, reabsorption of mobilized fluoride into areas of bone formation, or the relative insolubility of fluorapatite is the most important factor contributing to accumulation of the element in the skeleton.

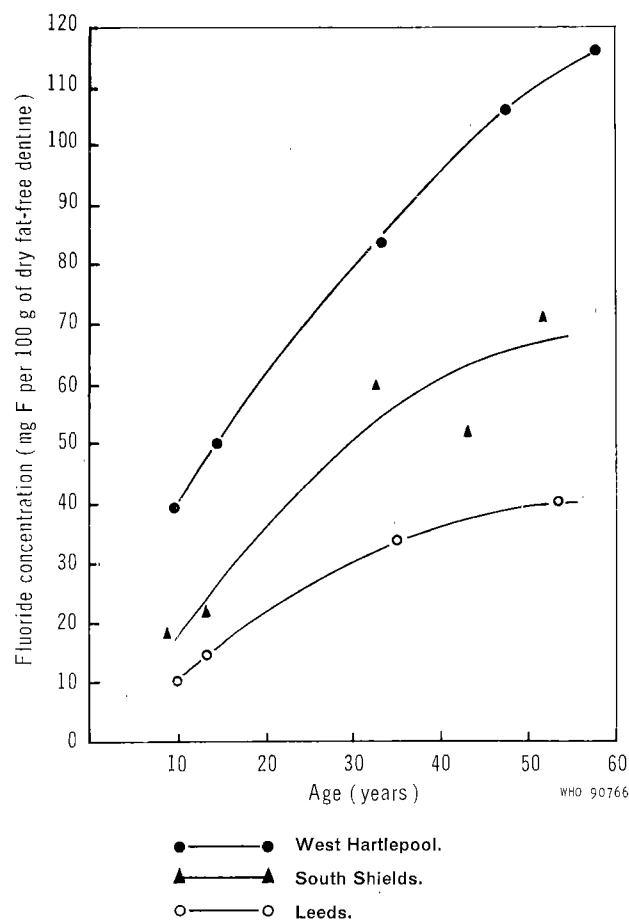
Teeth

Cementum, like bone, appears to absorb high levels of fluoride, but no information is available about its change in fluoride concentration with age.

The levels of fluoride in dentine and enamel are considerably lower than those in bone from the same individual. A relatively rapid increase in the average fluoride concentration is found during the early years of a tooth's life, but this rate of accumulation gradually declines as the tooth ages. Jackson & Weidmann (1959) demonstrated this pattern in both dentine and enamel of teeth from districts where different concentrations of fluoride were present in the water supply. The trends with age are shown in Fig. 6 and 7. The general pattern has been confirmed by Armstrong & Singer (1963).

The non-uniform distribution of fluoride in both dentine and enamel greatly reduces the value of results obtained by analysis of average samples. After the tooth has fully formed, fluoride is chiefly incorporated at tissue surfaces. There is no information about the way the fluoride level in the

FIG. 6
RELATIONSHIP BETWEEN AGE AND DENTINAL FLUORIDE
IN HUMAN PREMOLARS^a



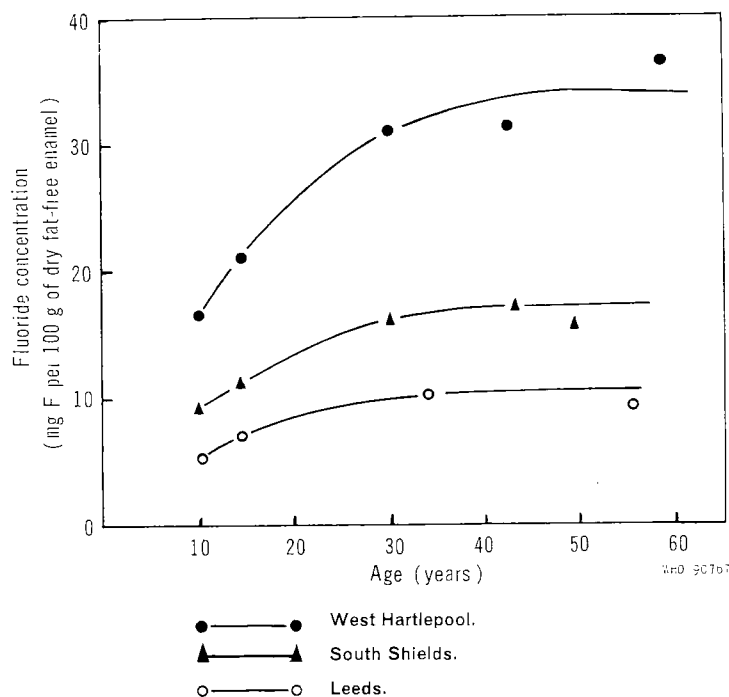
^a After Jackson & Weidmann (1959).

pulpal surface of permanent dentine changes with age. Since fluoride seems to be taken up more readily by secondary than by primary dentine, alterations in its concentration at the pulpal surface will probably to some extent depend upon the rate and amount of secondary dentine formation.

Fluoride in the outer regions of enamel might be expected to increase in concentration both with the level of ingestion and with age. The former relationship has been clearly established both in permanent (Brudevold, Gardner & Smith, 1956) and in deciduous teeth (Weatherell & Hargreaves, 1966). Both the level of fluoride in the enamel surface and the penetration

of the element into subsurface regions and concentration in the tissue interior relate to the fluoride content of the drinking water. The change in concentration in the enamel surface with age is not clearly established. In deciduous teeth from regions with low and with high fluoride in the drinking water, no correlation between age and the fluoride content of enamel surfaces was seen (Weatherell & Hargreaves, 1966). The situation in respect of the surface enamel of permanent teeth is also uncertain. Brudevold, Gardner & Smith (1956) suggested that there was some increase with age in the fluoride content of the outer layer of enamel. Recent studies of the labial surfaces of permanent incisors from a low-fluoride district revealed, however, that some teeth from individuals in their fifties or sixties contained surprisingly little fluoride. This suggested the possibility that a few microns of the tooth surface, with its associated high level of fluoride, might have been lost for one reason or another during the long period of the teeth in the mouth. Recent work (Hallsworth & Weatherell, 1969) showed that when a tooth surface had been worn away in the mouth, the high concentration of fluoride in the outer region of enamel was not restored, even in

FIG. 7
RELATIONSHIP BETWEEN AGE AND ENAMEL FLUORIDE
IN HUMAN PREMOLARS^a



^a After Jackson & Weidmann (1959).

West Hartlepool (England) where the fluoride concentration in drinking water was 2 ppm.

The extent to which fluoride accumulates in both bones and teeth with age, and the factors which govern it, are not clearly established. Such information might be important from several points of view. Observations about the life-long absorption and retention of fluoride will provide information to supplement and support knowledge about the skeletal incorporation and accumulation of other bone-seeking elements. Studies of fluoride levels in the surfaces of teeth relate directly to the many investigations into the role of fluoride in caries prophylaxis, and evidence about the loss of surface fluoride might at the same time provide information about the resistance of enamel surfaces to wear and erosion. Until recently, various technical problems have made many studies difficult. With the greatly improved methods for fluoride estimation and tissue sampling now available, there is every hope that the work of the next few years will resolve many of the uncertainties which at present obscure the picture of fluoride distribution and incorporation in the hard tissues.

4. DISTRIBUTION IN PLACENTA AND FOETUS (I. Gedalia)*

The placenta is the organ across which exchanges of gaseous, nutritive and excretory products take place between the foetal and maternal tissues, i.e., between their respective bloodstreams which histologically are in close proximity. The placental tissue is permeable even to high-molecular compounds like gamma-globulins, but generally an inverse relation exists between the molecular weight of substances and their ability to pass through the placenta (Villem, 1960). It has not been ascertained which mechanism is responsible for placental transfer—the ultrafilter theory, according to which the placenta acts as an inert, semipermeable membrane, or the vital function theory, according to which a preformed mechanism regulates a secretory process. The physiological characteristics of the placenta are not the same for all species of animals.

Studies on the placental transfer of fluoride have been induced by the demonstration of the influence of fluoride on the mineralization of teeth and on their resistance to dental caries. Of particular interest is the knowledge of the absorption and storage of fluoride in the human foetus and its relationship to the fluoride metabolism of the mother (Brzezinski, Bercovici & Gedalia, 1960).

Placental transfer of fluoride was demonstrated to occur under certain physiological conditions in the mouse (Ericsson & Ullberg, 1958; Ericsson, Ullberg & Appelgren, 1960), the rat (Lehman & Muhler, 1954;

* Since 1961, the author's studies have been aided by USPHS research grants D-1323-01-04 from the Institute of Dental Research, National Institutes of Health, Bethesda, Md. Some of the work quoted here was supported by the Mead Johnson Research Center.

Büttner & Muhler, 1958; Brzezinski et al., 1961), the dog (Knouff et al., 1936), the rabbit (Maplesden et al., 1960; Ericsson & Malmnäs, 1962), sheep (Bawden, Wolkoff & Flowers, 1964), and humans (Gardner et al., 1952; Held, 1954; Feltman & Kosel, 1955; Ziegler, 1956; Gedalia et al., 1961, 1964b; Ericsson & Malmnäs, 1962). In pregnant animals a certain amount of fluoride had to be present in the diet or drinking water before an appreciable amount of fluoride could be detected in the newborn (Lehman & Muhler, 1954; Büttner & Muhler, 1958; Brzezinski et al., 1961). Ericsson and co-workers (Ericsson & Ullberg, 1958; Ericsson, Ullberg & Appelgren, 1960), using an ^{18}F autoradiographic technique in pregnant mice, located the highest concentration of fluoride in the skeleton; the soft tissues were extremely low in fluoride, with the exception of the kidney and placenta.

Evidence with regard to the extent of placental transfer in humans has been conflicting (Gardner et al., 1952; Held, 1954; Feltman & Kosel, 1955; Ziegler, 1956). Past studies have established certain relationships between the daily fluoride intake by pregnant women and the fluoride content of the maternal blood, placental tissue and foetal blood at birth. Higher fluoride values were found in the maternal blood and placental tissue of pregnant women living in an area where the drinking water contained 1 ppm fluoride than in those living in a fluoride-free area (Gardner et al., 1952). The comparison of fluoride concentrations in foetal blood and placental tissue of women drinking practically fluoride-free water with those of women given fluoridated water or fluoride tablets has shown that in both groups the full-term placental tissue contained much more fluoride than the foetal blood (Feltman & Kosel, 1955). Ziegler (1956) compared the fluoride values in placental tissue, maternal blood and foetal blood of women drinking almost fluoride-free water with those of women given additional fluoride in milk. In the latter group there was a marked increase of fluoride concentration in the maternal blood and placental tissue, but in the foetal blood the fluoride value increased only slightly. All these studies indicated that fluoride accumulates in placental tissue, which may act as a partial barrier, protecting the foetus from toxic amounts (Gardner et al., 1952; Feltman & Kosel, 1955; Ziegler, 1956). However, Held (1954) reported about the same concentration of fluoride in maternal and foetal blood, as well as a similar increase in maternal and foetal blood fluoride levels after supplemental fluoride had been given, which he considered to imply that the placenta passively permits fluoride transfer to the foetus. In view of challenging reports on the importance of fluoride ingestion to pregnant women for the protection of the child's teeth, the fluoride relationships in placental tissues, maternal blood and foetal blood were re-investigated (Gedalia et al., 1961, 1964b; Ericsson & Malmnäs, 1962).

Table 12 shows that when the fluoride intake was low (drinking water containing about 0.1 ppm F), the fluoride content of the placental tissue was lower than that of the maternal or of the foetal blood, thus indicating little

TABLE 12
FLUORIDE CONCENTRATION IN PLACENTAL TISSUE, FOETAL BLOOD,
AND MATERNAL BLOOD AT LOW AND AT ELEVATED FLUORIDE INTAKE ^a

Material	Low fluoride intake		Elevated fluoride intake	
	Mean fluoride value (ppm)	SD	Mean fluoride value (ppm)	SD
Placenta	0.121	0.06	0.228	0.09
Foetal blood	0.165	0.07	0.175	0.05
Maternal blood	0.150	0.06	0.234	0.10

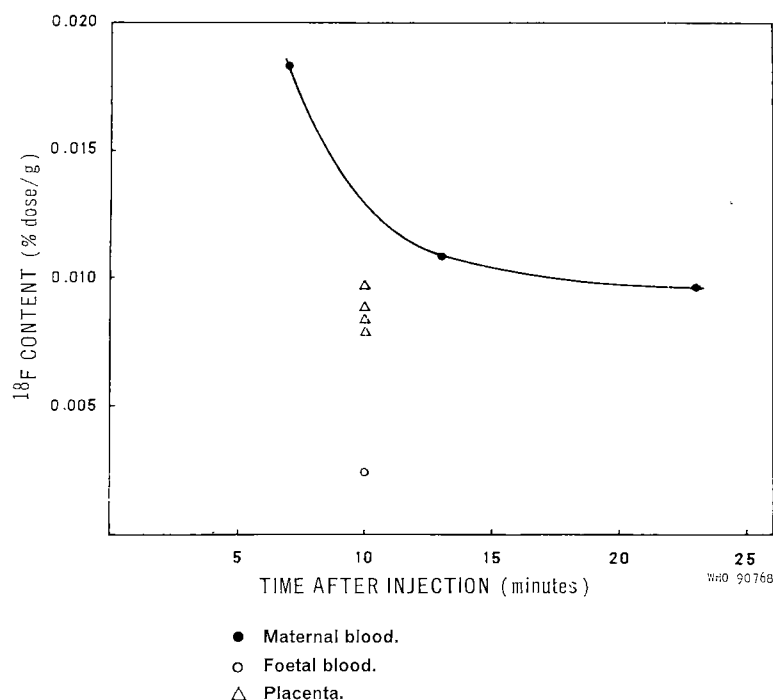
^a After Gedalia et al. (1964b).

obstruction to the passage of fluoride from the maternal to the foetal blood stream. On the other hand, when the fluoride intake was elevated, either from drinking water containing about 1 ppm F or through fluoride-tablet supplementation, the fluoride content of the placental tissue and of the maternal blood was higher than that of the foetal blood (Gedalia et al., 1964b). The limited permeability of the placenta to increased concentration of fluoride ions suggests that the placenta plays a role in the transfer of fluoride from mother to foetus (Gedalia et al., 1961, 1964b). However, all figures given for blood and placental fluoride content should be judged against the background of the micro-analytical difficulties and the degenerative changes taking place in the placenta towards the end of pregnancy. According to the ¹⁸F autoradiographic studies in pregnant mice (Ericsson & Ullberg, 1958; Ericsson, Ullberg & Appelgren, 1960), the full-term placenta contains areas of calcification which take up fluoride thus reducing the amount which enters the foetus. Such areas of calcification are probably responsible for the high fluoride content in the human placenta at the time of delivery.

The relatively high permissible body dose of ¹⁸F (Armstrong, Singer & Carlson, 1958) made it possible to study the fluoride transfer across the placenta of patients undergoing late therapeutic abortions in connexion with sterilization. Such investigations, supplemented by experiments on rabbits whose placentas are of the same type as those of humans, were carried out by Ericsson & Malmnäs (1962). In both humans and rabbits, the ¹⁸F content of the foetal blood was found to be very low 5-30 minutes after intravenous injection, always less than one-third of the simultaneous ¹⁸F concentration in the maternal blood. The ¹⁸F concentration of the placenta was between that of the maternal and that of the foetal blood (Fig. 8).

Recently the placental transfer of ¹⁸F was analysed autoradiographically and quantitatively in the late pregnancy of mice (1-2 days before expected parturition) (Ericsson & Hammarström, 1964). The foetal skeleton accumulated much less ¹⁸F than the maternal skeleton of these animals, owing to the slow diffusion of fluoride through the placenta and to the great homeostatic

FIG. 8
 ^{18}F CONTENT OF MATERNAL BLOOD, FOETAL BLOOD
 AND PLACENTA IN HUMANS^a



^a After Ericsson & Malmnäs (1962).

capacity for fluoride of the mammalian body (Carlson, Armstrong & Singer, 1960b). A sudden increase in maternal blood fluoride, such as that induced by the intake of fluoride tablets or by injection of ^{18}F in pregnancy, should therefore not produce any great rise in the fluoride concentration of the foetal blood.

Once fluoride enters the foetal circulation it is incorporated into foetal bones and teeth undergoing calcification. The fluoride is probably deposited as fluorapatite. In 1948, Martin published findings on the fluoride content of the femur, the pooled mandible and maxilla and the tooth buds from eight foetuses obtained from an area in Chicago which had a water supply low in fluoride at that time.

The results (Table 13) did not reveal any clear relationship between the amount of fluoride and the weight of the foetus. This finding agrees with more recent fluoride analyses of femurs, mandibles and teeth in 6-9 months old human foetuses from low-fluoride areas (Gedalia et al., 1964a)

TABLE 13
FLUORIDE CONTENT OF DRY FOETAL BONES AND
TEETH FROM A LOW-FLUORIDE AREA (CHICAGO, 1946-47) ^a

Foetus No.	Tissue analysed	Weight (g)	Fluoride (ppm)
1	Femur	4 790	29.0
	Mandible and maxilla		27.0
	Tooth buds		17.0
2	Femur	1 950	20.0
	Mandible and maxilla		19.0
	Tooth buds		13.0
3	Femur	1 125	16.0
	Mandible and maxilla		15.0
	Tooth buds		8.7
4	Femur	1 435	27.0
	Mandible and maxilla		24.0
	Tooth buds		13.4
5	Femur	1 300	24.0
	Mandible and maxilla		24.0
	Tooth buds		15.0
6	Femur	3 180	23.5
	Mandible and maxilla		20.0
	Tooth buds		15.0
7	Femur	4 660	14.0
	Mandible and maxilla		15.0
	Tooth buds		9.0
8	Femur	1 000	10.6
	Mandible and maxilla		9.3
	Tooth buds		8.0

^a After Martin (1948).

which do not indicate an appreciable increase in fluoride content of the calcified tissues with age of the foetus (Table 14).

TABLE 14
FLUORIDE CONTENT OF ASHED FOETAL BONES AND TEETH
FROM A LOW-FLUORIDE AREA
(ABOUT 0.1 ppm F, TEL-AVIV, 1961-63) ^a

Foetal age (months)	Number of cases	Mean fluoride value (ppm)		
		Femur	Mandible	Teeth
6	21	39.7	42.3	30.9
7	24	40.7	39.0	34.0
8	13	42.3	38.5	31.7
9	27	43.8	46.9	40.8

^a After Gedalia et al. (1964a).

In medium (Table 15) and elevated (Tables 16 and 17) fluoridated-drinking-water areas (Gedalia et al., 1964a; Blayney & Hill, 1964; Gedalia, Zukerman & Leventhal, 1965), the fluoride content of bones and teeth generally increased with advancing age of the foetus as a result of the

prolonged effects of the fluoride exchange and incorporation processes (Hodge, 1952).

The difference in the fluoride content of the foetal skeletal tissues from low (Tables 13 and 14), medium (Table 15) and elevated (Tables 16 and 17) fluoride areas is probably due to the different fluoride concentrations in the foetal blood from which the newly formed mineral crystals

TABLE 15
FLUORIDE CONTENT OF ASHED FOETAL BONES AND TEETH FROM
A MEDIUM-FLUORIDE AREA (ABOUT 0.55 ppm F, JERUSALEM, 1961-63) ^a

Foetal age (months)	Number of cases	Mean fluoride value (ppm)		
		Femur	Mandible	Teeth
6	31	59.0	47.0	32.6
7	20	71.6	53.5	43.0
8	7	79.4	66.0	57.9
9	34	92.5	78.8	69.7

^a After Gedalia et al. (1964a).

TABLE 16
FLUORIDE CONTENT OF ASHED FOETAL BONES AND TEETH FROM
AN ELEVATED-FLUORIDE AREA
(ABOUT 1 ppm F, NEGEV, SOUTHERN ISRAEL, 1961-64) ^a

Foetal age (months)	Number of cases	Mean fluoride value (ppm)		
		Femur	Mandible	Teeth
6	20	55.2	57.2	44.0
7	6	63.0	65.7	47.0
8	13	79.9	70.3	52.0
9	25	85.2	85.0	53.8

^a After Gedalia, Zukerman & Leventhal (1965).

TABLE 17
FLUORIDE CONTENT OF ASHED FOETAL BONES AND TEETH FROM
A FLUORIDATED-DRINKING-WATER AREA
(ABOUT 1 ppm F, EVANSTON, ILLINOIS, 1953) ^a

Foetus No.	Period of gestation (weeks)	Weight of baby (g)	Results of analysis (ppm)			
			Femur	Mandible	Maxilla	Tooth buds
1	28	880	78.9	155.6	125.5	45.8
2	35	2 126	95.1	92.7	82.0	
3	21	950	89.2	78.4	82.5	
4	36	2 416	121.6	120.1	111.8	

^a After Blayney & Hill (1964).

incorporate fluoride (Gedalia et al., 1964a; Gedalia, Zukerman & Leventhal, 1965). The similar foetal blood fluoride levels (Table 12) observed at low, medium and elevated fluoride intake (Gedalia et al., 1964b) may be the result of a rapid clearance of fluoride from foetal blood during the mineralization period of the foetal skeleton (Bawden, Wolkoff & Flowers, 1964). In adults living in places with different fluoride concentrations in the drinking-water supply (0.1-2.5 ppm), the plasma fluoride concentrations, too, were reported to show but small variations (Armstrong & Singer, 1959). The fluoride contents of the bones and teeth of fetuses from medium (Table 15) and elevated, either naturally (Table 16) or artificially (Table 17), fluoride areas of corresponding diets were not very different, thus confirming the limited permeability of the placenta in humans and rodents at increased fluoride intake (Gedalia et al., 1964b; Ericsson & Hammarström, 1964). Additional evidence of limited permeability is the inability to find mottled enamel, even in areas of severe endemic dental fluorosis, in any of the deciduous incisors (McClure, 1962) which are known to be almost completely calcified prenatally (Schour & Massler, 1940).

As to the fluoride content of the different foetal hard tissues, that of the femur is generally higher than that of the mandible or teeth at corresponding ages. Differences in the distribution of fluoride in various skeletal compounds in experimental animals have been attributed to their vascularity and rate of growth (Volker, Sognnaes & Bibby, 1941) which may perhaps account for the pattern of fluoride deposition in human foetal bones and teeth.

5. SUMMARY (W. D. Armstrong)

Application of adequate analytical procedures always reveals fluoride to be present in mammalian body-fluids and tissues, and further, that particularly large amounts occur in calcified tissues. Blood plasma is the most convenient and reliable indicator of the concentration of fluoride in body-fluids. Results have been presented which show that the mean plasma fluoride of normal humans is in the range 0.14-0.19 ppm and that the mean equilibrium plasma fluoride content is not elevated above this range in persons using water containing up to 2.5 ppm fluoride. Undoubtedly the plasma fluoride content of individuals rises slightly for brief periods of time following ingestion of fluoride in food and water, as has been demonstrated in animals and humans in studies with radiofluoride, but the degree of plasma fluoride elevation under these conditions, as deduced from the same studies, is so small as to be difficult to define by chemical analytical procedures. Even in patients who have been treated daily with amounts of fluoride which are fiftyfold larger than those which could be obtained from properly fluoridated water, the elevation of plasma fluoride content is limited in degree.

The fluoride content of soft tissues, with the exception of tendon, appears to be of the same order of magnitude as that of plasma, and there is evidence, derived from chemical analyses and distribution of radiofluoride in tissues, that intracellular fluids have a lower ionic fluoride content than extracellular fluid. As is the case with plasma, the soft tissues are markedly unresponsive in reflecting an increase of fluoride intake by an elevation of fluoride content. Fluoride intakes which produce acute intoxication will, however, result in clearly demonstrable elevations in the fluoride content of most soft tissues.

The fluoride contents of calcified tissues usually stand in the following descending order: cementum, bone, dentine and enamel. Calcified tissues, whether normal or ectopic, have a propensity for fixing fluoride, and a linear relationship between the fluoride content of the skeleton of humans and that of the drinking water has been demonstrated. This relationship exists because water is the most variable agent of fluoride intake and is frequently the most important quantitative source of fluoride in the dietary.

Fluoride is incorporated into lattice of the mineral of bones and teeth, and possibly also at crystal surface locations. Incorporation of fluoride occurs at the time of deposition of the mineral and by hetero-ionic exchange after the crystal has formed. The degree to which the latter process occurs varies with the anatomical structure and physiological state of the calcified tissue. There is a good positive correlation between the rate of turnover of a calcified tissue and the fluoride content of that tissue. The circumstances of variations of degree of acquisition of fluoride by hetero-ionic exchange, and the influence of remodelling of a calcified tissue on its behaviour towards fluoride, account for the variations in fluoride content at different sites in the same bone or tooth. Thus, subperiosteal and cancellous bone have higher fluoride contents than the compact part of the diaphysis. In enamel, the fluoride concentration decreases in an exponential fashion from the outer surface towards the amelo-dentinal junction; and, in dentine, the highest fluoride content is found in the pulpal surface, the amounts decreasing as the enamel is approached. The higher amounts of fluoride in the nearer-the-surface layers of enamel and dentine are due to the continued acquisition of fluoride by the mineral of these layers, probably by hetero-ionic exchange, after the near maturation of each appositional layer. The outer 100-200 μ of enamel continues to acquire fluoride from the oral fluids after eruption of the tooth and certainly, in this case, hetero-ionic exchange is the predominant process by which the fluoride is deposited in the superficial enamel layer.

While there is clear evidence that the fluoride content of the bones of humans tends to increase with age and with fluoride intake, it is not yet certain whether the bones reach, or approach, an upper limit in their fluoride concentration except, possibly, under most unusual circumstances of fluoride intake which produce frank osteofluorosis. A considerable fraction

of the fluoride in bones with markedly elevated fluoride contents is retained for a very long time after withdrawal of a high-fluoride-containing dietary. However, the apparent prolonged retention of fluoride in the skeleton could be due to resorption and redeposition in the same bony structure.

The placenta allows fluoride to reach the foetal circulation to some extent, and the same factors involved in fluoride deposition in bones during extra-uterine life operate in the foetal bones.

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Excretion of fluorides^{*}

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1. INTRODUCTION

Fluoride, a prototype bone-seeker, in its skeletal deposition also serves as an excellent example of a cumulative element. Because excessive, prolonged fluoride exposure leads not only to high skeletal concentrations but also to characteristic ill-effects, e.g., crippling fluorosis, more than ordinary significance is attached to evidence concerning F elimination.

Fluoride is excreted in the urine, deposited in the skin which is shed, lost through the sweat, and excreted in the faeces. Fluoride occurs in traces in milk, in saliva, in hair and, presumably, in tears. Fluoride is probably not exhaled in the breath, although definitive data are lacking.

The principal route of fluoride excretion is *via* the urine. With astonishing rapidity, quantities of F appear, generally reflecting the daily intake but governed by other factors, several of which are known, such as (a) the total intake, (b) the form in which the fluoride is taken into the body, (c) whether the individual is relatively unexposed or regularly exposed to fluoride, and (d) the health status of the individual, especially with regard to advanced kidney disease. These factors, as well as the urinary excretion of fluoride in pregnancy and during the process of mobilization of fluoride from skeletal stores, are discussed in some detail in this chapter.

Efforts have been made to include all human data by direct citation or by reference. Animal data are included to substantiate human experience or to furnish evidence in the absence of human data.

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2. EXCRETION ROUTES OTHER THAN URINARY

Faecal Excretion

About 10% of the total daily fluoride excretion can usually be accounted for in the faeces.

An individual ingesting an average diet in the USA and not drinking fluoridated water usually excretes less than 0.2 mg in the faeces (range 0.01-0.5 mg) per day. When diets contain relatively insoluble fluoride compounds, e.g., bone meal, cryolite, insoluble calcium salts, or fluoride precipitants such as aluminium or calcium compounds, larger amounts of fluoride pass through the gastrointestinal tract and are excreted in the faeces. Under such circumstances the percentage excreted of the total fluoride ingested may be considerably larger, as much as 30% or more (Largent, 1961; Rich, Ensink & Ivanovich, 1964). Animal studies confirm this principle (Lawrenz & Mitchell, 1941; Lawrenz, Mitchell & Ruth, 1939; Messner, Weinreb & Gedalia, 1965).

Part of the fluoride in the faeces is almost certainly undissolved, unabsorbed fluoride. With insoluble fluorides, the smallness of the ingested particles enhances absorption; for instance, fluoride in rock-phosphate powder, which is known to have a low solubility, ingested in a finely dispersed form as may happen in fertilizer plants, was recovered in the faeces only to the extent of 13-19% of the total intake. Part of the faecal fluoride probably represents fluoride that has been absorbed and re-excreted *via* the gastric and intestinal juices. Soluble fluorides given intravenously appear in the intestinal contents, as has been shown by the animal studies of Wallace-Durbin (1954) using radiofluoride and also by the faecal analyses carried out after oral administration of soluble fluorides by Wagner & Muhler (1957). Re-excretion into the gut presumably accounts for the extra faecal fluoride observed after inhalation of HF (Largent, 1959). In subjects who inhaled 1 to nearly 5 ppm HF in air, the faecal fluoride level was increased 3- to nearly 10-fold. Urinary fluoride analyses for these same individuals showed that 4 to over 9 mg were excreted per day during the period of HF exposure. It therefore seems reasonable to assume that the total fluoride taken into the body by these subjects ranged perhaps from 8 to 18 mg per day, of which 0.2 to 0.7 mg per day appeared in the faeces (see p. 148).

Several investigators have reported on fluoride concentrations in human faecal samples taken under a variety of conditions of fluoride intake. For example, when the F intake was 0.4-0.5 mg, faecal excretion ranged from 0.03 to 0.12 mg F daily; when the intake was 4-19 mg (as NaF or CaF₂ solution), faecal excretion increased to 0.19-0.33 mg (Largent, 1961; Machle, Scott & Largent, 1942; McClure et al., 1945). Ham & Smith (1954b) reported human faecal values ranging from 0.09 to 0.15 mg per day on a normal diet. Selected data from Largent's study show that faecal excretion

TABLE 1
FLUORIDE BALANCE IN HUMANS ^a

F in water supply (ppm)	Age (years)	Length of residence (years)	Time of observation (days)	F ingested daily (mg)			F excreted daily (mg)			Balance (mg/day)
				Fluid	Food	Total	Faeces	Urine	Total	
2	35	10	96	2.4	1.2	3.6	0.4	2.9	3.3	+0.3
5.5	55	29	60	3.8	1.3	5.1	0.6	4.5	5.1	0
6.1	57	34	133	6.7	1.0	7.7	0.4	8.1	8.5	-0.8
8	57	19	140	11.3	2.5	13.8	1.4	10.4	11.8	+2.0
20	30	8	45	20.8	1.5	22.3	1.4	12.3	13.7	+8.6

^a Selected data from Largent (1961).

was 0.6 mg per day or less when the water contained up to 6 ppm, but was greater when the water contained 8 or 20 ppm (Table 1).

Excretion in Sweat

Some fluoride is lost from the body in sweat; during excessive perspiring appreciable amounts may be excreted in this way.

Individuals living in a comfortable environment probably lose a little fluoride from the body daily in the sweat; the amount is unknown. When subjects were maintained at an ambient temperature of 84-85°F (about 29.5°C) and a relative humidity of about 50%, about 25% of the fluoride excreted per day appeared in the sweat, although in the investigation in question sweat was collected during only 8 hours out of the 24 (McClure et al., 1945).

Under hot, moist conditions McClure et al. (*op. cit.*) found up to 46% of the ingested fluoride in the sweat. Perspiration samples collected during one or two hours from the same subject ingesting 0.4-0.5 mg F daily contained 0.02-0.06 mg of F. Largent (1961) found that excretion rates of fluoride in the sweat sometimes nearly equalled those in the urine. Crosby & Shepherd (1957) found that under conditions of profuse sweating "up to 50% of the total fluoride excreted appeared in the sweat". Individual variability may be considerable. In Largent's two subjects one "appeared to excrete only extremely small amounts of fluoride through the skin" (Largent, 1961); during fluoride-ingestion periods sweat contained 0.3-1.8 ppm F, and the subjects responded differently to an increase in the fluoride intake level (see also McClure et al., 1945). Crosby & Shepherd (1957) observed that within one hour after ingestion of a fluoride dose, higher levels occurred in sweat, probably because of the temporarily increased fluoride level of the blood. The concentrations in sweat may be higher than those in plasma, although no investigation has deliberately compared plasma and sweat concentrations in a single experiment. In two individuals ingesting normal

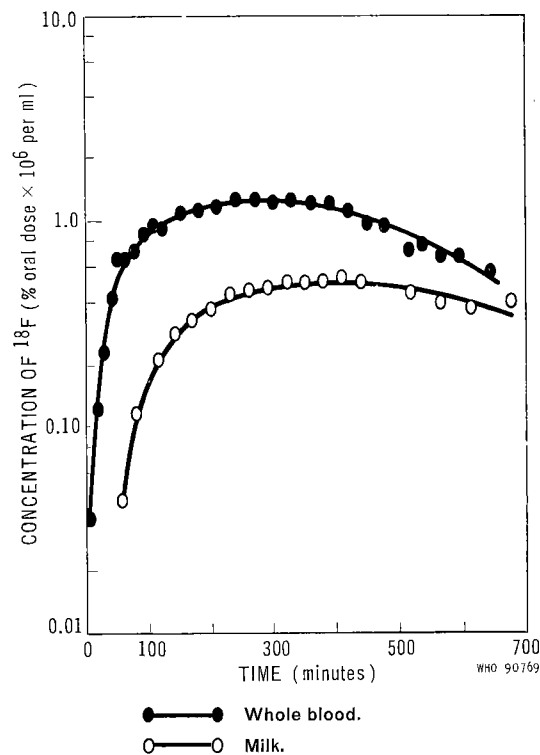
amounts of fluoride, the sweat concentrations ranged from 0.3 to 0.7 ppm in one and from 0.5 to 0.9 ppm in the other (Crosby & Shepherd, *op. cit.*), values higher than the normal plasma levels of 0.1-0.2 ppm (Singer & Armstrong, 1960).

Although there is some evidence that the concentration in sweat is higher when additional fluoride is ingested, the significance of sweat as a route of excretion has not been established. To a limited extent sweating can be considered to provide an autoregulation of the fluoride balance when fluctuating climatic conditions considerably vary the intake of fluoride from drinking water.

Excretion in Milk

Fluoride is a natural constituent of human milk. Negligibly small fractions of the daily fluoride intake are excreted in milk.

FIG. 1
CONCENTRATION OF ^{18}F IN THE BLOOD AND MILK OF A COW
AFTER INGESTION OF RADIOACTIVE SODIUM FLUORIDE ^a



^a After Perkinson et al. (1955).

The concentrations in human milk range from less than 0.1 to about 0.2 ppm. This range is so nearly that of blood plasma that it is tempting to describe the concentration of fluoride in milk as being similar to that in blood. Perkinson et al. (1955) showed that radiofluoride given orally to a dairy cow was excreted thereafter in the milk in concentrations similar to but lower than those in the blood (Fig 1). No such data are available on humans. Elevated fluoride concentrations in the drinking water or supplemental fluoride intake may increase somewhat the fluoride content of milk in lactating women (Held, 1955). Held reported that the F content of human milk was increased by about 15-40% by daily supplements of 5 mg F. Just as human or animal plasma fluoride levels show little increase when drinking water concentrations are elevated, so fluoride concentrations in cow's milk increased only slightly when fluoride concentrations in the feed were raised. Suttie, Miller & Phillips (1957) showed that cattle receiving feed normally containing 3-5 ppm F excreted milk containing 0.1 ppm F, and as the level in the feed increased to 50 ppm F the milk concentration increased to about 0.4 ppm F, thus being still within safe limits for human consumption.

Trace quantities of fluoride in milk are bound to fat and to the albumin-globulin fraction, whereas casein contains about one-fourth of the total fluoride in the whole milk (Ericsson, 1958a). Fluoride in milk is not completely diffusible.

Fluoride administered in milk to rats or humans was absorbed slightly more slowly than when given in water, but ultimately as completely. These facts should not prevent milk from being used as a fluoride vehicle when drinking water cannot be fluoridated; however, long-term clinical experiments should be continued. It is a matter of conjecture whether the small amounts of fluoride in the mother's milk are of importance to tooth and bone development of the newborn. Analyses of human milk have been reported by Bercovici, Gedalia & Brzezinski (1960) and by Büttner (1962). Fluoride concentrations in normal cow's milk and in the milk of several species of animals absorbing unusual amounts of fluoride have been summarized by Hodge & Smith (1965, pp. 149, 150).

Excretion in Saliva

Only a negligible fraction of the total fluoride intake can be accounted for in the saliva.

Less than 1% of the activity of ingested radiofluoride was recovered from human saliva samples (Carlson, Armstrong & Singer, 1960). The normal concentrations (McClure, 1941) are presumably very similar to those in blood. Only fragmentary information is available on salivary flow rates and fluoride contents in man. A subject who took 8 mg of fluoride showed detectable fluoride in the saliva only after 2 hours (Büttner & Muhler, 1962). In normal young adults whose drinking water contained

1 ppm F, no correlation was found between the paraffin-stimulated flow rates of parotid saliva and the F contents (Busch & Shklair, 1965). Radio-fluoride given intravenously to a cat appeared in the submaxillary saliva within a minute (Wills, 1940).

Fluoride excretion in stimulated saliva did not differ appreciably from that in unstimulated in spite of much higher flow rates (Hattiyasy, 1957). Salivary F concentrations range between 0.04 and 0.5 ppm (mean values of 0.1-0.2 ppm) (Dvir, Gedalia & Sulitzeanu, 1962; Gedalia, Yardeni & Gershon, 1963; Martin & Hill, 1950; McClure, 1941) irrespective of drinking-water concentrations up to 1.8 ppm F (McClure, 1941). Dvir and associates (*op. cit.*) and Martin & Hill (1950) examined the fluoride content of pooled and individual saliva samples from children drinking artificially and naturally fluoridated water. Human saliva from the parotid duct contained slightly lower ^{18}F concentrations than plasma at various times after ingestion of ^{18}F (Carlson, Armstrong & Singer, 1960). The secretion rate and buffer capacity of human saliva increased very slightly after ingestion of 5-10 mg F (Ericsson, 1959).

The distribution and reactions of the fluoride ion in enamel-saliva environment have been followed in radioactive studies with ^{18}F (Ericsson, 1958b). Fluoride is practically completely diffusible in saliva; no appreciable binding to organic components or precipitation occurs.

The trace amounts of fluoride in saliva seem to play a minor role in the accumulation of surface-enamel fluoride; however, the long-term bathing effects on the surface enamel by the continuous saliva flow should not be dismissed (Brudevold, Gardner & Smith, 1956).

It has been postulated that fluoride from the saliva may be incorporated into dental calculus during precipitation of the latter (Gedalia, Yardeni & Gershon, 1963). The mean fluoride contents of saliva samples from calculus-bearing and calculus-free groups were similar, 0.18 and 0.16 ppm, respectively. No tendency to dental calculus deposition could be detected in persons with an increased fluoride content in the saliva.

Analyses of human saliva have been tabulated by Hodge & Smith (1965, p. 146).

3. URINARY EXCRETION

Relation to Circumstances of Fluoride Intake

The urinary fluoride level is widely regarded as one of the best indices of fluoride intake. In discussing the significance of urinary fluoride concentrations, however, at least two groups of individuals, with differing circumstances of intake, should be recognized.

1. Individuals whose normal intake is fairly constant. In such individuals, urinary fluoride concentrations may fluctuate because variable amounts are consumed in the usual mixed diet or because different amounts of water are

drunk. Over periods of months, however, intake, urinary excretion, and skeletal concentrations tend to establish what are at least superficially steady states. For most populations, the urinary concentrations are relatively low, 1 or 2 ppm or less.

Certain groups, however, sustain unusual exposures—for example, industrial exposures, high drinking-water concentrations, or excessive water consumption due to high environmental temperatures. In these groups, urinary fluoride concentrations are much higher but presumably will ultimately also reflect steady states.

2. Individuals who at irregular intervals experience considerable but brief fluoride exposures. Such individuals remain “relatively unexposed” in the sense that their bony tissues are by no means “saturated”. When the customary normal intake is temporarily markedly exceeded, therefore, the rapid processes of distribution and excretion (*a*) deposit roughly half of the additional fluoride in the skeleton and (*b*) remove the balance promptly from the body *via* the urine.

Excretion in Consistently Exposed Individuals

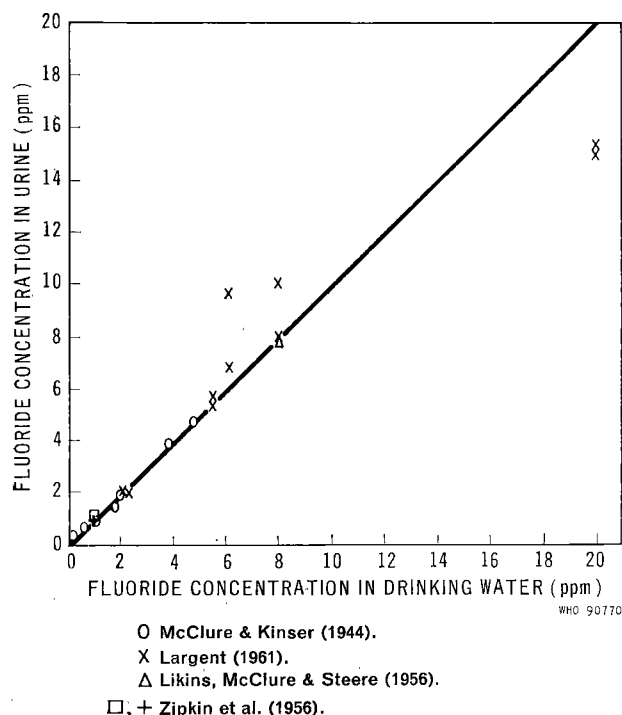
Urinary fluoride levels as a function of intake

Human urinary fluoride concentrations depend upon—and, in fact, are nearly equivalent numerically to—the drinking-water concentrations.

The extraordinary linearity of the relation between the concentration of fluoride in the drinking water (up to 8 ppm) and the concentration of fluoride in the urine is shown in Fig. 2. Since the first statement in the well-known paper of McClure & Kinser (1944), which included observations on individuals from communities in which the water supplies contained naturally up to 5 ppm F, this relation has been repeatedly confirmed. The urinary F concentrations of individuals in communities where the water contains the higher concentrations exhibit considerable scatter. In the USA, the normal individual consuming water that contains little or no fluoride usually excretes urine with a fluoride concentration ranging from 0.2 to 0.5 ppm (Gedalia, 1958; Jirásková & Ruzicka, 1959). In a community with water fluoridated at 1 ppm, the normal urinary concentrations often range from 0.5 to 1.5 ppm. The quantitative, near equality of the urinary concentration to the drinking-water concentration is a notable feature of Fig. 2. Persons who have for a long time been resident in communities where the water contains fluoride, and who are presumably in a steady state of fluoride balance, ultimately excrete each day an amount of fluoride essentially equivalent to the amount taken in. Skeletal storage of fluoride accounts for some of each day's intake, and is ultimately matched by the F mobilized from skeletal stores.

An individual who drank a litre of water per day and excreted a litre of urine of the same F concentration would be in exact fluoride balance. This

FIG. 2
RELATION BETWEEN FLUORIDE CONCENTRATION IN THE URINE
OF HUMANS AND THAT IN THE WATER SUPPLIES USED



proportion is a misleading oversimplification. Food accounts for nearly half the total water intake, and in the absence of profuse sweating almost half the total water loss occurs insensibly through the lung. The coincident equality of urinary concentration and drinking-water concentration therefore reflects the normal pattern of drinking-water consumption—urine excretion in the presence of a fluoride steady state. The reliability of the urinary fluoride excretion as an index of total exposure has conferred an unusual usefulness and significance on urinary fluoride analyses in any industrial exposure.

The steady state

Evidence that a steady state does in reality exist can be found from balance studies such as those conducted by Largent (1961). In Table 1, for example, are given data on three individuals who drank water containing 2, 5.5 and 6.1 ppm F, respectively, for 10, 29 and 34 years, and who were in over-all fluoride balance during extended periods of observation (96, 60 and 133 days, respectively). One individual was in precise balance, the other

two nearly so ($+0.3$ mg/day and -0.8 mg/day, respectively). Two individuals (Table 1) ingesting higher concentrations of fluoride, 8 and 20 ppm, were both in positive balance. The individual who drank water containing 20 ppm, even though in residence for eight years, had not yet reached a steady state.

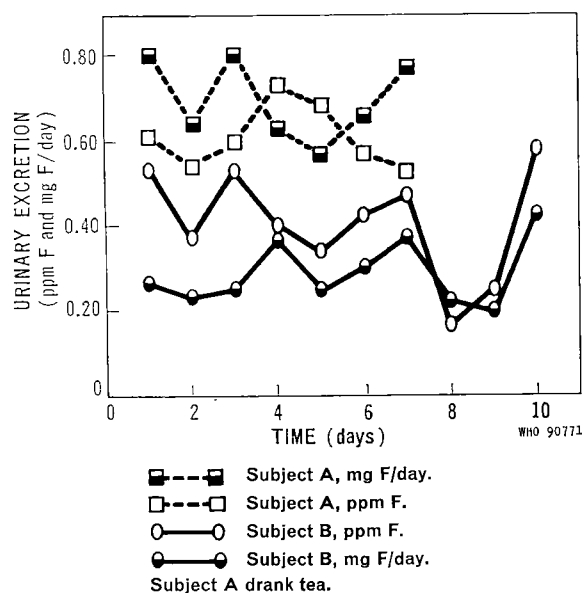
In other balance studies, weekly urinary F excretion over a period of more than four months showed considerable fluctuations but an over-all trend towards a steady state (Machle, Scott & Largent, 1942). In two additional balance studies, no indication of a steady state was gained. In short-term studies Ham & Smith (1954b) found, in female subjects on a normal diet, reasonably constant urinary fluoride concentrations, with small positive fluoride balances. The urinary fluoride excretion of patients on large therapeutic doses of fluoride (60 mg F per day) was reasonably consistent from period to period; each patient was in positive balance during most of the study (Rich, Ensink & Ivanovich, 1964).

In animals given daily dosages of fluoride for long periods there is also a linear relation between fluoride intake and urinary fluoride excretion (Hodge & Smith, 1965, p. 159).

Individual variation

Urinary fluoride concentrations are characteristically variable, from hour to hour, from day to day, and from individual to individual. Only by

FIG. 3
DAY-TO-DAY VARIATIONS IN URINARY FLUORIDE EXCRETION ^a



^a Data from Büttner, Schülke & Soyka (1961).

studies of sufficient duration can the underlying constancies be revealed. Fluoride is so rapidly excreted that the urine sample collected in the three hours following fluoride intake will contain an appreciable amount of the total ultimately excreted; conversely, an individual consuming a very large amount of fluid may excrete a dilute urine with a lower *concentration* of fluoride. In Fig. 3, the day-to-day variations in the urinary fluoride excretion of two subjects are shown both in mg per day and in ppm (Büttner, Schülke & Soyka, 1961). The lowest and highest values show a threefold variation for Subject B. The habits of the individual are important: for example, in the above-mentioned study, Subject A drank tea (see also Harrison, 1949) and, since tea contains about 1 ppm F, he excreted more fluoride than Subject B, who did not drink tea and whose drinking water must have been low in fluoride, since the total excretion was in the range 0.2-0.5 mg per day.

Necessity for 24-hour urine samples

The difficulties of collecting a 24-hour urine sample necessitate the consideration of the practical question of whether spot samples could be used instead. To answer this question, samples from workmen in magnesium foundries, hydrogen fluoride plants, high-octane-gasoline refineries and other industries have been collected in such a way that concentrations in 24-hour samples could be compared with those in spot samples taken during the same period. Tabular summaries of these studies and also similar data for non-industrial populations are offered by Hodge & Smith (1965, p. 53). The conclusion is clear-cut: 24-hour samples are more reliable and should be collected if the data for a single individual are to form the basis for decisions and interpretations. Fluoride analyses of spot samples, although varying in the same individual from time to time, and in different individuals at the same time even though exposures were presumably comparable, give reasonably reliable averages for groups, and can be used for industrial hygiene control or population surveys (see also Gedalia, 1958).

Büttner, Schülke & Soyka (1961) could find no consistent difference either in the fluoride concentration (ppm) or in the total amount of fluoride (mg) in 24-hour samples of urine when they compared the excretion of children in a control period with that in a period during which a stannous fluoride dentifrice was used.

Fluoride intake in animals may also be estimated by urine analyses (Shupe et al., 1963).

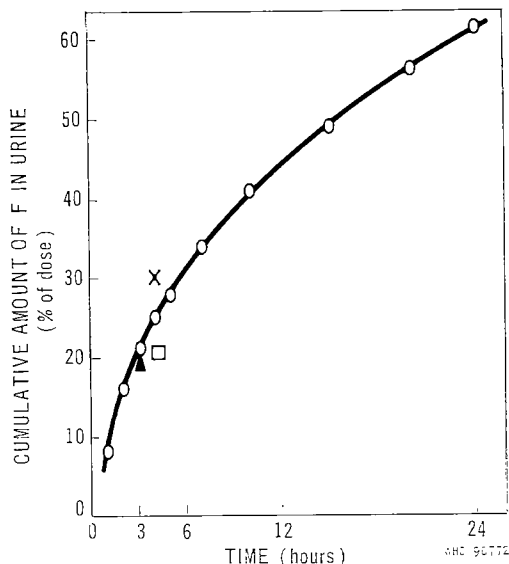
Excretion in Relatively Unexposed Individuals

Rate

The rapidity of urinary excretion is one of the most important characteristics of the behaviour of the fluoride ion in the body. Even small amounts,

e.g., 1.5 mg (Hodge & Smith, 1965, p. 157) or 5 mg (Zipkin, Lee & Leone, 1957), taken in a glass of water, are absorbed and excreted so rapidly that 20% of the fluoride can be found in the urine after three hours (Fig. 4).

FIG. 4
CUMULATIVE EXCRETION OF FLUORIDE IN THE URINE OF MAN
FOLLOWING THE ORAL ADMINISTRATION OF SODIUM FLUORIDE



- Average from 8 subjects drinking water containing 1 ppm F and given a single dose of 5 mg F (Zipkin, Lee & Leone, 1957).
- ▲ Average from 6 subjects drinking water containing 0.06 ppm F and given a single dose of 1.5 mg F (Hodge & Smith, 1965, p. 157).
- × Average from 2 subjects drinking water containing 1 mg F labelled with ^{18}F (Carlson, Armstrong & Singer, 1960).
- Average of 7 trials with 5 subjects drinking water containing 1 mg F labelled with ^{18}F (Ericsson, 1958a).

Using ^{18}F , Carlson, Armstrong & Singer (1960) and Ericsson (1958a) found that up to 30% of a 1-mg dose was detectable in the urine in 4 hours. Zipkin, Lee & Leone (1957) noted that extra fluoride appeared in the urine with the greatest rapidity in the first hour; thereafter the rate dropped rapidly until after about eight hours the control rate of approximately 0.1 mg per hour was again approached (see also Jirásková & Ruzicka, 1959). The very rapid rate of excretion is one of the most important protective factors in severe fluoride poisoning: usually, either death occurs within four hours or the individual recovers. The critical period is short (*a*) because F is rapidly removed from the bloodstream and the extracellular fluid *via* the kidney, and (*b*) because skeletal deposition is also extremely rapid. Together, these

processes effectively lower fluoride levels if the absorbed dose is not overwhelming.

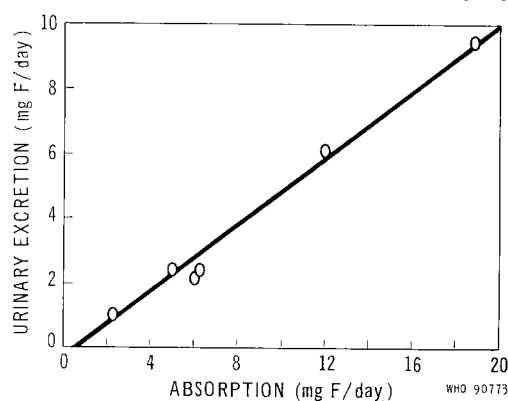
The interplay of urinary excretion and skeletal deposition and the rapidity of these processes are responsible for the findings of McClure, who drank various community water supplies, ranging in F concentration from 0.1 to 3.8 ppm, and found that his urine soon (i.e., in a day or so) showed practically the same F concentration as the drinking water (McClure & Kinser, 1944).

Fluoride given in milk appears in the urine somewhat more slowly than when given in equal amounts in the drinking water. Absorption from both vehicles is ultimately comparable (Ericsson, 1958a). Harrison (1949) showed that the daily urinary fluoride excretion was related to the amount of tea consumed, and that urinary F was lessened if milk was added to the tea.

Relation to deposition of fluoride in bone

In the relatively unexposed individual about half of a single dose of fluoride is excreted in the urine in the ensuing 24 hours and about half is deposited in the skeleton. In a classical study continued over a period of many months, Largent (1959) collected samples of everything he ate and drank, and of everything he excreted, and so was able to make definitive measurements of the retention of fluoride when daily doses of 1-18 mg of fluoride were ingested. Periods of observation at each dose level ranged from a month to several months. This fluoride balance study, unsurpassed in quality and quantity of data, led to a conclusion of unequivocal clarity (Fig. 5). Fluoride retention followed closely a simple straight line indicating storage of 50% of the absorbed fluoride. In Largent's experiments the

FIG. 5
RELATION BETWEEN ABSORPTION AND URINARY EXCRETION OF FLUORIDE^a



^a After Machle & Largent (1943).

fluoride was taken as sodium fluoride or calcium fluoride in solution, as solid bone meal, as solid cryolite and in other forms. Examination of a summary of his data (Largent, 1961) shows that if the fluoride is in solution, about 50% is retained on the average. From daily doses of *solid* cryolite furnishing 12-36 mg F, fluoride was retained to the extent of 34-45%. Similar values were reported for F retention (40-50%) from *solid* bone meal in doses giving 1.5-6 mg F daily (Largent, 1961; McClure et al., 1945). Urinary F levels appear to be related to the amounts absorbed rather than to the amounts ingested (Table 2). Retention figures not significantly

TABLE 2
FLUORIDE CONCENTRATIONS IN URINE
AFTER ABSORPTION OF ABNORMALLY
LARGE QUANTITIES OF F ^a

Average amount absorbed (mg F per day)	Average urinary concentration (mg F per litre)
3.98	3.1
8.37	6.7
11.95	5.6
12.90	7.8
18.67	11
23.65	16
23.86	17

^a Data from Largent (1959).

different from those quoted above were reported by Ham & Smith (1954b), who observed that adult women retained 20-52% of about 1 mg of fluoride taken as tea.

Animal studies confirm the general dimensions of these retention data.

Younger individuals, who have a greater proportion of their skeleton available to the circulation and who are actively laying down bone mineral, excrete a lower percentage of a given dose of fluoride than do adults. Infants excrete 32-50% (average 40%) of the F ingested daily (Ham & Smith, 1954a). Longwell (1957) reported that children in the London area aged 5-6 years excreted half as much F in the urine (0.16 mg per 24 hours) as did those aged 10-12 years (0.35 mg per 24 hours). Gedalia (1958) found that the urinary fluoride content of children 1-3 years old was only half that of children 4-6 years old, and that, in general, the urinary F content increased with age from 1 to 12 years (see also Auermann, 1962). The clearest indication of the difference between the behaviour of the skeleton of the child and that of the adult is to be found in the analyses of urine samples in Montgomery County, Md., before and after the water supply was fluoridated (Zipkin et al., 1956). Immediately after fluoridation of the water, the urinary fluoride concentration in adults aged 30-39 years showed an increase from the pre-

fluoridation value of about 0.3 ppm and, in a month or so, approximated to the 1-ppm concentration in the drinking water, the values remaining between 0.9 and 1.0 ppm thereafter. In contrast, the urinary F concentration in children aged 5-14 years increased from 0.3 ppm to only 0.6 ppm after three months and slowly rose to about 0.8 ppm after two years and 0.9 ppm after three years. A similar slow increase in the urinary concentration in children was shown in the city of Grand Rapids, Mich., after water fluoridation was instituted there (Zipkin et al., *op. cit.*). That the urinary fluoride concentration is not characteristically lower in children who have been all their lives on fluoridated water can be seen from the data obtained in Aurora, Ill., where boys in each age-group from 8 through 17 years had average urinary fluoride levels of 0.9 ppm (Zipkin et al., *op. cit.*). Similar evidence can be drawn from the study of the urinary concentrations following the defluoridation of a community water supply that had originally been elevated in fluoride. When the 8 ppm F in the water supply of Bartlett, Tex., was reduced to 1 ppm, the urinary fluoride concentrations in the adults diminished somewhat more rapidly than did those in the children (Likins, McClure & Steere, 1956). This is the obverse of the changes seen in the Montgomery County study.

Mechanism of Urinary Excretion

The extraordinary rapidity of excretion of fluoride from the body can be highlighted by considering the fact that a milligram of fluoride, consumed at time zero, absorbed and presumably distributed through the normal body halogen pool, 100-150 grams of chloride, is handled by the kidneys so expeditiously that nearly a third of the milligram has been excreted in the urine after four hours. As Chen et al. (1956) point out: "It would be unnecessary to assume that a special excretory mechanism were involved if the renal clearance of fluoride could account for the prompt appearance in the urine of a sizeable fraction of a small oral dose". Carlson, Armstrong & Singer (1960) observed renal clearances in two human subjects following the ingestion of 1 mg of fluoride labelled with ^{18}F . The fluoride clearances (*a*) were always greater than the chloride clearance, (*b*) increased with urine flow, and (*c*) were always less than the creatinine clearances. Resorptions of 51 and 63% were observed, as compared with the 99.5% or greater resorption of chloride in a normal individual. Renal blood flow is so copious that this somewhat less efficient resorption of fluoride accounts for the very rapid rate of excretion. Studies on the dog conducted by Chen et al. (1956) and Carlson et al. (1960) gave comparable findings (e.g., Table 3). When carrier-free ^{18}F was administered, the ^{18}F concentrations in the urine were 3-14 times greater than those in the plasma. The clearance of ^{18}F exceeded that of chloride by 8- to 179-fold. Tubular resorption ranged from 23% to 78% in the several tests made. The addition of carrier F to the continuous infusion

TABLE 3
RENAL EXCRETION OF FLUORIDE IN THE DOG^a

Conditions	Length of period (min)	¹⁸ F concentration (U/P ratio)	Urine flow (ml/min)	¹⁸ F clearance (ml/min)	Clearance ratio		Tubular resorption (%)
					¹⁸ F/Cl	¹⁸ F/creatinine	
Carrier-free ¹⁸ F	5-15	3-14	0.4-3	2-10	8-179	0.2-0.8	23-78
Carrier + ¹⁸ F	10-15	14-30	0.2-0.5	4-13	26-230	0.2-0.5	46-77
After chlorothiazide	9-10	0.9-15	1.5-1.8	2-24	0.2-262	0.06-0.9	14-94

^a Selected data from Carlson et al. (1960).

did not alter the general picture: the urinary fluoride to plasma fluoride ratios ranged from 14 to 30; fluoride clearances were 26-230 times greater than chloride clearances; tubular resorption ranged from 46% to 77%. After administration of a powerful diuretic, chlorothiazide, chloride excretion increased; the general picture, however, was unchanged: the ¹⁸F concentrations in the urine were 0.9-15 times those in the plasma, the clearance of ¹⁸F ranged from 0.2 to 262 times that of chloride, and the tubular resorptions ranged from 14% to 94%. Thus, in the dog as in man, the rapid urinary excretion of F is accounted for by normal kidney mechanisms. It is not necessary to assume that any fluoride is secreted by the tubules, because throughout this study the clearance of ¹⁸F was always less than that of creatinine (Table 3).

Fluoride is thus shown to be removed from the circulation by glomerular filtration, and the rapidity of excretion can be traced to the less efficient tubular resorption.

Excretion in Kidney Disease or Injury

Only fragmentary data are available on this aspect of urinary fluoride excretion. Elderly women without kidney disease excreted urine with the same F concentration as younger, pre-menopausal women (Sircovitch, Gedalia & Zukerman, 1964). Preliminary studies on elderly patients with advanced kidney disease showed considerable variation from patient to patient before and after fluoridation; no trend appeared to indicate that these nephritic patients consistently excreted less fluoride than elderly patients with normal kidney function (Smith, Gardner & Hodge, 1955). Schlesinger (personal communication) collected urine samples from a group of children with serious kidney disease who were drinking water containing 1 ppm fluoride and found that the urinary fluoride concentrations were in the normal range. Yudkin, Czerniejewski & Blayney (1954) compared the concentrations of fluoride in urine samples from nephritic and normal individuals living in an area where the drinking water contained 1 ppm F;

similar comparisons were made in areas where the water contained 0.1 ppm F. The urine of both the normal and the nephritic individuals contained about 0.3 ppm F in the low-fluoride area; in the fluoridated area, the normal individuals excreted urine containing about 1 ppm F, whereas the average value for eight nephritic patients was 0.64 ppm. Urine samples from 11 nephritic patients (non-protein nitrogen values: 40-100 mg per 100 ml), aged 53-83 years, residents of Charlotte, N.C., contained lower F concentrations (average 0.10 ppm) than were found in 12 pooled samples (range 0.16-0.40 ppm F) from normal individuals, aged 25-55 years, in the same city (Smith, unpublished data). Two patients with clinical evidence of renal dysfunction given doses of 6 mg fluoride daily excreted less fluoride in their urine than would normally be expected (Largent, 1961).

From the limited data cited, it appears that urinary fluoride concentration and amount excreted tend to be lessened in renal failure. From another source—namely, fluoride analyses of human bone—confirmatory evidence can be drawn. Call et al. (1965) found elevated F concentrations only in the bones of patients suffering from chronic kidney disease. Linsman & McMurray (1943) cite the case of a patient who died of renal disease and was found at autopsy to have 7500 ppm F in the vertebral ash. Maes, Dufaux & Vandenbroucke (1960) describe a similar case in which a patient suffering from chronic interstitial nephritis and exposed in the course of his employment to F-containing dusts had 11 600 ppm F in the ash of a tibial bone sample taken by biopsy. A reasonable explanation of the course of events can be made. If renal function is impaired to the extent that fluoride excretion is slowed and reduced, absorbed fluoride will tend to remain elevated longer in the blood, thereby offering an opportunity for more F to deposit in bone. In patients with depressed water excretion, the reduction in F excretion may or may not cause a decrease in urinary F concentration. Some support for these concepts may be drawn from animal experiments. Nephrectomized rats were found to have slightly higher plasma ^{18}F levels than normal rats, and increased skeletal deposition of fluoride (Carlson, Singer & Armstrong, 1960).

Kidney disease or injury of lesser severity does not appear to reduce urinary F excretion detectably.

Rabbits with kidneys so severely damaged by uranium that they were nearly moribund nevertheless excreted fluoride effectively and maintained over a 30-day period the same fluoride balance as was found in normal, unpoisoned rabbits (Smith, Gardner & Hodge, 1955).

Excretion in Pregnancy

Gedalia, Brzezinski & Bercovici (1959) reported that, in regions with 0.5-0.6 ppm F in the drinking water, urinary fluoride levels decreased steadily from the fifth month of pregnancy to the eighth month, and rose slightly

thereafter but not to the initial level. In fact, it was 2-3 months after delivery before urinary fluorides were again at the usual pre-pregnancy concentration.

Shortly before delivery the fluoride levels in maternal blood (Gedalia et al., 1961) and saliva (Lerman et al., 1962) were lower than those in the blood of non-pregnant women or in the saliva of the same women in the fourth month of pregnancy. The fluoride content of the urine was found to be lower shortly after delivery than shortly before delivery (Bercovici, Gedalia & Brzezinski, 1960). After delivery, the urinary fluoride levels of non-lactating women were about the same as those of lactating women (Bercovici, Gedalia & Brzezinski, *op. cit.*). The additional storage of fluoride is probably to be accounted for by the increased availability of the maternal skeleton as a result of the hormone-induced bone changes in the normal cycle of parturition. The total amount of fluoride stored from the fifth through the ninth month of pregnancy, as estimated from the changes in urinary fluoride concentration, may be 30 mg. If the mother's bone mineral is taken to be 3000 g, the additional fluoride would contribute a 10-ppm increase in bone fluoride concentration—an undetectable increase. If the normal fluoride content of the young adult skeleton is estimated at about 750 ppm in persons drinking water containing 0.5 ppm, the skeleton would contain about 2 g of fluoride. An increase of 30 mg, therefore, only amounts to 1.5% additional F. It seems probable that calcification of the skeletal system in the foetus has little to do with this additional fluoride storage. A 3000-g foetus may contain about 30 g of total skeletal mineral. Since the bone of the newborn usually contains 50-100 ppm fluoride, less than 3 mg of fluoride would be accounted for in the foetal bone mineral.

Mobilization of Fluoride from Skeletal Stores

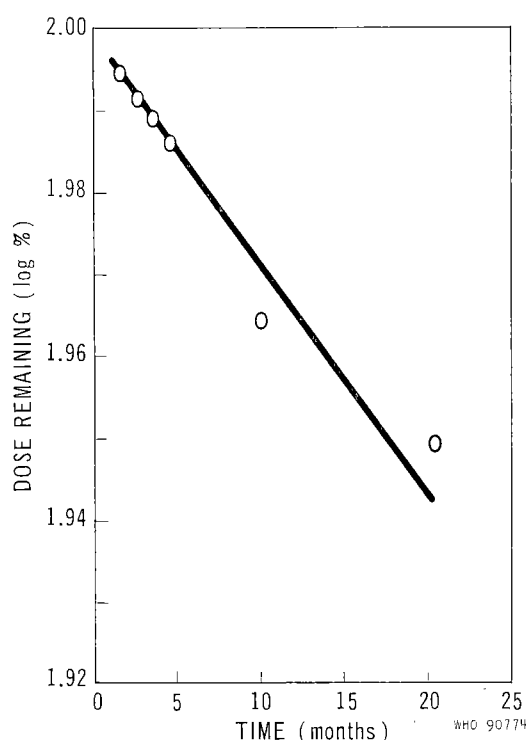
Fluoride deposition in the skeleton of man is not an irreversible process. If an individual who has been living in a community where the drinking water contains high amounts of fluoride—for example, 8 ppm—moves to a locality where there is little fluoride in the drinking water, excess fluoride appears in the urine for some time. Roholm (1937) showed that when a workman who has been exposed to high concentrations of F in dust—and who has stored considerable fluoride in his skeleton—leaves his employment, his urinary fluoride levels will be elevated for long periods, in fact for years. The process of mobilization of fluoride from the skeleton, first identified by Roholm, presumably involves the loss of fluoride both from the surface and from the interior of the crystals of the bone mineral. If the plasma fluoride and extracellular fluoride levels decrease somewhat, exchangeable fluoride from the crystal surfaces is replaced by hydroxyl ions and is freed to enter the circulation and be lost from the body *via* the urine. In the con-

stant process of osteoblastic-osteoclastic reworking of the human skeleton, the osteones are torn down and all the mineral in the crystals thus dissolved is again available to the circulation. From these crystals all the fluoride, including that in internal lattice positions, may enter the extracellular fluid and be excreted or redeposited on the crystal surfaces.

Rats treated with parathormone lost bone as expected, but neither the bone F concentration (Gedalia & Kletter, 1964) nor the plasma F concentration (Singer & Armstrong, 1960) was altered—a fact that points to an excretion of F which had been mobilized from the bone without disturbing the homeostatic control.

A striking example of the regularity of the mobilization processes is found in the data assembled by Largent (1959) following the two-year period during which he took 2 to nearly 20 mg F per day in balance periods of 1 to 3-4 months. Because he had measured his total F intake and excretion

FIG. 6
MOBILIZATION OF SKELETAL FLUORIDE IN MAN^a



^a The subject's skeleton was estimated to contain 1.7 g of fluoride retained from prior experimental periods of known fluoride ingestion (Largent, 1947). The amounts mobilized during successive intervals after completion of the ingestion experiments were calculated from the reported urinary excretion data (*loc. cit.*).

over the two-year period, he could calculate at the end of this time that his skeleton contained about 1.7 g of fluoride more than when he had begun the study. In Fig. 6 the logarithm of the percentage remaining of the 1.7 g of fluoride is plotted against specified times after Largent ceased the experimental ingestion of fluoride. From the line as drawn, if it reliably indicates a simple exponential process of mobilization, an estimate can be made that about 8 years would be required to remove half the fluoride from the skeleton. The graph is of interest because Largent's data furnished one of the first estimates of the turnover time of the adult human skeleton. It is tempting to draw another line connecting the percentages of F remaining after 10 months and 20.5 months. If this line should represent a stable mobilization rate, the loss of F from the skeleton immediately after stopping F doses was relatively greater, perhaps because of exchange processes. Largent (1959) showed, for three additional subjects, that after termination of an elevated intake of fluoride, a nearly linear trend of decreasing fluoride excretion ensued for a long period. One of these subjects had been ingesting 12 mg of fluoride daily as NaF in a metabolism experiment; another had been taking 3 mg F daily; and the third had for a long time been resident in a community in which the drinking water contained 4 ppm F. Largent described these processes by exponential equations.

Likins, McClure & Steere (1956) measured the urinary fluoride excretion of children and adults in Bartlett, Tex., before and after the water supply was defluoridated from its natural level of 8 ppm to a concentration of 1.0-1.5 ppm. As expected, the urinary excretion of fluoride decreased both in the children and in the adults, but more slowly in the children, presumably because of the greater availability of the young skeleton to the circulation and, consequently, the longer time for exchange and other reactions to establish new equilibrium conditions.

Observations of mobilization of fluoride have repeatedly been made in experimental animals such as rats, and in two studies on cattle. The data are summarized in Hodge & Smith (1965, p. 544).

Excretion in Bone Disease

Little or no information has been recorded about the urinary fluoride levels in bone disease. It has been observed that individuals suffering from crippling fluorosis or exhibiting fluoride osteosclerosis exhibit higher than normal urinary fluoride levels for long periods after removal from excessive fluoride exposure. Urinary fluoride excretion has been measured in four patients diagnosed as suffering from primary osteoporosis and in one patient with Paget's disease (Rich, Ensinn & Ivanovich, 1964). The urinary fluoride values (mg/day) ranged from 0.14 to 0.76 (probably normal) except in one individual, who excreted only 0.05 mg/day.

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Physiological effects of small doses of fluoride

G. N. JENKINS¹ — P. VENKATESWARLU² — I. ZIPKIN³

1. INTRODUCTION (I. Zipkin)

Primary interest in this chapter is centred on present-day knowledge of how the body handles fluoride at different doses up to about 4 ppm in the water supply for extended periods of time. Three areas of discussion are pursued.

First, Professor Venkateswarlu reviews our present knowledge of the concentration of fluoride in body-fluids and in soft tissues, as well as the relationship of fluoride to intermediary carbohydrate, lipid and protein metabolism. The effect of fluoride on hormones, vitamins and enzymes is also discussed. Secondly, the author of this introduction discusses the effects of fluoride on the chemical and physical structure of bone, and the mechanisms affecting the deposition and mobilization of skeletal fluoride. Finally, Professor Jenkins collates the information available on the effect of fluoride on the chemistry and morphology of teeth, and on suggested mechanisms to explain the cariostatic effect of fluoride.

Wherever possible the human is the subject of relevance. Where fluoride data for man are unavailable, corollary studies on experimental animals are presented.

2. EFFECTS ON BODY-FLUIDS AND SOFT TISSUES

(P. Venkateswarlu)

Fluoride accumulates to easily measurable levels in calcified tissues and it is also present in soft tissues, but in only minute traces which are difficult

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to determine. For this reason the bulk of the earlier work on fluoride metabolism was confined to the hard tissues. More recently, the development of fairly dependable micromethods for fluoride determination and the availability of radiofluoride (^{18}F) have given impetus to the study of fluoride metabolism in the soft tissues and body-fluids.

For want of space this review is restricted to the metabolism of inorganic fluorides. An excellent review of the biological effects of organic fluorides has been published by Hodge, Smith & Chen (1963).

In the following text, F denotes the fluoride ion.

Mechanisms of Transport and Homeostasis of Fluoride

Gastrointestinal absorption of fluoride

The process of gastrointestinal absorption of F appears to be governed by an interplay of anatomical, physiological and biochemical factors. From different groups the following findings have been reported. F absorption in the ruminants was observed to be confined mostly to the rumen (Perkinson et al., 1955). The greater gastrointestinal absorption of F in the rabbit than in the rat was attributed to the longer gastrointestinal tract of the rabbit (Largent, 1948). F transport was not found to vary with the anatomical site of the small intestine (Venkateswarlu, 1962). Wagner (1962a) observed that 80% of the administered F was absorbed through the rat gastric mucosa in 15-20 minutes when the pyloric sphincter was ligated. Curiously, molybdenum was reported to depress gastric absorption and increase intestinal absorption of F in the adult rat, while it diminished intestinal absorption in the young rat (Stookey, Crane & Muhler, 1962). These findings need confirmation.

Soluble fluorides are fairly well absorbed in contrast to insoluble fluorides. Nevertheless, at low concentrations the less soluble fluorides can be very nearly as effective sources of F as the more soluble fluorides. Al^{+++} , Ca^{++} and Mg^{++} which can form insoluble complexes with F decrease absorption of F. Citrate and other substances which can complex Ca^{++} and thereby counteract the influence of Ca^{++} present in the gastrointestinal juices facilitate F absorption. Absorption of physiologically inert and covalently bound F compounds (KPF_6 , $(\text{C}_2\text{H}_5)_4\text{NPF}_6$, KBF_4) is greater than that of the physiologically active simple fluorides (NaF , Na_2SiF_6 , SnF_2) (Zipkin & Likins, 1957). A possible explanation of this finding is that while a fraction of ionic fluorides can be complexed by intestinal Ca and phosphate ions by precipitation as CaF_2 or adsorption on insoluble calcium phosphate and thus rendered unavailable for absorption, covalent fluorides cannot be so complexed and seem to be wholly available for (passive) absorption. The PO_3F ion in, for example, $\text{Na}_2\text{PO}_3\text{F}$ is only gradually split by enzymes in the digestive tract and while its F content is metabolically available its absorption is much less influenced by calcium salts (Ericsson, 1967).

Intestinal transport of F seems to be passive (Stookey, Dellinger & Muhler, 1964), in contrast to that of chloride (Armstrong, Venkateswarlu & Singer, 1961), and regulated by plasma F level. A low plasma F level, such as might be expected in an actively growing young animal with relatively little prior exposure to F (Savchuck & Armstrong, 1951; Zipkin & McClure, 1952) or in starvation (F depletion) (Stookey, Crane & Muhler, 1964), may facilitate F absorption, whereas an elevated plasma F level, such as could occur in continuous ingestion of F (Lawrenz, Mitchell & Ruth, 1940) or in renal failure (bilateral nephrectomy) (Stookey, Crane & Muhler, 1963), may decelerate the intestinal absorption of F. However, these probabilities remain to be substantiated by actual determination of plasma F levels in the different situations.

The active transport of F from the serosal to the mucosal side of everted intestinal sacs of rats has been reported (Parkins, 1966).

Distribution of fluoride in blood

At the normal pH of 7.4, the erythrocyte: plasma ratio of ^{18}F is 0.66 : 1 in the dog; the ratio decreases with increase in pH and *vice versa*. This pH-dependent migration of ^{18}F is similar to the chloride shift, although there are some quantitative differences in the migration of fluoride and chloride ions (Carlson, Armstrong & Singer, 1960a).

Less than 5% of plasma ^{18}F is non-diffusible. In fluoridated serum, F migrates to a large extent with the serum albumin, and only a small proportion migrates freely towards the anode, whereas F is completely absent from the serum globulin fractions (Sforzolini, Cusma & Mastrantonio, 1964). Ca has been shown to act as a mediator in binding ^{18}F to albumin (Carlson, Armstrong & Singer, 1960a).

Homeostasis, adaptation and mobilization

Following any mode of ingestion of F, the F in blood plasma will be elevated above the normal resting level, and homeostatic mechanisms will operate to restore the level to normal.

The disappearance of ^{18}F from blood following intravenous injection of ^{18}F has been studied in rats. The nature of the ^{18}F disappearance curve suggests that it may be a composite of several curves, possibly reflecting equilibration of ^{18}F in extracellular and intracellular fluids, skeletal deposition and urinary excretion (Wallace-Durbin, 1954). The ^{18}F disappearance curve in ruminants has been found to be resolvable into four components similar to comparable curves reported for ^{45}Ca (Perkinson et al., 1955). Similar plasma ^{18}F disappearance curves have been reported in humans (Carlson, Armstrong & Singer, 1960b).

The efficiency of regulation of the plasma F level has been demonstrated in humans (Singer & Armstrong, 1960). The mean plasma F content of

four populations using drinking water containing 0.15, 1.1, 1.1 and 2.5 ppm F was approximately the same, the values being 0.14, 0.15, 0.19 and 0.16 ppm F respectively. A slight, but significant, elevation of the plasma F level (0.26 ppm) was noted in a population drinking water containing 5.4 ppm F. No significant or prolonged rise of plasma F level was detected following ingestion of 1 mg of F; and little diurnal variation of plasma F was observed beyond a very slight rise following the intake of food. In the dog, after one year of daily administration of 1 g and 5 g UF_4 per kg body-weight, the blood F level was reported to be 0.25 ppm and 1.25 ppm respectively (F. A. Smith, cited by Voegtlin & Hodge, 1949). In subjects with metabolic bone diseases, administration of doses of NaF 50 to 100 times greater than the normal F intake did not increase the plasma F level more than four- or five-fold and, after withdrawal of NaF, the F levels reverted to the normal pretreatment level (Armstrong et al., 1964).

While the rise in plasma F level is controlled by skeletal sequestration and by urinary, dermal and faecal (gastrointestinal) excretion, there seems to be a self-regulatory mechanism whereby an elevated plasma fluoride level such as can result from renal failure (Stookey, Crane & Muhler, 1963) or from continuous ingestion of fluoride restrains intestinal absorption of F (Lawrenz, Mitchell & Ruth, 1940).

While homeostatic mechanisms seem to operate well in certain species (the rat and the human), they seem to be less efficient in others—for example, cattle and chickens. In chicks receiving a natural basal diet containing 25 ppm F and the same diet with 300, 600 and 900 ppm supplementary F, for two weeks from one day of age, the plasma F levels were reported to be 0.23, 2.1, 3.1 and 5.2 ppm respectively (Suttie, Phillips & Faltin, 1964). However, to evaluate correctly the differences in efficiency and pattern of F homeostatic mechanisms in various species, it would be necessary to have data on plasma F levels, determined preferably by the same method, in different species receiving identical doses of F over similar periods of time.

Acquisition of fluoride by soft tissues

The F content of soft tissues and body-fluids is extremely low in comparison with that of hard tissues. This fact could be explained in more than one way.

First, it is possible that the avidity of the hard tissues for the circulating F is so strong and the renal excretion of F operates to such an extent that the fluoride level in plasma and other body-fluids is kept too low to boost the soft-tissue fluoride level appreciably. If this hypothesis were correct, the fluoride levels in blood and soft tissues would be higher in species devoid of hard tissues than in species with hard-tissue structures after a similar degree of fluoride ingestion. Unfortunately, no reliable data in respect of this are as yet available. According to Carlson, Singer & Armstrong (1960), more

^{18}F appeared in the soft tissues of nephrectomized rats than in those of control animals after intraperitoneal administration of the isotope.

Secondly, it is possible that physico-chemical characteristics of cellular membranes do not favour significant intracellular transport of F. For example, barley root membrane is permeable to the Cl ion, which is actively absorbed against a concentration gradient of 1 in 300, but is not permeable to the F ion despite a very favourable concentration gradient (Venkateswarlu, Armstrong & Singer, 1965). In studies of the *in vivo* intestinal absorption of initially equal concentrations of Cl and F in the lumen, a more than 3 to 1 discrimination in favour of Cl as against F has been observed (Armstrong, Venkateswarlu & Singer, 1961). Rat gastric mucosal membranes acquire less F than Cl from the plasma and also subsequently secrete less F than Cl in the gastric juice (Venkateswarlu, 1962).

Administration of 1 mg F daily for 90 days to weanling rats failed to demonstrate any increase in the F contents of heart, liver and kidney even as fluoride saturation of the skeleton was approached (Wagner, Stookey & Muhler, 1958). However, in the absence of data on the plasma F levels, it is not possible to say whether the absence of rise of F in the soft tissues was due to the lack of attainment of an adequate level of circulating F or to the very limited permeability of the cell membrane to the F ion.

Normally, the F levels of most soft tissues are below 1 ppm but slightly in excess of the plasma F levels (0.1-0.2 ppm). Aorta and placenta have more F than other soft tissues (Ericsson & Ullberg, 1958; Gardner et al., 1952; Sita & Venkateswarlu, unpublished data; Smith et al., 1960), presumably because they develop zones of calcification in which circulating F can be trapped (Ericsson & Hammarström, 1964). Increased deposition of Mg in soft tissues seems to facilitate increased retention of F in these tissues (Foster et al., 1960; Griffith, Parker & Rogler, 1963a).

Besides intracellular Ca and Mg, intracellular colloids could also play a role in the acquisition of F by soft tissues. Although intracellular proteins carry a net negative charge at physiological pH, the possibility of their binding anions like F has been indicated. Since, in the binding process, the negativity of the protein is decreased, a simultaneous uptake of a greater number of protons than the number of anions is postulated (Engel et al., 1961). The role of Ca in the binding of F by albumin has already been mentioned.

Despite decreased skeletal retention and increased excretion of F in older rats, their soft tissues have been reported to contain more F than the soft tissues of younger animals (Wagner, 1962b). Whether, owing to the decreased rate of sequestration of circulating F by the adult skeleton, the plasma F level in the older rats is elevated, thus facilitating progressive accumulation of F in the soft tissues, or whether the F-binding constituents of the tissues (Mg, Ca, or proteins with a predilection for F) could increase with the age of the rats is not known and requires investigation.

It has been observed that, following ^{18}F administration, relatively vascular tissues such as liver, spleen and intestine acquire more ^{18}F per gram of tissue than the less vascular tissues like skin and muscle. ^{18}F concentrations, with the exception of those in the kidney and salivary glands, paralleled the blood ^{18}F level at all times. Thyroid did not accumulate ^{18}F and its ^{18}F concentration did not exceed the blood ^{18}F level at any time in the course of 9 hours of the experiment (Wallace-Durbin, 1954).

Hein et al. (1956) reported that thyroid, kidneys and adrenal glands (all blood-rich organs) contained the highest concentration of ^{18}F 30 minutes after oral administration or 10 minutes after intravenous administration of the isotope.

The distribution of ^{18}F in the soft tissues of rats on low-fluoride and high-fluoride diets was studied at 80 and 120 minutes after intraperitoneal injection of ^{18}F . To add another dimension to the study of fluoride metabolism, a labelled chloride (^{36}Cl) was administered simultaneously with ^{18}F and the relative distribution of the isotopes was determined (Venkateswarlu, 1962). In the following, $^{18}\text{F}_{\text{tw}}$ and $^{36}\text{Cl}_{\text{tw}}$ denote the ^{18}F and ^{36}Cl counts per minute per gram of tissue water (tw) while $^{18}\text{F}_{\text{pw}}$ and $^{36}\text{Cl}_{\text{pw}}$ denote the ^{18}F and ^{36}Cl counts per minute per gram of plasma water (pw).

No difference was found in the values of the ratio $^{18}\text{F}_{\text{tw}}/^{18}\text{F}_{\text{pw}}$ for corresponding tissues in rats on low-fluoride and high-fluoride diets. The ratio was less than 1.0 in all tissues except the kidney and the tail tendon. The magnitude of the ratio for the kidney is undoubtedly due to the role of the organ in concentrating fluoride from the plasma in the process of formation of urine.

In this study, the stomach is a particularly interesting organ in that it concentrates chloride and secretes HCl. The results indicated that the stomach wall and the gastric juice had a larger content of ^{36}Cl than of ^{18}F , compared with their plasma levels, suggesting a discrimination in favour of chloride. Gastric wall was the only tissue among those examined in which the ratio under discussion was found to be less than 1.0 in all examples. This ratio appeared to be even lower in the gastric juice, suggesting preferential secretion of chloride. This finding is in contrast to that with the kidney and urine, in which the ratio was in favour of the fluoride ion.

Although the $^{18}\text{F}_{\text{tw}}/^{18}\text{F}_{\text{pw}}$ and $^{36}\text{Cl}_{\text{tw}}/^{36}\text{Cl}_{\text{pw}}$ ratios in tendon could be influenced by loss of water during dissection of the tissue, the ratio $(^{18}\text{F}_{\text{tw}}/^{18}\text{F}_{\text{pw}})/(^{36}\text{Cl}_{\text{tw}}/^{36}\text{Cl}_{\text{pw}})$ would not be affected in similar circumstances. In the case of animals sacrificed 80 minutes after isotope administration, the latter ratio was definitely less than 1.0. However, in those animals which were allowed to live for 120 minutes, the ratio was more than 1.0, indicating a preferential uptake and sequestration, progressing with time, of ^{18}F by the tendons. These observations are of interest in relation to the several publications in the literature describing hypercalcification of certain tendons

in humans who had consumed, for long periods of time, drinking water with a high fluoride level (Shortt et al., 1937; Singh, Jolly & Bansal, 1961).

However, if such a calcification of the tendon (an organic matrix) is dependent on its prior acquisition of F, we do not as yet know exactly how F so acquired leads to subsequent calcification of the tendon. Recently attention has been called to the presence of organically bound F in the organic matrix of tooth enamel as distinct from the inorganic F in its mineral phase (Little, Casciani & Rowley, 1968). The significance, if any, of the organically bound F in the enamel is not known.

Acquisition of F by hard tissues

The bulk of the F administered by any route, on entering the extracellular fluid, is invariably sequestered by the skeletal structures, which have a variable propensity for acquiring fluoride.

The author of this section found no significant difference in the acquisition of intraperitoneally administered ^{18}F by corresponding hard tissues of rats which had ingested and retained significantly different amounts of fluoride (F in skeletal ash varying from 24 to 1727 ppm) (Venkateswarlu, 1962). Thus the previous fluoride load in the bones, within wide limits, is not of consequence in determining the amount of ^{18}F which can be acquired subsequently by the bones.

The ^{18}F thus acquired represents an increment of fluoride deposition and not an "exchange", because it was subsequently shown that bones of high F content did not exchange their fluoride with that of plasma in two hours following intraperitoneal injection of carrier-free radiofluoride (^{18}F) in saline (Armstrong & Singer, 1963a).

No more will be said here about the acquisition of F by hard tissues since this subject is extensively dealt with by Weidmann and Weatherell in Chapter 4, section 3, and by Zipkin in section 3 of this chapter.

Urinary excretion of fluoride

This aspect is covered in Chapter 5 (Hodge, Smith & Gedalia).

Placental transfer of fluoride

This aspect is covered by Gedalia in Chapter 4, section 4.

Mammary and salivary secretion of fluoride

These aspects are covered in Chapter 5 (Hodge, Smith & Gedalia).

Metabolic Interrelationships of Fluoride in Body-Fluids and Soft Tissues

Fluoride and general metabolism

Fluoridation of public water supplies for the control of dental caries has raised some questions about the health safety of concentrations of fluoride

in communal water in the range 1-2 ppm. The epidemiological and experimental evidence for the safety of water fluoridation has been impressive and very massive.

Clinical studies in areas where the water is fluoridated and clinical and post-mortem studies in areas where the waters have a high natural fluoride content are reported in Chapter 8.

Some concern was expressed about reports of depression of blood pressure, increased heart rate and increased femoral blood flow in dogs following administration of 1 and 5 mg F by stomach tube (Richardson, Muhler & Bishop, 1955). However, it was subsequently demonstrated that the mere introduction of the stomach tube itself caused the decrease in blood pressure (Caruso & Hodge, cited by Smith (1962)).

Reports of renal changes in rats following ingestion of water containing 1, 5 or 10 ppm (Ramseyer, Smith & McCay, 1957) also caused some concern. But these observations could not be confirmed, and later it was established that the changes reported to be caused by F ingestion were no different from those normally seen in the kidneys of aged rats and were not readily traceable to F ingestion (Bosworth & McCay, 1962). Fluoride seemed to be implicated in the etiology of urinary stones, but no experimental evidence was obtained (Herman, 1956; Spira, 1956). Moreover, Venkateswarlu, Singer & Armstrong (1958) observed a slightly lower frequency of urinary stones in rats receiving a urinary-stone-inducing diet plus 10 ppm F in drinking water than in control rats which received the same diet, but no supplemental fluoride.

Renal handling of F was the same in normal rabbits as in rabbits in which chemical nephritis had been induced. Elderly patients with normal kidney function and a similar age-group of patients with long-standing kidney disease showed no difference in the range of urinary F concentrations (Smith, Gardner & Hodge, 1955). Thus kidney disease does not seem to accentuate the problem of renal handling of fluoride in an individual. The decrease in intestinal absorption of F as a homeostatic mechanism after nephrectomy has already been mentioned.

The question of iodine metabolism and possible interference by another halogen such as F is discussed in Chapter 7.

It is pertinent to mention here the reports from India of the incidence of skeletal fluorosis in areas with 3-5 ppm F in the drinking water (Pandit et al., 1940). Some of these reports have still to be critically evaluated and re-established. In all probability the F intake *via* water and food is very high in these localities because of the increased intake of water and salt to correct their heavy losses from the body in the tropical climate. Some samples of rock salt (75% NaCl) which have been used extensively as cooking salt till recently in regions such as the Punjab in India have been shown to contain F in concentrations as high as 200 ppm, corrected for 25% insoluble residue in the rock salt (Sita & Venkateswarlu, 1967). Daily consumption of 15-20 g

salt (NaCl), in the form of rock salt, could result in the daily ingestion of 3-4 mg F over and above the F consumed in food, beverages (tea) and drinking water (sometimes contaminated with F sediments). However, quite contrary to expectations, the fluoride in the rock salt was not found to be biologically available. There was no increase in the skeletal F in rats receiving rock salt in the diet over that found in appropriate control rats (Raman, Sita & Venkateswarlu, unpublished data). Data on precise F intake and on faecal and urinary excretion of individuals from endemic areas of fluorosis would be most useful for a critical appraisal of the association of skeletal fluorosis with drinking waters containing relatively low levels of F.

Fluoride and carbohydrate metabolism

In certain circumstances F was found to depress respiration and stimulate fermentation in micro-organisms, and in other circumstances quite the opposite effects were observed (Runnstrom, Borei & Sperber, 1939). Stimulation of fermentation could be associated with depression of polysaccharide synthesis. In the presence of F (10-11 ppm), less ^{14}C -glucose was incorporated in the polysaccharides (Weiss, Schnetzer & King, 1964). At higher concentrations (20-200 ppm), CO_2 production was inhibited, presumably owing to a block of the enolase system resulting in accumulation of hexose phosphates (Nilsson, 1930). Such inhibition could be nullified by the addition of phosphate acceptors such as adenylic acid (Runnstrom & Hemberg, 1937).

In acute F poisoning of rabbits (2 hours after subcutaneous injection of 250 mg NaF per kg body-weight), glycogen decreased in liver and skeletal muscle and remained unaltered in the myocardium. Phosphorylase decreased in skeletal muscle only. In chronic fluorosis (oral doses of 50, 30 and 10 mg NaF per kg, daily for 3 months), mild to severe degeneration and almost complete disappearance of phosphorylase in the necrotic foci were found in the myocardia and liver, but no significant changes were observed in the skeletal muscle even with these extremely high doses (Iwase, 1958). In this context, one may recall the inhibitory effect of F on the role of HGF (the hyperglycaemic-glycogenolytic factor) and on epinephrine in the activation of muscle phosphorylase (Cori, 1950).

Zebrowski, Suttie & Phillips (1964) administered ^{14}C -glucose and ^{14}C -palmitate to rats which had received 0.1% NaF in the diet for 30-45 days, and observed that, in comparison with the controls, fatty acid catabolism was decreased and glucose catabolism unaffected, but glycogen turnover was depressed.

The degree of F exposure to which the above-mentioned experimental animals were subjected is never encountered in practical human nutrition and endemic fluorosis.

In a very severe case of skeletal fluorosis with spastic paraplegia, a glucose-tolerance test was found to be normal (Murthi, Narayana Rao & Venkateswarlu, 1953), as were the blood sugar levels of several fluorotic patients (Shortt et al., 1937). In a study of chronic experimental fluorosis in monkeys (Pandit & Narayana Rao, 1940), glycosuria was not observed, although the animals had developed a very advanced and severe form of osteosclerosis.

Fluoride and lipid metabolism

Study of the interrelationship of F and lipid metabolism assumes considerable importance in view of the implication that F plays a role in the incidence of arteriosclerosis (Exner & Waldbott, 1957). Epidemiologically, there is no evidence to support such an implication (Leone et al., 1954). In fact, Bernstein et al. (1966) found a higher frequency of roentgen-visible calcification of the aorta in low-fluoride than in high-fluoride areas.

Increasing the fat (Crisco,¹ corn oil, lard, cotton-seed oil) from 5% to 20% in F-supplemented rations aggravated the growth-retarding effect of fluoride in the rat and chick and also resulted in higher retention of F in the whole carcass, as well as in the femur and soft tissues (Bixler & Muhler, 1960; Büttner & Muhler, 1958a; Miller & Phillips, 1955). The higher retention of F, however, had no effect on serum cholesterol levels (Büttner & Muhler, 1958b).

The *in vitro* oxidation of fatty acids is reported to be inhibited by fluoride (Johnson & Lardy, 1950). As mentioned earlier, fatty acid catabolism was reduced in rats receiving 0.1% F in the diet for 30-45 days (Zebrowski, Suttie & Phillips, 1964); but 0.1% F in the diet amounts to an ingestion of 500-700 mg F per day, which is more than 100 times the 2-5 mg F ingested by any person *via* diet and water containing 1 ppm F.

Fluoride and protein metabolism

The urine of a fluorotic monkey was reported to darken on standing. The darkening was attributed to the presence of homogentisic acid (method of identification not mentioned) suggestive of an interference with the metabolism of phenylalanine and tyrosine (Pandit & Narayana Rao, 1940). However, F (10^{-3} M/litre final concentration, more than 100 times the tissue F concentration) added to guinea-pig-liver homogenates was not found to have any inhibitory effect on the *in vitro* oxidation of tyrosine (Venkateswarlu, 1955). Also, the patterns of urinary excretion of phenolic acids in fluorotic patients did not differ from those in normal humans (Saini et al., 1964).

¹ Proprietary name of a hydrogenated vegetable oil used for culinary purposes.

Other instances of fluoride-protein interrelationship—for example, the binding of F by dietary proteins in the gastrointestinal tract, by plasma proteins, by tendon, and by the intracellular proteins—have already been discussed, and the topic of fluoride and enzymes will be dealt with in some detail later in this section.

Fluoride and mineral metabolism

Fluoride and calcium. A number of observations indicated interactions between calcium and F. The distribution of F in the body was shown to conform to some extent to the pattern of distribution of calcium. A positive relation between F deposition and metastatic calcification was observed when calcification-inducing diets were fed to rats and guinea-pigs (Stookey & Muhler, 1963). Tissues such as aorta, which gradually accumulate calcium, also seemed to acquire increasing amounts of F. Placenta seemed to accumulate F readily in its zones of calcification. Calcium could mediate the binding of F by plasma proteins (Carlson, Armstrong & Singer, 1960a). Dietary Ca, by reducing F absorption, protected the organism against F intoxication (Lawrenz & Mitchell, 1941; Narayana Rao, 1942).

However, in natural conditions in humans, even on a restricted Ca diet, the daily ingestion of 7 and 14 mg F has no effect on the calcium balance (Wagner & Muhler, 1959). Larger doses of F used in the treatment of cases of osteoporosis promote a positive calcium balance and give fair clinical results (Purves, 1962; Rich & Ensink, 1961). F in physiological doses has no influence on serum or salivary Ca and P levels (Knappwost & Tochtermann, 1955). In the third trimester of pregnancy, both Ca and F are found to be lowered in serum, possibly coinciding with an active uptake of the elements by the foetus (Gedalia et al., 1963).

Fluoride and strontium. An alarm was created by a proposition that F ingestion could enhance the skeletal acquisition and retention of ^{90}Sr from radioactive fall-out (Kerwin, 1958). Analysis of human teeth, which keep once acquired mineral concentrations more stable than the bones, revealed no relationship between F and Sr in the tooth or any correlation of the tooth levels with F and Sr levels in drinking water (Steadman, Brudevold & Smith, 1958). Experiments on rats revealed that F ingestion does not increase retention of ^{89}Sr and *vice versa* (Muhler, Stookey & Wagner, 1959; Rogers, Wagner & Stookey, 1961). The metabolisms of Sr and F are not inter-related to warrant the above suspicion.

Fluoride and magnesium. In rats receiving a high-lipid diet, the F : Mg ratio in soft tissues was closely related to that in mineralized tissues. In rats receiving high Mg and high F besides high lipid, the F : Mg ratio increased in soft tissues and decreased in mineralized tissues (Foster et al., 1960). Whether this increase in F with respect to Mg could, to any extent,

explain the increased toxicity of fluoride when ingested in conjunction with a high-lipid diet is an open question. Presumably Mg-dependent physiological enzyme mechanisms are decelerated by increasing F levels—for example, inhibition of enolase could result in less efficient utilization of carbohydrates.

Incorporation of 0.08% F and 0.25% Mg in chick rations caused a greater growth depression than did F alone. Chicks on Mg plus F developed a characteristic leg weakness which was not seen on Mg or F alone. Raising the Ca level of the diet did not prevent the interaction of Mg and F. Plasma alkaline phosphatase was elevated by F; enolase activity in muscle homogenates was not impaired (Griffith, Parker & Rogler, 1963b). In chicks receiving supplemental Mg, increase in dietary F elevated Mg in plasma, bone, liver and kidney, but not in heart or muscle (Griffith, Parker & Rogler, 1963a).

In the dog, addition of F to the diet (200 ppm) prevented the development of gross aortic lesions and calcification of soft tissues inducible by a low-Mg diet (30 ppm). The mechanism of protection is not clear. Such protection was not detected in rats. While the lowering of the serum Mg level induced by low-Mg diet was not influenced by F in the dog, the level was further lowered by F in the rat (6-7 weeks' experiment) (Chiemchaisri & Phillips, 1963). However, in short-term experiments in the guinea-pig, the lowering of serum Mg on low-Mg diet was minimized by 450 ppm F in the diet (Thompson, Heintz & Phillips, 1964)—a finding which indicates the need for further investigations. Mg-F interrelationships may have something to do with the lower incidence of aortal calcifications found in a high-fluoride area (Bernstein et al., 1966).

The effects of Mg and/or ascorbic-acid deficiency, with and without additional F in the diet, on the metabolism of connective tissues were investigated in guinea-pigs. Blood serum hexosamine was normal in fluorosis and Mg deficiency. In aorta, hexosamine was elevated and hydroxyproline depressed in guinea-pigs on low-ascorbic-acid diet, high-F/low-Mg diet and low-Mg/low-ascorbic-acid diet (Thompson, Heintz & Phillips, *op. cit.*).

Fluoride and iron. When iron (^{59}Fe) was administered by stomach tube to rats raised on iron-deficient or iron-supplemented diets, an enhanced uptake of ^{59}Fe by the blood was observed in rats receiving additionally either a prior dietary supplement of F (200 ppm) for 76 days or an oral dose of F, sufficient to complex the ^{59}Fe , immediately after the iron was administered (Ruliffson, Burns & Hughes, 1963). It is difficult to explain the results on the basis of F facilitating the intestinal absorption of Fe, because Fe and F would appear to interfere with each other in intestinal absorption (Ruliffson & Hopping, 1963; Venkateswarlu, 1962). However, Stookey, Crane & Muhler (1964), who examined the entire gastrointestinal tract of the rat in F absorption studies, observed that the concomitant presence of

Fe enhanced F absorption. Whether F facilitates absorption of Fe in the stomach is not known. Whether the higher level of ^{59}Fe in blood could be a result of inadequate transport and utilization of plasma ^{59}Fe under the influence of F cannot be answered in the absence of data regarding the specific activities of blood Fe. Decreased *in vitro* incorporation of ^{14}C -glycocoll and ^{59}Fe in protoporphyrin by blood from rabbits receiving toxic doses of F has been proposed as the underlying cause of the hypersideroplasmatic hypochromic anaemia observed in fluorotic rabbits (Benard, Gajdos & Gajdos-Torok, 1958). The entire Fe metabolism as influenced by toxic doses of F would seem to merit further careful investigation, although ingestion of water containing about 1 ppm F is decidedly not known to have any adverse effect on normal Fe metabolism.

Fluoride and molybdenum. It has already been stated that some workers have found that Mo increases F absorption and, if confirmed, this might explain the reported increase of F levels in the bone and soft tissues of animals receiving a dietary supplement of Mo (Stookey & Muhler, 1962). However, recent reports throw doubt on this concept of an interaction of F with Mo (Adkins & Kruger, 1966; Büttner, 1961, 1963; Ericsson, 1966; Goodman, 1965). A combination of Mo and F was reported to be significantly more effective in reducing caries than F alone (Büttner, 1961; Jenkins, 1967; Malthus, Ludwig & Healy, 1964; Stookey, Roberts & Muhler, 1962). Molybdate itself may possess some anti-caries potentiality through its slight powers of inhibiting salivary acid production at pH 5 and reducing the *in vitro* solubility of calcium phosphate and enamel (Jenkins, 1967).

Fluoride and phosphate. Phosphate enhances fluoride absorption in the intestine presumably by counteracting the inhibitory action of intestinal calcium on F absorption (Bixler & Muhler, 1960). The formation of a fluorophosphate complex facilitating F transport has been also considered as a possibility. Absorption of fluorophosphate is interfered with less than that of F by calcium ions and for this reason, where calcium intake is very variable, monofluorophosphate has been suggested as a suitable form for fluoride in some caries preventive measures (Ericsson, Santesson & Ullberg, 1961).

Prolonged administration of F (20 mg/kg) to pups was reported to increase the serum organic phosphate and decrease the inorganic phosphate (Andreeva, 1957). In fluorotic rats, as compared with controls, subcutaneously administered ^{32}P was acquired more by muscle (not liver, brain, or thyroid) and bone. This has been interpreted as resulting from fluoride stimulation of the metabolic turnover of phosphate in muscle and bone. The animals were reported to excrete more urinary phosphate and the authors propose dietary supplementation with phosphate to correct for the alleged phosphate loss in fluorine intoxication (Gabovich, Bukhovets & Ermakova,

1960). Again, ingestion of 20 mg F per kg body-weight is never encountered in natural conditions. The interactions between fluoride and phosphate in enzyme mechanisms will be discussed later. An increase in dietary phosphorus from 0.14% to 0.71%, dietary calcium remaining constant, was reported to increase the appetite of growing rats and result in a distinctly heavier dry fat-free skeleton, although total retention and distribution of F in bones, teeth and soft tissues was not altered (Lawrenz & Mitchell, 1941).

Fluoride and sulfate. Sulfate presumably increases the intestinal absorption of F by counteracting, like phosphate, the inhibitory effect of intestinal Ca on F absorption (Stookey, Crane & Muhler, 1964).

Fluoride and chloride. The relative absorption of ^{18}F and ^{36}Cl by barley roots (Venkateswarlu, Armstrong & Singer, 1965), the relative intestinal absorption of ^{18}F and ^{36}Cl in the rat (Armstrong, Venkateswarlu & Singer, 1961), and the relative metabolism and transport of intraperitoneally administered ^{18}F and ^{36}Cl in rats on low- and high-F diets (Venkateswarlu, 1962) have been already discussed.

F at a level of 0.1% in chick rations was reported to be associated with hyperatrophy and histological changes in the proventriculi, and it was suggested that the production of HF instead of HCl could be the cause (Gardiner et al., 1959). No data on the HF or HCl content of the gastric juice were reported. It is pertinent here to point out an earlier discussion of a report that the gastric mucosa in the normal rat acquired a higher $^{36}\text{Cl}/^{18}\text{F}$ ratio than did the plasma and, further, that the gastric juice acquired a greater $^{36}\text{Cl}/^{18}\text{F}$ ratio than did the mucosa, possibly owing to a preferential acquisition and secretion of Cl over F by the gastric mucosa.

In a recent report, Cl has been considered an absolute requirement for the active intestinal transport of F in the secretory direction (Parkins & Faust, 1968). These findings await confirmation.

Fluoride and iodide. This aspect is dealt with by Demole in Chapter 7, section 5.

Fluoride and the effects of the parathyroid hormone

There is a dearth of knowledge on F and hormone interrelationships. At present our knowledge is essentially restricted to information concerning F and the thyroid gland (referred to above), and to a few limited studies on F and the parathyroid hormone, to be described now.

In bilaterally nephrectomized rats, peritoneal lavage with fluoride resulted in a significant increase in osteoclast count and activity as reflected by the proliferation of the osteoclasts into the bone trabecular zones. Similar changes were observed in nephrectomized fluorotic rats (in which the bone mineral is rendered less soluble) when calcium-free fluid was employed for

peritoneal lavage. In both these situations (Talamage & Doty, 1962), where the plasma ionic calcium was lower than in the corresponding control rats, increased osteoclastic activity was observable only in parathyroid-intact rats and not in parathyroidectomized rats. Fluoride, possibly by lowering the plasma ionic calcium, indirectly stimulates the parathyroid glands. However, parathyroid function was reported to be normal in patients with chronic fluorosis (Singh et al., 1966).

In parathyroid-hormone-treated rats, the plasma F level was found to remain the same as that in the control rats, despite a rise in plasma Ca indicating mobilization of bone mineral and therefore release of bone F into the plasma. Failure to observe any rise in plasma F level was explained on the basis of efficient homeostatic mechanisms involving sequestration of plasma F by newly forming bone mineral and also renal clearance of F (Singer & Armstrong, 1964). It is possible that, even in parathormone-treated animals, skeletal sequestration of plasma F plays as dominant a role in the homeostasis of F as it does in normal animals. This proposition is borne out by the observation that acquisition of intraperitoneally administered ^{18}F by selected hard tissues was the same in parathormone-treated rats as in normal control rats (Venkateswarlu, Armstrong & Singer, 1966b).

Fluoride and vitamin metabolism

Some histological structures of the skeleton and teeth are adversely affected by fluorine intoxication and also by deficiency of vitamins A, C and D. This led to the comparison of fluorosis with various vitamin (and other nutritional) deficiencies and to considerable speculation as to the possibility of treating or preventing fluorosis by vitamin administration. The basic interrelationships remain to be understood; in no species has fluorine intoxication been cured or prevented unequivocally by administration of any one or any combination of the above-mentioned vitamins. Attempts to relate the ameliorative effect of green leafy vegetables on fluorosis in rats to any of the dietary factors present in them have been unsuccessful (Phillips, cited by Suttie & Phillips (1959)).

Of all the vitamins, ascorbic acid seems to have attracted the maximum attention in connexion with fluoride metabolism. The interrelationship between vitamin C and F seems to require critical evaluation because of the suggested role of vitamin-C therapy in ameliorating fluoride intoxication, particularly after Pandit and co-investigators (1940) reported from India the co-existence of severe forms of human fluorosis and vitamin-C deficiency in a certain endemic area. This observation has been unjustifiably equated with aggravation of fluorosis by vitamin-C deficiency. Later Pandit & Narayana Rao (1940) demonstrated the ameliorative effect of vitamin-C-rich foods on fluorosis in monkeys (not *vitamin C alone* as misunderstood in some reviews). How far other factors present in vitamin-C-rich foods

could contribute to the beneficial effects observed seems to have been unfortunately overlooked.

Wadhvani (1952) induced fluorosis in monkeys kept on normal and scorbutic diets, noted higher mortality, stiffness of limbs and restricted movements in the latter group and observed dramatic improvement following vitamin-C therapy. This observation was interpreted as indicating that recovery from fluorosis was brought about by vitamin-C therapy, but it could merely reflect recovery from the experimentally induced scorbutic state by vitamin-C supplementation. The vitamin-C content of the "normal" basal diet was not given. Swelling of joints, reduced vivacity, restricted gait due to painful joints, etc. are commonly encountered in scorbutic guinea-pigs.

Wadhvani (1954) further claimed to have observed amelioration in fluorotic patients after administration of heavy doses of ascorbic acid; he reported improvement in radiological pictures. The vitamin-C status of the patients was not assessed before instituting the vitamin-C therapy. Conditions similar to chronic osteoarthritis, with osteophytic outgrowths, pseudoankylosis, and periosteal thickening, in chronic vitamin-C deficiency have been described by others (Mouriquand et al., 1940). One cannot rule out that the afore-mentioned radiological improvement could again be due simply to recovery from a chronic scorbutic state, which was reported by Pandit et al. (*op. cit.*) to be very prevalent in the area from which Wadhvani's patients came. The "aggravated" condition of fluorosis with vitamin-C deficiency as encountered in endemic fluorosis (Andreeva, 1959; Ivanova, 1959; Pandit et al., 1940; Wadhvani, 1954) might very well be a complex superimposition of the signs and symptoms of scurvy on those of fluorosis. The co-existing condition of scurvy responds to vitamin-C therapy while the basic condition of fluorosis does not. Extra vitamin-C administration would not be expected to have a profound beneficial influence on frank fluorosis, unassociated with scurvy, as could be produced in experimental animals (Venkateswarlu & Narayana Rao, 1957).

There is no evidence to support the view that F ingestion enhances the body's need for ascorbic acid. In experiments with guinea-pigs, F (1 mg per 100 g body-weight for 24 days) was not found to increase the excretion of vitamin C from the body, nor was F (1 mg per kg body-weight for 24 days) found to cause increased utilization or destruction of vitamin C in the body-tissues (Venkateswarlu & Narayana Rao, 1957). The observation of a lowered vitamin-C content of soft tissues in the fluorotic guinea-pig reported by Phillips, Stare & Elvehjem (1934) should not be over-emphasised in the present context of practical human and animal nutrition; the level of F administered was extremely high (25 mg per kg body-weight daily) and is not encountered in spontaneous endemic fluorosis. Administration of extra vitamin C (10 times the optimal requirement) to fluorotic guinea-pigs did not produce any perceptible effects either on skeletal retention or on urinary and faecal

excretion of fluoride. However, Muhler (1958) reported slightly increased skeletal deposition of fluoride in guinea-pigs receiving extra dietary vitamin C as compared with guinea-pigs on minimal vitamin-C intake. Again, in apparent contradiction to these observations, we have a report (Gabovich & Maistruk, 1963) indicating that vitamin C lowers the rate of F deposition in the hard and soft tissues of the guinea-pig and presumably increases the rate of F excretion.

Fluoride in extremely high concentrations might be expected to interfere with the role of vitamin C in the body. From a study of the activity of cellular oxidative mechanisms it was postulated that symptoms of scurvy and fluorosis result primarily from disturbances of specific phases of cellular respiration (Phillips, Stare & Elvehjem, 1934). Hauck (1934) believes that the interrelationships, if any, between fluorine and vitamin C, would be in the nature of fluorine interfering with the metabolism of vitamin C rather than with its storage. The specific mechanism by which the fluorine interferes with the role of vitamin C is not well established so far. It would appear that even if fluorine interferes with the metabolic function of vitamin C, it does not result in the production of all the phases of scurvy. Of the several guinea-pigs that were dissected, none of the fluorotic animals (not restricted to scorbutic diet) ever showed the characteristic signs of scurvy—haemorrhages, particularly at the costochondral junction—which were found in all fluorotic and non-fluorotic guinea-pigs rendered scorbutic by a vitamin-C-deficient diet (Venkateswarlu & Narayana Rao, 1957).

The significance of the finding of Phillips & Chang (1934) that fluoride ingestion causes an increased biosynthesis of vitamin C in the rat, which has been confirmed by Venkateswarlu & Narayana Rao (*op. cit.*), remains to be understood precisely.

Supplementary doses of vitamin D were found to have no therapeutic effect on experimental chronic fluorosis in young rats (Lindemann, 1965).

Fluoride and enzymes

General observations. Little is known about the *in vivo* effects of F at the low levels occurring naturally in body-fluids and soft tissues on enzymes and the various facets of general metabolism of the living organism. Low levels of fluoride have been reported to promote growth, flowering and higher yields of various plants. Perhaps such development of plants has been due to the stimulation of photosynthesis (Navara, 1963) by traces of fluoride and improvement in soil organic matter (Leroux, 1940) and nitrogen fixation (Fedorov, 1947) by soil micro-organisms under the influence of F. Traces of F stimulated the growth of embryonal cultures of chick heart and kidney (Hintzsche, 1954). In tissue-culture experiments employing murine leukaemic lymphoblasts (Albright, 1964), human HeLa and Minn. EE cells (Armstrong et al., 1965) and bone (Proffit & Ackerman, 1964), F was found

to interfere with cell growth and metabolism only at levels higher than 10 ppm (5×10^{-4} M), which far exceeds the natural level of fluoride—below 0.2 ppm (10^{-5} M)—in body-fluids, thus providing a safety factor of 50 or more.

10^{-4} M NaF was found to inhibit parathyroid-induced resorption of five-day-old mouse calvaria explants in tissue culture. Such a concentration of F was not found to impair osteoid formation (Goldhaber, 1967). Subsequent radioactive studies showed that F concentrations of 10^{-6} – 10^{-4} M activated, and higher concentrations inhibited, *in vitro* collagen synthesis. While resorption of preformed collagen in the explants was inhibited by F, resorption of poorly calcified collagen newly synthesized by the explant in the tissue culture was not inhibited by F. It was concluded that F does not directly inhibit the collagen-degrading enzymes but, by interaction with the (pre-existing) mineral phase associated with the preformed collagen, somehow protects the collagen matrix from such enzymes (Golub, Glimcher & Goldhaber, 1968). In this context, one may recall the view that proteolytic enzymes do not attack the tooth protein matrix until after it has been demineralized. It has been concluded that Ca salts protect the collagen from enzyme attack (Evans & Prophet, 1950). In tissue culture of bone, F probably reinforces or enhances a similar protective action of the mineral phase against the collagen-degrading enzymes as postulated in the case of dental tissues.

A decrease in proteins and Mg-dependent enzymes such as alkaline phosphatase and certain esterases in the serum of rats ingesting water containing 100 ppm F for 50 days was reported (Rieckstniece, Myers & Glass, 1965). A slight decrease in the total plasma proteins and some variations in the quantitative pattern of plasma protein fractions in rats receiving 10–20 mg F per kg body-weight for six weeks were also reported (Moore & Wagner, 1968). However, whether the observed alterations are due to a specific effect of F or to the effects of a generalized toxic condition remains to be clarified. For example, liver glucose-6-phosphate dehydrogenase was found to be decreased in fluoride-fed rats. However, by regulating the food intake it was shown that the decrease in enzyme activity in fluoride-fed rats was a consequence of a direct effect of F on the pattern of food intake. The primary effect of excessive dietary fluoride was therefore considered to be on the regulation of food intake rather than on the control of tissue enzyme synthesis (Carlson & Suttie, 1966).

Fluoride and enzyme activation. At certain optimal concentrations, fluoride is reported to activate a variety of physiological processes—for example, yeast respiration (Borei, 1942); *Escherichia coli* fermentation (Opienska-Blauth, Kanski & Stobinska, 1949); enzymatic decomposition of nitromethane (rabbit liver) (Egami & Itahashi, 1951); synthesis of citruline (rat liver) (Cohen & Hayano, 1947); vibrio adenosinase (Agarwala et al.,

1954), pancreatic diastase (pig) (Wachsmann & Grutzner, 1902); respiration of gastric mucosa (frog) (Davies, 1949). Not much is known about the mechanisms of activation of these processes by F. It is likely that F, through its ability to complex certain metals, unmask active surfaces of the enzyme previously covered by Ca or some other heavy metal. When a particular metabolic pathway is blocked by F, the cellular organization could stimulate and exploit an alternative pathway for cellular function which may appear as activation by F of the enzyme concerned. F could indirectly activate an enzyme. For instance, by inhibiting the phosphorylase-rupturing enzyme, F contributes to the conservation of tissue phosphorylase, a situation suggestive of activation of phosphorylase by F (Sutherland, 1951).

Fluoride and enzyme inhibition. Mechanisms of inhibition by fluoride of several enzyme systems have been investigated using fluoride concentrations several times higher than those present in normal body-fluids. To what extent the mechanisms as revealed by using such high concentrations of F would be operative at low levels of fluoride cannot be predicted. Nevertheless these studies lend an insight into the possible mechanisms, which seem to be many.

Fluoride can partly bring about enzyme inhibition by being adsorbed on (and thus blocking) the active sites of the enzyme required for formation of enzyme-substrate complex. This could be the reason why in some instances there is less inhibition of enzyme when F is added after the substrate (Marcuse & Runnstrom, 1943). In the presence of fluoride, a shift in the absorption spectrum of certain enzyme proteins—catalase (Ogura et al., 1950), cytochrome C (Borei, 1945)—has been noted; this could possibly mean direct combination of F with the protein. In some instances—yeast fermentation (Lipmann, 1929)—the combination is competitive and reversible, while in others—pancreatic lipase (Murray, 1929)—it is non-competitive and irreversible. Surprisingly lipases lose sensitivity to F on purification (Gyotoku, 1930); the action of F must be through some impurity or a labile component of the enzyme which is not directly involved in the enzyme function.

Susceptible isoenzymes are inhibited to various degrees by F, perhaps owing to differences in the order of reaction and in the affinity of F for the enzyme. Aerobic glycolysis is inhibited by F in Jensen rat sarcoma, but not in rat testis (Dickens & Simer, 1929). Human liver esterase is inhibited by F, but pancreatic and intestinal esterases are not (Gomori, 1955). Further, the activator requirement of the isoenzymes could be different. Mg-activated animal and plant glutamine synthetases are inhibited by F more than the corresponding Co-activated synthetases. F binding (Denes, 1954) with the metal activator is greater in the Mg-activated synthetases.

It was once presumed that F inhibition of some enzymes could be explained on the grounds that magnesium fluorophosphate inhibits competi-

tively the activating effect of Mg^{++} . However, in the case of succinic dehydrogenase (Slater & Bonner, 1952) and enolase (Peters, Shorthouse & Murray, 1964), it has been shown that fluorophosphate is not inhibitory, although a mixture of F plus phosphate certainly is. F and phosphate seem to reinforce each other's affinity for the enzyme and bring about a greater inhibition than when present alone.

In cellular oxidation, involving the cytochrome system, F is believed to interfere with reduction of cytochrome in intact systems and oxidation of cytochromes in cell free systems. Fe in the porphyrin moiety of cytochrome C forms no complex with F at the physiological pH. Observations of combination of F and cytochrome oxidase separately with cytochrome C suggest competitive inhibition by F (Borei, 1945).

The fluoride inhibition curve of acid phosphatase differs from other inhibition curves. It is reversed by higher concentrations of F and augmented at low pH. The nature of inhibition was influenced by ionic strength, specific cations and protein concentration. Multivalent organic anions protected the enzyme against F inhibition (Reiner, Tsuboi & Hudson, 1955). Alkaline phosphatase can be protected against irreversible inactivation by F through prior incubation with alanine at pH 8.8 (Nguyen-van-Thoi, Roche & Roger, 1946).

No difference has been found in the intracellular concentration of enzymes in control bacteria and in bacteria subcultured for 20 days in increasing quantities of F (Williams, 1964). However, 1 ppm F slightly inhibited acid production by streptococci and lactobacilli although, at this concentration of F, the inhibition was small (Bibby, Van Kesteren & Volker, 1942). Following a prior prolonged exposure to F, acid production by lactobacilli was inhibited even in the absence of fluoride. Either some change in protein due to exposure to fluoride or continued adherence of F to protein could be one of the causes of low acid production (Clapper, 1947).

In instances where no effect of fluoride on bacterial metabolism can be observed, the problem of permeability requires recognition. At lower pH values, when the inhibition increases, undissociated HF seems to penetrate yeast cells, thus raising the pH outside the cells (Malm, 1940). While F plus glucose causes no inhibition of dephosphorylation in *Esch. coli*, F plus glucose plus succinate causes 80% inhibition; presumably succinate facilitates F permeability (Aubel, Grunberg-Manago & Szulmajster, 1949). Starvation also increases F permeability in yeast cells (Malm, 1947).

The enzymatic and other mechanisms by which fluoride inhibits dental caries are dealt with by Jenkins in section 4 of this chapter.

As mentioned earlier, we are still a long way from understanding precisely the biochemical mechanisms underlying the role of F, if any, in biological systems, particularly at the low levels F is present in body-fluids and soft tissues.

TABLE 1
PHYSIOLOGICAL PROCESSES AND ENZYMES INFLUENCED SLIGHTLY OR
MODERATELY BY LOW CONCENTRATIONS OF F (10^{-4} M/LITRE AND BELOW)^a

Physiological process or enzyme involved	Source or material	Concentration of F (M/litre)	Reference
<i>Activation by F</i>			
Glucose fermentation	<i>Esch. coli</i>	10^{-5}	Opienska-Blauth, Kanski & Stobinska (1949)
Embryonal culture	Chick heart	1.25×10^{-5}	Hintzsche (1954)
Photosynthesis	Kidney bean (<i>Phaseolus vulgaris</i>)	3×10^{-5}	Navara (1963)
Fermentation	Yeast	5×10^{-5}	Slater (1951)
Respiration	Cancer tissue	10^{-4}	Sellei & Jany (1931)
Nitrogen fixation	<i>Azotobacter</i>	10^{-4}	Fedorov (1947)
Lipase	Liver	5×10^{-5}	Loevenhart & Peirce (1906-7)
<i>Inhibition by F</i>			
Calcification	Hypertrophic cartilage slices (rat)	10^{-5}	Robison & Rosenheim (1934)
Citric acid production	<i>Aspergillus niger</i>	5×10^{-5}	L'vov & Toupizina (1938)
Acid production	Streptococci and lactobacilli	10^{-5}	Bibby, Van Kesteren & Volker (1942); Clapper (1947)
Activation of acetate	Kidney and liver homogenates	10^{-4}	Aisenberg & Potter (1955)
Esterase	Pig liver	5×10^{-7}	Peirce (1913-14)
Acid glycerophosphatase	Sheep brain	6×10^{-6}	Mattocks & Holtan (1949)
Carbonic anhydrase	Rat incisor	10^{-5}	Kondo & Kuriaki (1961)
Adenosine triphosphatase	Cardiac muscle	10^{-5}	Hegglin, Grauer & Munchinger (1949)
Phosphomonoesterase	Serum	10^{-5}	Fleury, Courtois & Plumel (1950)
Phosphatase	Coconut kernel	10^{-4}	Sadasivan (1951)
Pyrophosphatase	Yeast	10^{-4}	Webb (1948)
Pyrophosphatase	Fire-fly	10^{-4}	McElroy, Coulombre & Hays (1951)
Glutamine synthetase	Animals and plants	10^{-4}	Denes (1954)
Acid phosphatase	Human saliva and prostate gland	10^{-4}	Smith, Armstrong & Singer (1950)
Isocitric dehydrogenase	Rat liver	10^{-4}	Doberenz et al. (1964)

^a The F concentration in normal body-fluids and soft tissues is 5×10^{-5} to 5×10^{-5} M/litre. "Ionic" F in plasma is 5×10^{-7} to 2×10^{-6} M/litre.

Some of the physiological processes and enzymes reported to be activated or inhibited slightly or moderately by levels of fluoride below 2 ppm (10^{-4} M per litre) are shown in Table 1. It may be recalled that the F content of body-fluids is believed to be 0.1-0.2 ppm and of soft tissues to be around 1 ppm.

Is Fluorine an Essential Dietary Element ?

The beneficial role of fluorine in the control of caries has been amply demonstrated by epidemiological studies, by the success of fluoridation of public water supplies as a mass caries-control measure, and by the reduction of experimental caries in animals by fluoride in the dietary. In the light of these experiences it is logical to ask: Is fluorine an essential element ?

This question has not been satisfactorily answered because of the difficulties in the preparation of a fluoride-free diet and because of the lack of adequately sensitive methods for the determination of minute traces of fluoride. Passing reference may be made to investigations in this area by Sharpless & McCollum (1933), Lawrenz, cited by Mitchell & Edman (1945), McClendon (1944), McClendon & Gershon-Cohen (1953, 1954) and Maurer & Day (1957), and to a discussion by Muhler in Chapter 2, section 3.

In a more recent study a diet extremely low in fluoride was prepared to assess the fluoride requirement of the rat (Venkateswarlu, 1962). Two groups of rats received the low-F diet; one of these groups received double-distilled water and the other water containing 10 ppm F. There was a slight suggestion that the reproductive capacity of the animals in the low-F group was less than that of those in the other group. However, the difference between the two groups may have been only a chance result. It was originally intended to observe the rats through 3 to 5 generations before attempting to reach a decision as to the biological requirement for fluoride in the rat, but the experiment had to be abandoned because few animals reproduced beyond the third generation, and very few of the offspring were adequately nursed by the mothers. The cause of these unexpected developments may perhaps be traced to the artificial diet. Either some unknown deficiency was inadvertently created, or some toxic factor was produced in the preparation and storage of the diet in the refrigerator. Muhler (1951) attempted to prepare a fluoride-low synthetic diet and abandoned the experiments because the purified diet would not sustain reproduction. Maurer & Day (1957) found that only approximately half of the offspring born to female rats fed on a special F-low diet (F less than 0.007 ppm) could be successfully weaned, but from the observations they were able to make they concluded that fluorine was not essential in the rat. It may be well to point out that their conclusions were based on F analyses using a macrodistillation and titration method "sensitive to no less than" 1.0 μg F, whereas the F levels in the present writer's investigations (Venkateswarlu, *op. cit.*) were determined by microdistillation (Singer & Armstrong, 1959) and a micro-analytical spectrophotometric method (Venkateswarlu, Armstrong & Singer, 1966a) capable of detecting as little as 0.1-0.2 μg F. In other words, it cannot be ruled out that some of the bones which were reported by Maurer & Day to contain no F might actually have contained some.

In another recent study (Doberenz et al., 1964) on the effects of a minimal F diet on rats, an increase in plasma isocitric dehydrogenase level with a concomitant decrease in the enzyme level in the liver was reported. 10^{-4} M F was found to inhibit liver isocitric dehydrogenase *in vitro*. The report, however, does not include observations on the *in vitro* effect of F on plasma isocitric dehydrogenase activity. The F content of the diet, determined by a macro-method, comparable in sensitivity to that of Maurer & Day, was

reported to be less than 0.005 ppm and the mean F content in 91-day-old rat tibiae was 2.9 ppm.

In section 3 of this chapter some observations and hypotheses on the role of F in the formation and maintenance of apatitic structures are described. If these can be confirmed in experimental animals raised on fluoride-free diets, the essential nature of F for the development of calcified tissues would appear to be established.

3. EFFECTS ON THE SKELETON OF MAN (I. Zipkin)

Fluoride is a unique ion in that it continues to deposit in the calcified structures after the other constituents of bone have already reached a steady state. Thus, the major constituents—calcium, phosphorus, magnesium, carbonate and citrate—reach their maximum concentration early in life and remain essentially unchanged, even after administration of large amounts of the ion in question. Fluoride, on the other hand, showed a tenfold increase in bone following ingestion of each of four drinking waters with fluoride contents of <1.0, 1.0, 2.6 and 4.0 ppm, respectively.

About 96% of the fluoride found in the body is deposited in the bones, so that it becomes important to study the effect of this ion on the physical and chemical structure of bone as well as on its morphology and physiology. These effects of fluoride will be discussed as they relate to long-term exposure of man to levels of water-borne fluoride up to 4 ppm. The effects of levels as high as 8 ppm in the drinking water are discussed in detail by Leone in Chapter 8, section 2. Various factors affecting the deposition of fluoride in bone have been reviewed by Weidmann and Weatherell in Chapter 4, section 3.

The physiological effects of water-borne fluoride on the skeleton are a resultant of the effects on the chemistry, morphology, histopathology, X-ray density, and integrity or structure of both the inorganic and the organic phase of bone. In addition, the interplay of bone remodelling, fluoride deposition and mobilization may also influence skeletal physiology or function following fluoride exposure. It will be indicated that the various parameters mentioned do not interfere with the normal physiology of the skeleton in man ingesting water containing up to 4.0 ppm F and indeed up to 8.0 ppm F.

Effect of Fluoride on the Chemistry of Bone

Current theories propose that calcification of bone is preceded by a nucleation process in the early deposition of calcium and phosphorus on the chief organic matrix of bone, collagen, to form the mineral phase generi-

¹ The terms "apatite" and "apatitic" refer to compounds deviating from hydroxyapatite in theoretical calcium and phosphate content. They may contain other ions such as fluoride, carbonate or water within the lattice work and give X-ray diffraction patterns very similar to those of pure hydroxyapatite. The crystal structure of hydroxyapatite has recently been redetermined and refined from both X-ray and neutron diffraction data by Kay, Young & Posner (1964).

cally called hydroxyapatite, or $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$.¹ Statistically, the hydroxyls of any crystal of apatite in calcified structures may be partially or completely substituted isomorphically by fluoride. Thus, mixed crystals of hydroxyapatite and fluorapatite may be present. In addition, the mineral phase of hydroxyapatite is presumed to be oriented along the collagen fibres, which are said to act as a sort of model or template for the deposition of the mineral. It would be important, therefore, to study various factors which might change the character or structure of collagen, since they might thereby alter the nucleation process and thus the deposition of mineral.

The chemical changes in bone with fluoride deposition will be related later in the text to crystallinity of the inorganic phase of bone—namely, apatite.

Inorganic constituents

The ash, fluoride, calcium, phosphorus, magnesium, sodium, potassium, and carbonate content of the iliac crest, rib and vertebra of individuals exposed to drinking water containing up to 4.0 ppm F for 10-87 years prior to demise has been reported by Zipkin, McClure & Lee (1960). Data from this study are given in Table 2.

Calcium, phosphorus and potassium in the bone ash were unaffected by mean concentrations of bone fluoride as high as 0.8%. There was a small decrease in sodium as the fluoride increased. The carbonate content decreased about 10%, whereas the magnesium increased about 15%, when the fluoride showed an eightfold increase.

At autopsy the bones of individuals whose drinking water contained 0.5 ppm F and who had been exposed to low concentrations of fluoride in

TABLE 2
ASH, FLUORIDE, CALCIUM, PHOSPHORUS, MAGNESIUM, SODIUM,
POTASSIUM, CARBONATE AND CITRATE CONTENT OF SELECTED HUMAN BONES
AS RELATED TO FLUORIDE CONCENTRATION OF THE DRINKING WATER^a

Constituent	Percentage of constituent ^b in bones					
	Iliac crest		Rib		Vertebra	
	< 1.0 ppm ^c	4.0 ppm ^c	< 1.0 ppm ^c	4.0 ppm ^c	< 1.0 ppm ^c	4.0 ppm ^c
Ash	53.5	58.6	56.0	57.4	49.8	52.3
F	0.08	0.69	0.08	0.70	0.10	0.80
Ca	38.8	38.4	38.8	38.4	37.8	37.8
P	17.5	17.7	17.5	17.7	17.5	17.5
Mg	0.50	0.57	0.50	0.60	0.50	0.61
Na	0.73	0.70	0.79	0.68	0.73	0.68
K	0.09	0.08	0.09	0.10	0.18	0.12
Carbonate	5.83	5.20	5.41	5.19	5.31	4.55
Citrate	2.23	1.56	1.92	1.39	1.95	1.30

^a After Zipkin, McClure & Lee (1960).

^b Ash is expressed on a dry, fat-free basis. All other values are expressed on an ash basis.

^c Concentration of F in drinking water.

the air showed normal values for Ca, P, F and ash (Call et al., 1965) similar to those reported previously by Zipkin, McClure & Lee (*op. cit.*). The highest levels of bone fluoride found in subjects with the most severe kidney disease (Call et al., *op. cit.*) were within the range for normal individuals drinking water containing less than 0.5 ppm F.

Organic constituents

The effect of fluoride on mucopolysaccharide and collagen turnover has been reported. No significant changes were seen in the incorporation of ^{35}S following a 10-fold increase in fluoride incorporation in the rat, which indicated that there was no synthesis of new polysaccharide in the femur, mandible, or pelvis (Zipkin, 1969). Rats receiving 10 ppm F for one month or for 10 months showed no change in total collagen, and only a group drinking water containing 50 ppm F for one month showed any significant decrease in total collagen synthesis of the calvaria (Peck, Zipkin & Whedon, 1965). New collagen synthesis was depressed in the calvaria of rats receiving 10 ppm F for 10 months or 50 ppm F for 2 or 4 weeks. Labelled proline was used as the marker in these experiments. In human subjects suffering from bone resorptive diseases, doses of 15-30 mg F per day for one year have been reported to reduce collagen synthesis, as measured by the *in vitro* incorporation of ^{14}C -proline by iliac crest bone specimens obtained at autopsy (Nichols & Flanagan, 1966). Bone organ culture experiments have also indicated diminished ^{14}C -proline uptake when the culture medium contains 10-20 ppm F (Golub, Glimcher & Goldhaber, 1968b; Proffit & Ackerman, 1964). In addition, degradation of collagen in bone organ cultures has been reported with medium containing as little as 0.2 ppm F, by direct chemical determination of collagen (Golub, Glimcher & Goldhaber, 1968b) and by histological techniques (Goldhaber, 1967).

Data have also been published on the relation of fluoride to the concentration of citrate in human bone (Zipkin, McClure & Lee, 1960). As shown in Table 2, the citrate content of the iliac crest, rib and vertebra decreased about 30 % as the fluoride increased about eightfold.

Effect of Fluoride on the Physical Structure of Bone

The effect of the consumption of drinking water containing up to 4.0 ppm F on histology, X-ray findings and bone density, as well as on the crystallinity of the apatite structure, will be discussed. The X-ray studies are discussed at greater length in Chapter 8, section 5.

Histology

The histology of bones of individuals exposed to low levels of fluoride in drinking water has been reported by Geever et al. (1958) and Weidmann,

Weatherell & Jackson (1963). The iliac crest, rib, vertebra and, in some cases, sternum of individuals drinking water containing <1.0, 1.0, 2.6 and 4.0 ppm F for at least 10 years prior to autopsy contained approximately 0.05% F, 0.15% F, 0.25% F and 0.40% F, respectively, on a dry, fat-free basis. Some 100 bone specimens as well as the intervertebral cartilage were examined histologically (Geever et al., *op. cit.*) for focal calcification of the periosteum and adjacent tendons and fascia, as well as for osteoclasia, osteophytosis and cortical thickness. Marrow sections were examined for haematopoiesis and for the degree and extent of trabeculation. Incidental changes due to aging were observed in all groups and were not significantly related to the fluoride intake.

Weidmann, Weatherell & Jackson (*op. cit.*) examined histologically the ribs of individuals drinking water containing <0.5, 0.8, and 1.9 ppm F for width of the cortex and number and thickening of the cortical trabeculae. No differences were seen in resorption areas of the trabeculae or of the compacta among the three groups.

Roentgenology

In an extensive paediatric study by Schlesinger et al. (1956) children drinking water containing 1.2 ppm F in Newburgh, N.Y., were examined at intervals over a period of ten years. Roentgenograms were taken of the right hand, both knees and the lumbar spine, and bone density as well as bone age was estimated. No differences of any significance could be found in any of the roentgenographic studies, including the bone-density and skeletal maturation (estimations of bone age) assessments. Previously, McCauley & McClure (1954) also found no adverse effect on the rate of ossification and maturation of the carpal bones of children aged 7-14 years continuously exposed to levels of water-borne fluoride as high as 6.2 ppm F.

Using step wedge techniques, Odland, Warnick & Esselbaugh (1958) found no difference in the bone density of the os calcis or the phalanx 5-2 of adolescents from two areas in Montana, one with 1 ppm F and the other with a negligible concentration of fluoride in the drinking water. Large differences in caries prevalence, however, were observed in the two areas.

Increased bone density or osteosclerosis was not apparent roentgenographically when the concentration of fluoride in the drinking water was less than 4 ppm (Geever et al., 1958; Morris, 1965; Stevenson & Watson, 1957), when the urinary fluoride concentration was less than 10 ppm (Largent, Bovard & Heyroth, 1951) or when the bone contained less than about 5000 ppm fluoride on a dry, fat-free basis (Weidmann, Weatherell & Jackson, 1963; Zipkin et al., 1958). Indeed, the fluoride content of bone has been used as a differential diagnosis between rheumatoid spondylitis and crippling fluorosis (Steinberg et al., 1955, 1958).

The increased bone density observed among 10-15% of individuals drinking water containing 8 ppm F (Leone et al., 1955) and the unusually high frequency of osteoporosis seen in Framingham, Mass. (Leone et al., 1960) among individuals consuming an essentially fluoride-free drinking water prompted a number of clinical studies on the effect of high levels of fluoride on resorptive bone disease (Rich, Ensinn & Ivanovich, 1964; Purves, 1962; Cohen & Gardner, 1964; Nagant de Deuxchaisnes & Krane, 1964; Higgins et al., 1965; Hodge & Smith, 1968). The effect of fluoride on bone density and on calcium balance in these studies has been equivocal. Field studies relating senile osteoporosis to water fluoride are reported elsewhere in this monograph.

No data appear to be available on the fluoride concentrations in resorbing human bone. Steinberg et al. (1958) reported that the bones of individuals in both fluoride and non-fluoride areas with various types of arthritis contained normal amounts of fluoride. It would appear that a conservative approach should be taken to the subject of elevated and prolonged administration of fluoride in various bone dyscrasias, as re-emphasized recently by Sognnaes (1965).

McClure (1944) has reported that bone-fracture experience and height-weight data of high school boys and young selectees of the Armed Forces were not related to fluoride levels of up to 6.0 ppm in the drinking water.

Crystallinity of bone apatite

The observation that an increase of fluoride in bone decreases the concentration of a number of chemical constituents, such as citrate, that are usually assigned to the surface of the apatite crystals of bone rather than within the lattice, prompted studies on the size and strain of apatite crystals as influenced by fluoride. For this purpose, X-ray diffraction techniques, as reviewed by Carlström (1955), using powdered specimens of bone were employed by Zipkin, Posner & Eanes (1962), Schraer et al. (1962) and Eanes et al. (1965). The theoretical considerations involved in the techniques employed for human bone were reported by Posner et al. (1963).

The X-ray diffraction patterns of powdered bone are poorly resolved when compared with authentic samples of well-crystallized hydroxyapatite. This lack of resolution is due to the small size and the imperfection of biological apatites. An increase in the degree of resolution using a template method (Posner et al., *op. cit.*), however, indicated an increased crystallinity associated with an increase in bone fluoride.

Relation to age. Robinson & Watson (1955) have made the most comprehensive study on the relation of crystal size to age in the human. They reported an increase in size under the electron microscope from an essentially non-crystalline unresolvable particle of less than 50 Å in the infant to crystals 1500 Å by 500 Å by about 100 Å in the senile subject. Enamel

apatite is much more crystalline than the apatite of bone and may exceed 10 000 Å in length and 1000 Å in thickness. Larger crystals per unit mass would provide less surface than smaller crystals or, phrased otherwise, a reduced specific surface. Since most reactions occur at surfaces, large crystals would be more stable and less chemically reactive. The increase in crystal size with age would be expected to play an important role in the decrease in turnover exchange reactions in aging bone.

Relation to fluoride deposition in bone. Since the crystals of bone apatite are exceedingly small, show strain and imperfections and hence give a poorly resolved X-ray diffraction pattern (Zipkin, Posner & Eanes, 1962), it was not possible to measure the width at half maximum (β) of any of the characteristic peaks of apatite as an index of line broadening. The crystallinity, as previously mentioned, is inversely proportional to the degree of line broadening, so that a lower β value calculated from an over-all template (Posner et al., 1963) represents a higher degree of crystallinity.

Fig. 1 shows an unresolved X-ray diffraction powder pattern of a sample of iliac crest containing 0.224% F.

The increased resolution of a sample of iliac crest containing 0.873% F can be seen in Fig. 2.

The inverse relationship between fluoride content and β values for a large number of bones from individuals drinking water containing up to 4.0 ppm F is shown in Table 3.

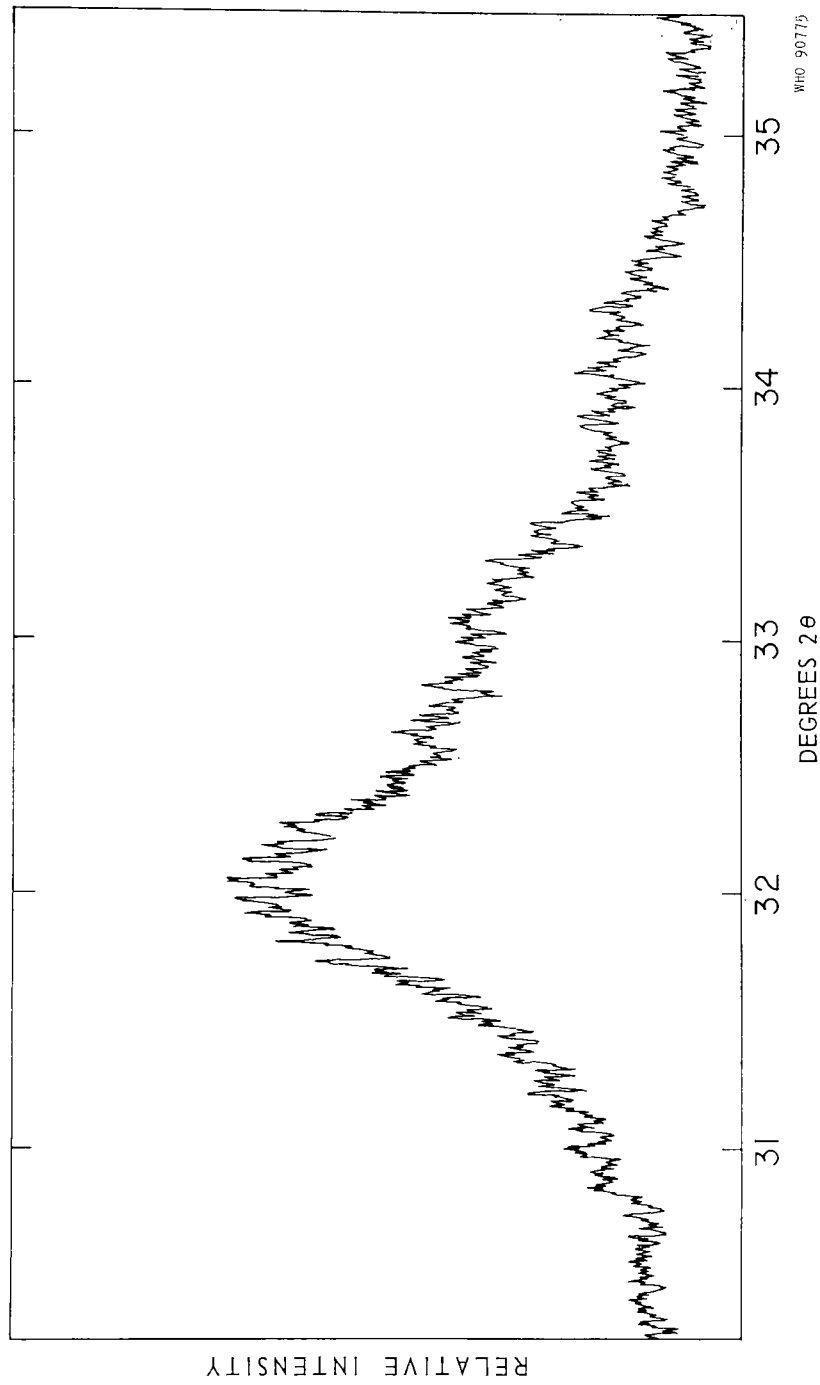
TABLE 3
RELATION BETWEEN PERCENTAGE OF FLUORIDE IN HUMAN BONE
AND "CRYSTALLINITY" INDEX

Bone	New York City	Grand Rapids, Mich.	Colorado Springs, Colo.	Lubbock, Tex.
Iliac crest				
Mean F	0.064 (12) ^a	0.205 (3) ^a	0.504 (10) ^a	0.711 (3) ^a
β ^b	0.959	0.786	0.740	0.667
Rib				
Mean F	0.082 (13)	0.273 (2)	0.495 (12)	0.842 (1)
β	0.919	0.775	0.738	0.600
Vertebra				
Mean F	0.088 (11)	0.280 (4)	0.606 (11)	0.802 (4)
β	0.950	0.853	0.691	0.600
Mean				
Mean F	0.078 (36)	0.253 (9)	0.533 (33)	0.773 (8)
β	0.942	0.837	0.733	0.625

^a The figures in parentheses indicate the number of comparisons.

^b β is inversely related to crystallinity and a decrease in β is a measure of an increase in crystal size or state of perfection, or both.

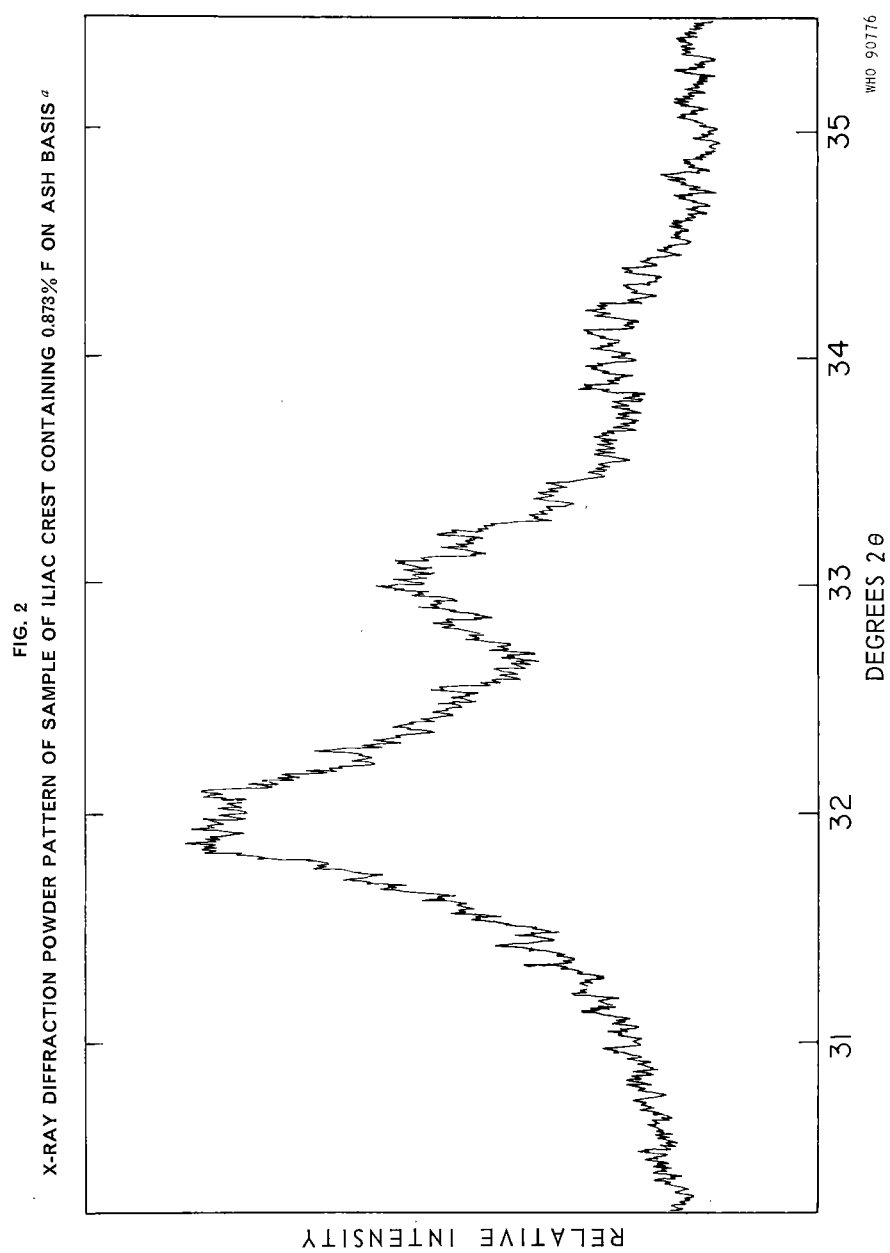
FIG. 1
X-RAY DIFFRACTION POWDER PATTERN OF SAMPLE OF ILIAC CREST CONTAINING 0.224% F ON ASH BASIS^a



$\beta = 0.80^\circ 2\theta$.

^a Reproduced, with permission, from Zipkin et al. (1982).

WHO 90775



While line broadening in X-ray diffraction patterns does not distinguish between the contribution of size or strain to crystallinity, low angle scattering studies by Eanes et al. (1965) have indicated that the enhancement of crystallinity is due in the main to increased size of the bone apatite crystals.

Increased crystallinity as a result of increased deposition of fluoride has also been demonstrated recently by Schraer et al. (1962) in rats and by Zipkin, Eanes & Shupe (1964) in cattle. Recent studies have also indicated increased bone crystallinity in the mouse (Zipkin, Sokoloff & Frazier, 1967) and in the chicken (Zipkin and associates, unpublished data) following administration of fluoride.

The enhancement of the crystal texture of bone apatite by fluoride may be due either to a direct effect of fluoride on the nucleation process to form large crystals of hydroxyapatite or to a displacement of such ions as carbonate (Bachra, Trautz & Simon, 1965; Grøn et al., 1963) and citrate (Patterson, 1954) which have been shown in *in vitro* studies to disturb crystallization of apatitic compounds.

Relation to chemistry of bone. It is generally agreed that Na, K and citrate do not occupy positions within the lattice of biologically synthesized apatites. The position of magnesium is still equivocal and recent evidence indicates that carbonate may at least partially substitute for phosphate in the apatite crystal (LeGeros, Trautz & LeGeros, 1965).

The increase in bone crystallinity may affect the concentration of those ions which are surface-oriented. As already mentioned, fluoride reduces the over-all surface per unit of mass of bone crystals thus delimiting the concentration of those ions found there, such as Na, K and citrate. It is also possible that such ions as calcium and phosphate as well as magnesium and carbonate may be adsorbed on the surface of the crystals as well as being incorporated within the lattice. It has also been postulated that magnesium is present as a cation complex $(\text{MgOH})^+$ on the surface of the apatite crystal. The increase in magnesium with an increase in fluoride may be due to the formation of some ion complexes of Mg (OH, F) as a separate phase.

The decrease in carbonate with fluoride may be due to an increase in crystal perfection, thus enhancing the elimination of carbonate from the lattice, where it is presumed to substitute for phosphate. On the other hand, it is also conceivable that a simple displacement of surface-adsorbed carbonate occurs.

It is most difficult to resolve clearly the possibilities outlined since the crystals are small and show strain and imperfections, and hence only poor diffraction patterns are produced. Even when good resolution of the X-ray diffraction pattern is obtained, other techniques such as infra-red and solubility measurements as well as the electron microscope must be used. Present methods do not yet unequivocally establish the locus of an ion as extra- or intra-crystalline.

Possible Mechanisms for the Deposition and Mobilization of Skeletal Fluoride

The deposition of fluoride in bone and its mobilization therefrom may be considered the resultant of at least two major processes. Vital metabolic processes of bone remodelling must play an important role in fluoride deposition, although major emphasis will be placed on physico-chemical considerations. Possible calcium phosphate precursors of apatite will be discussed as proposed by the workers in Dallemagne's laboratory (Dallemagne, 1964), by Brown et al. (1962) and by Hirschman & Sobel (1965). Conflicting views on the possible existence of a so-called "calcium defect" apatite will also be discussed. It thus seems feasible that more than one mineral phase may exist in calcifying structures.

Metabolic considerations

The ultrastructural organization of bone has been reviewed recently by Pautard (1964), and its relation to the formation and resorption of the skeleton has been discussed by Hancox & Boothroyd (1964).

It is generally believed that some of the apatite crystals are oriented along the collagen fibres (Glimcher, 1959) and that the mucopolysaccharide ground substance may also play a role in the nucleation and growth of the apatite crystals (Sobel, 1955). It is apparent, therefore, that factors affecting the organization of the organic matrix may alter the deposition of apatite and thus the incorporation of fluoride. Newly forming matrix is associated with active osteoblasts, while resorption of bone has been associated with osteoclasts at the resorbing site. Osteoblastic and osteoclastic activity may thus play a role in either "freeing" or "burying" active sites or surfaces of the apatite molecule. As bone ages and reaches a more or less "steady state" in the remodelling of its Haversian systems, less fluoride may be deposited. In addition, little fluoride is released from rat bone following discontinuance of fluoride administration (Zipkin, 1960) and this may in part be due to "buried" and relatively inaccessible apatite as a result of the decreased metabolic turnover of bone. The loss of fluoride is relatively small and may also be explained on physico-chemical grounds, which will be developed later.

Fluoride is not deposited uniformly throughout a given bone and may be related to the rate of growth and the degree of vascularization in various parts of the same bone. Thus, more fluoride is deposited in the epiphyseal than in the diaphyseal portions of bone (Zipkin & Scow, 1956).

The deposition of fluoride in non-physiological calcifications such as urinary calculi does not appear to follow the orderly processes of accretion and remodelling found in bone (Zipkin, Lee & Leone, 1958). Indeed, the concentration of fluoride in the calculi was higher than that in the bones of individuals with essentially the same fluoride exposure. In addition, the

fluoride content of the urinary tract calculi of individuals from low-fluoride areas was not significantly different from that of calculi from individuals whose drinking water contained 2.6 ppm F. The bones, on the other hand, show about a five- to six-fold difference in fluoride concentration. Fluoride has been reported to be associated with a decrease in aortic plaque in human subjects (Bernstein et al., 1966), and both animal and *in vitro* models have been devised to study this phenomenon (Zipkin, I. et al., unpublished data). Factors affecting the biological deposition of fluoride are more completely discussed by Weidmann and Weatherell in Chapter 4, section 3.

Physico-chemical considerations

Fluoride appears to be associated with the mineral phase of bones and teeth almost exclusively, so that the deposition and mobilization of fluoride may be considered to simulate an *in vitro* reaction of fluoride with hydroxyapatite. It would seem worth while at the outset, therefore, to consider the various formulae which have been proposed for the mineral phase of bone and to remember that none has been unequivocally proved to represent the true inorganic compound or compounds of bone. The major source of difficulty lies in the observation that "young" bone rarely attains the theoretical Ca/P weight ratio of 2.15 for hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$.

As bone "matures" the Ca/P weight ratio approaches and may exceed that of stoichiometric hydroxyapatite. The composition of hydroxyapatite is variable, that is, the percentages of Ca and P may differ from the theoretical percentages, but the apatitic materials still give X-ray patterns virtually identical to that of stoichiometric hydroxyapatite. Hence, a number of formulae have been introduced to account for the non-stoichiometry of hydroxyapatite.

Synthetic apatitic calcium phosphates can be prepared with lower Ca/P ratios which give diffraction patterns similar to $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ (Winand, Dallemagne & Duyckaerts, 1961; Armstrong & Singer, 1965). Reactions of fluoride with some of the proposed structures of the apatite series of compounds will be presented later.

1. Proposed formulae for the inorganic phase of bone:¹

(a) $\text{Ca}_{10-x}\text{H}_x(\text{PO}_4)_6(\text{OH})_{2-x}$, where $x = 0-2$ (proposed by Winand, Dallemagne & Duyckaerts (1961)). If $x = 1$ then $\text{Ca}_9\text{H}(\text{PO}_4)_6\text{OH}$ or $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ results which has been designated α -tricalcium phosphate hydrate by Dallemagne and co-workers. It has been postulated that both hydroxyapatite and $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ may co-exist as separate phases.

¹ von K hl & Nebergall (1963) have proposed a general formula for synthetic apatites that does not necessarily include bone, but considers carbonate—namely: $\text{Ca}_{10-x-y}(\text{HPO}_4)_x(\text{CO}_3)_y(\text{PO}_4)_{4-x-y}(\text{OH})_{2-x-y}$. When x and y are zero, stoichiometric hydroxyapatite results.

(b) $\text{Ca}_{10-x}\text{H}_{2x}(\text{PO}_4)_6(\text{OH})_2$ (proposed by Posner, Stutman & Lippincott (1960)). When $x = 0$, then $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ or hydroxyapatite results. The authors suggest that protons between the oxygens of phosphate ion make up the defect in Ca so as to balance the molecule electrostatically. This view has been contested by Winand and associates (*op. cit.*) and Brown et al. (1962) on the ground that more than one apatitic phase may exist with a lower Ca/P ratio rather than merely a single phase of defect hydroxyapatite, thus accounting for the lower Ca/P ratio.

(c) Neuman & Neuman (1958) have proposed the formula $\text{Ca}_{10-x}(\text{H}_3\text{O})_{2x}(\text{PO}_4)_6(\text{OH})_2$, where hydronium ions (H_3O^+) may provide electro-neutrality by compensating for missing Ca.

(d) Octacalcium phosphate, $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$, has been proposed by Brown (1966) as a calcium phosphate compound which because of its structural relationship to hydroxyapatite may co-exist with it in bone, particularly at initiation of deposition of the inorganic phase.

(e) Armstrong & Singer (1965) have suggested that the bone mineral is made up of microcrystals of hydroxyapatite with surface-coated ions derived from extracellular body-fluids (Ca, Mg, Na, K, carbonate, citrate and phosphate). It would be highly important to be able to determine by direct measurements the intra- and extra-crystalline proportions of these ions.

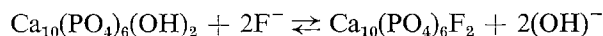
Thus, formulae have been proposed which require either hydrogen (formula b), hydronium ions (formula c), hydrogen with an accompanying loss of hydroxyl (formula a and formula of von K hl & Nebergall) or loss of hydroxyl (formula of von K hl & Nebergall) to maintain electrostatic balance. In addition, separate phases of " -tricalcium phosphate hydrate" or octacalcium phosphate have been postulated to exist or layer with hydroxyapatite. And it has also been suggested that bone mineral is represented by microcrystals of hydroxyapatite with a variety of adsorbed ions. The relative proportions of surface and intracrystal components were calculated from theoretical equations (Armstrong & Singer, 1965).

2. Reactions of fluoride with apatitic calcium phosphates:

(a) *Substitution.* Since the fluoride ion is about the same size and shape as the hydroxyl ion, it can readily exchange isomorphically, with only a small decrease in the *a* axis from 9.42   for hydroxyapatite to 9.37   for fluorapatite (Carlstr m, 1955) as shown by X-ray diffraction data. Infra-red data by Elliott (1964) have also demonstrated the substitution of hydroxyl in synthetic hydroxyapatite by fluoride. The *c* axis remains unchanged at about 6.88  . The substitution of hydroxyl by fluoride is probably statistical in that some apatite crystals may show complete substitution, while, in others, mixed crystals may exist—namely, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH}, \text{F})$.

McCann (1953) and Neuman et al. (1950), as well as other workers cited by Hodge & Smith (1965), have clearly demonstrated that, at low levels, fluoride reacts with synthetic apatites (McCann, *op. cit.*) as well as with bone

(Neuman et al., *op. cit.*) to form fluorapatite. This reaction can be expressed by the following equation:



The equation is reversible in that less fluoride was incorporated in ashed bone (Neuman et al., *op. cit.*) at high hydroxyl-ion concentrations.

At the levels of fluoride found in the circulating body-fluids, only fluorapatite is found in bone, and X-ray diffraction analysis of bone has not revealed the presence of CaF_2 . No CaF_2 is formed, according to McCann, unless the concentration of F in the solution in contact with the solid exceeds about 100 ppm.

The findings of Neuman et al. (*op. cit.*) on the *in vitro* exchange capacity of bone for fluoride have been borne out by a number of subsequent *in vivo* studies (Zipkin et al., 1956; Zipkin, unpublished data). Neuman and co-workers found that when they passed a solution containing 2 ppm F (as NaF) through a column of ashed bone the fluoride concentration of the bone reached 1.04%, representing a maximum substitution of about one in four of the X positions of $\text{Ca}_{10}(\text{PO}_4)_6\text{X}_2$ where X represents OH. The effluent gradually increased in fluoride concentration until the affluent and effluent values were equal, so that the reaction followed the course of a typical exchange absorption.

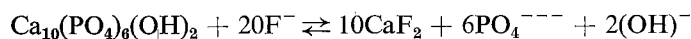
When 1 ppm F was introduced into a municipal drinking supply (Zipkin et al., 1956), almost three years were required for the urine of children 5-14 years of age to reach 1 ppm, indicating a slow equilibration or exchange absorption with young bone. The urine of adults reached 1 ppm F within one week, indicating a rapid attainment of a steady state in the case of older bones. The slow approach to the steady state in children may play a more important role than the physico-chemical exchange reaction.

In young rats on a 60% restricted intake of diet (Zipkin, unpublished data) to produce smaller bones, the concentration of fluoride was higher than in the larger bones of *ad libitum* fed rats, but the total fluoride deposited was the same, indicating again that bone may be acting as an ion-exchange column. In rachitic rats (Zipkin, Likins & McClure, 1959), the bones were smaller and the concentration of fluoride in them was about $2\frac{1}{2}$ times higher than in the larger bones of *ad libitum* fed rats, but the total fluoride deposited was less. It appears that either the ion-exchange capacity of the rachitic bone was exceeded or the bone was sufficiently altered physiologically to disturb its exchange capacity (Zipkin, Likins & McClure, *op. cit.*).

It has been postulated by Slatkine (1962) and Baud & Slatkine (1965) that the *in vivo* substitution of OH ions in the mineral bone substance by F ions does not entail a transformation of hydroxyapatite into fluorapatite, as generally assumed, but rather corresponds to a conversion of "carbonato-hydroxyapatite" to "carbonatofluorapatite", as suggested by McConnell (1962). LeGeros, Trautz & LeGeros (1965) interpreted their infra-red data

to indicate that carbonate substituted for phosphate but not for OH in the apatite lattice.

While low levels of fluoride convert hydroxyapatite to fluorapatite, elevated levels produce CaF_2 . Thus, treatment of synthetic hydroxyapatite or enamel slabs with concentrations of fluoride as high as 2% NaF produced characteristic lines of CaF_2 on diffraction, according to the following equation:



(b) *Seeding mechanisms.* Several short communications have indicated that low levels of fluoride may enhance the precipitation of apatites from metastable solutions. Hydroxyapatite alone increased the rate at which calcium and phosphate ions precipitated from solution, and as little as 0.2 ppm F even more markedly increased this rate of seeding (Brudevold & Messer, 1961). In other studies, Spinelli, Amdur & Brudevold (1964) reported that 3-10 ppm F increased the amount of precipitation of apatite from *in vitro* calcium phosphate systems. They suggested that conversion of hydroxyapatite to fluorapatite by replacement of hydroxyl by fluoride played only a minor role in the precipitation process. Precipitation and recrystallization of the hydroxyapatite under the influence of fluoride was presumed to explain the observed phenomena.

Taves & Neuman (1964) also reported that fluoride at the levels found in human serum enhanced calcification in solutions with concentrations of calcium and phosphate ions lower than normally required.

Conversion of calcium phosphate compounds to hydroxyapatite

Current theories postulate that more than one microcrystalline phase exists in calcified structures, particularly in very young bone (Brown et al., 1962; Hirschman & Sobel, 1965; Dallemagne, 1964; MacGregor & Brown, 1965). Dallemagne (1964) has proposed a hydrated tricalcium phosphate which on aging may be converted to hydroxyapatite. Brown et al. (1962) have suggested that octacalcium phosphate, $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$, may be the early mineral phase of bone associated in a lamellar fashion with hydroxyapatite. On the basis of an *in vitro* study using a child's bone powder, MacGregor & Brown (1965) reported: "It would appear then that in the child the solid phase in equilibrium with body fluids is octacalcium phosphate (OCP), $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$, while in the adult the Ca/P ratio of the equilibrating solid phase is more nearly that of HA". Most importantly, it has been further suggested that fluoride enhances the conversion of OCP to hydroxyapatite (Newesely, 1965; Brown, 1966).

The low Ca/P ratios found in the *in vitro* calcification of rachitic epiphyseal cartilage by Hirschman & Sobel (1965) were thought to resemble those in the composition of newly deposited bone mineral *in vivo* and cor-

responded to the Ca/P ratio of either octacalcium phosphate or a "defect" apatite.

Since both tricalcium phosphate hydrate and octacalcium phosphate have lower Ca/P ratios than the more stable hydroxyapatite or fluorapatite, mixtures of these initial microcrystals would account for the low Ca/P ratio. It may, thus, not be necessary to invoke the existence of a single phase of a "defect" or low Ca/P apatite. The presence of tricalcium phosphate hydrate or octacalcium phosphate in calcified tissues has also been indicated since both produce pyrophosphate on heating whereas hydroxyapatite does not. Heating bone as well as dentine and enamel yields pyrophosphate (Dallemane, 1964). Furthermore, older bone yields less pyrophosphate on heating than younger bone (Posner et al., 1965).

Solubility data

It is difficult to establish the solubility characteristics or solubility products of hydroxyapatite and fluorapatite since these compounds may exist with varying non-stoichiometric ratios of calcium to phosphorus. They are sparingly soluble and may also occur in various crystal sizes. In addition, it is difficult to prepare well-crystallized hydroxyapatite. The following solubilities are quoted in moles per 100 ml:

Calcium oxalate	5.2×10^{-6}
Calcium fluoride	2×10^{-5}
Magnesium fluoride	1.2×10^{-4}
Hydroxyapatite	6.6×10^{-6}
Fluorapatite	1×10^{-6}

E. C. Moreno of the American Dental Association (personal communication), on the basis of certain thermodynamic data, has estimated the solubility products for hydroxyapatite and fluorapatite to be:

$$\text{Hydroxyapatite: } [\text{Ca}]^{10}[\text{PO}_4]^6[\text{OH}]^2 = 4.85 \times 10^{-113}$$

$$\text{Fluorapatite: } [\text{Ca}]^{10}[\text{PO}_4]^6[\text{F}]^2 = 1.44 \times 10^{-119}$$

As postulated by others, the solubility and solubility products of fluorapatite are less than those for hydroxyapatite—a finding which has been related to the cariostatic effect of fluoride. Recent data indicate that fluorapatite and hydroxyapatite have similar solubility in saliva (McCann & Brudevold, 1966).

Essential role of fluoride in calcification

All skeletal and dental tissues examined so far in the vertebrate phyla contain fluoride. In addition, it has been suggested that fluoride may be necessary for the conversion of early metastable calcium phosphates to

fluorapatite, the most stable of the apatites (Brown, 1966; Newesely, 1965). These observations have led to the interesting and provocative hypothesis expressed by Newesely (1961)—and reiterated by Perdok (1963)—that the formation of bone whose mineral component has an apatitic structure or structures would not be possible under biological conditions without the presence of small quantities of fluoride.

Perdok (1963) has further postulated that hydroxyapatite and the organic matrix may be firmly held to each other by hydrogen bonds between the nitrogens and the carbonyls of the amino-acid structures and the hydroxyls of the apatite. More importantly, he suggests that the strength of hydrogen bonds increases when fluoride is substituted for hydroxyl leading to a more stable structure. These hypotheses await proof, but provide challenging ideas. Perhaps newer physical techniques, such as nuclear magnetic resonance, will provide evidence for the bonding energies within the environs of the fluoride atom in calcified structures.

Summary

1. The effects of fluoride on the skeleton have been reviewed as they relate to the long-term ingestion of water containing up to 4 ppm F.

2. No changes have been observed in the concentration of the major components of bone, Ca and P, as a result of fluoride deposition. Small decreases were observed in the sodium and potassium concentration in the bones of individuals drinking water containing 4 ppm F as compared with those drinking water containing trace quantities of fluoride. The carbonate content decreased about 10% and the citrate about 30%. The ash and magnesium content increased slightly but consistently.

3. Histological studies have indicated that no pathology attributable to fluoride was seen in the rib, iliac crest or vertebra of individuals with an eightfold range in fluoride concentration in the bone. Examinations were made for focal calcification of the periosteum and adjacent tendons, for osteoclastic and osteophytic activity and for cortical thickness. Marrow sections were also examined for the degree of haematopoiesis and trabeculation.

4. No changes have been observed in roentgenograms taken of the hands, knees, and lumbar spine, in bone density and in skeletal maturation of children exposed for at least 10 years to fluoridated water supplies.

5. Fluoride has been observed to enhance the crystal texture of the mineral phase of bone, and this finding has been related to the decrease in the citrate content of bone with increasing fluoride concentration as well as to the possible increase in chemical stability of the resultant crystals.

6. The deposition of fluoride in bone has been related to metabolic and cellular processes of bone remodelling involving osteoblastic and osteoclastic activity with the consequent possibilities of "freeing" and "burying" fluoride-deposition sites.

7. At low levels of fluoride concentration, the predominating reaction with the apatite, e.g., of bone, is presumed to be substitution for the hydroxyl. At high levels of fluoride concentration, it is postulated that CaF_2 forms. The latter compound has not been seen in bone, but has been identified after topical application of fluoride to enamel surfaces.

8. In addition to substitution of hydroxyl by fluoride in the apatite of bone, it appears that fluoride may enhance the rate of seeding of apatite. In other words, precipitation of apatite occurs at a lower concentration of calcium and phosphate ions than would be expected.

9. It has been suggested that the early microcrystals to appear in calcifying structures are probably not hydroxyapatite, but may be a hydrated tricalcium phosphate or octacalcium phosphate. Both these compounds have a Ca/P ratio lower than that calculated for hydroxyapatite. It has also been postulated that there is one phase in bone, namely, an apatite with missing calcium, that leads to a non-stoichiometric or so-called "defect" apatite.

10. It has been stressed that (a) there is no direct evidence of the state of fluoride in calcified structures, (b) more than one "species" of fluoride may exist, (c) a number of different calcium phosphates may co-exist, and (d) information is lacking on the state of the bonds between the organic and inorganic phases of bone.

4. MECHANISM OF EFFECTS IN THE MOUTH (G. N. Jenkins)

The concentration of fluoride in the dental tissues follows a similar pattern to that of bone described in Chapter 4, section 3. The age of the subject and the fluoride intake in food and drink are the chief influences, but in enamel, from which cells and a circulation are absent, the uptake almost ceases after the age of about thirty. The F concentrations in deciduous teeth are consistently lower than those in permanent teeth formed under the same conditions.

It is now well established that, again as in bone, the distribution of fluoride within the tooth is not uniform. The fluoride levels in the outer layers of enamel are five to ten times higher than those in the inner layers. This probably arises partly because the outer layers are in contact with tissue fluid for a longer time after formation and before eruption than are the inner layers and partly because, after eruption, the outer enamel is in contact with the saliva. The secondary dentine, which forms slowly throughout life and has relatively prolonged contact with the tissue fluid of the pulp, has a higher F concentration than the more rapidly formed primary dentine. The rise in fluoride concentration with age in dentine is due partly to a rise in the concentration in the primary dentine and partly to the fact that the proportion of secondary dentine in the whole tooth increases with age.

Other effects of fluoride on the composition of teeth are discussed more logically after considering the relation between the fluoride concentration and the solubility of enamel and its caries resistance.

Fluoride Concentration of Enamel in Relation to Caries

Quite soon after the relation between fluoride and caries had been demonstrated, Armstrong & Brekhuis (1938) reported that enamel from non-carious teeth contained a higher concentration of fluoride (0.0111 %) than did the surviving enamel from carious teeth (0.0069 %), but that the concentration in dentine did not differ in the two groups. This difference in enamel fluoride levels was of high statistical significance and referred to teeth varying in caries resistance but formed in areas without a high fluoride concentration in the water. For some years, these figures were quoted as being the only established chemical difference between enamel with high and enamel with low resistance to caries. A set of figures published later by McClure (1948) failed to confirm them, however. After it had become clear that the fluoride concentration of enamel was influenced by the age of the subject, Armstrong & Singer (1963b) reported that the teeth analysed in the earlier study came from subjects who differed sufficiently in average age to account for the difference in fluoride concentration in the enamel. There is still no explanation of why the fluoride concentration of the dentine showed no difference, however, since it is also affected by age.

Effect on carbonate and citrate of enamel

The question of the relation between the fluoride of enamel and caries resistance was re-opened by Nikiforuk (1961), who investigated the concentration on the *outer surface* of enamel in caries-resistant and carious teeth from individuals in towns with and without a high fluoride concentration in the drinking water. In view of the finding discussed in section 3 of this chapter that fluoride affected the carbonate and citrate concentrations of bone, Nikiforuk studied also the concentrations of those substances in the inner and outer enamel. His first published results (Table 4) suggested that the outer enamel of non-carious teeth from Toronto (0.1 ppm in water) contained a significantly higher fluoride concentration (136 ppm) than that from the surviving part of carious teeth (83 ppm). In Brantford, Ontario, where the fluoride content of the water is 1 ppm, the corresponding figures were 357 ppm and 229 ppm. Smaller differences in the opposite direction were found for the carbonate content of outer enamel (lower in the "fluoride town" and higher in the carious teeth). When the ratio of carbonate to fluoride was compared in the outer enamel from the four sets of teeth, very striking differences were obtained, the values ranging from 0.53 for non-carious enamel from a town with 1 ppm F to 2.45 for carious teeth from a town without fluoride. Later estimations (Nikiforuk, 1965) on larger

TABLE 4
MEAN VALUES FOR CARBONATE AND FLUORIDE CONCENTRATIONS IN OUTERMOST
LAYERS OF CARIOUS AND NON-CARIOUS TEETH^a

	CO ₂ (%)		F (ppm)		CO ₂ : F ratio $\left(\frac{\% \text{ of CO}_2}{\text{ppm F}} \times 100\right)$	
	Cariou	Non- cariou	Cariou	Non- cariou	Cariou	Non- cariou
Deciduous (from fluoride town)	2.09	1.90	202	369	1.05	0.51
Deciduous (from non-fluoride town)	2.02	2.03	84	139	2.40	1.46
Permanent (from fluoride town)	1.86	1.79	285	425	0.65	0.42
Permanent (from non-fluoride town)	1.84	1.83	176	183	1.04	1.00

^a After Nikiforuk (1965).

numbers of teeth confirmed the difference in the fluoride level and in the carbonate to fluoride ratio of carious and non-carious teeth from a fluoride town, but not from a town with no fluoride in the water. There was a correlation between fluoride and citrate on the surface enamel, but it was much weaker than that in bone. Brudevold, McCann & Grøn (1965) stated that their analyses failed to confirm the conclusions of Nikiforuk on the interrelation between carbonate and fluoride in enamel, but their results did, in fact, show a similar trend where the F concentration of the water supply varied from 0.1 to 1.6 ppm; it was at higher intakes that the relationship broke down.

Mode of Action of Fluoride in Dental Caries

The theories on the mode of action of fluoride that have attracted most attention are (a) that fluoride reduces the solubility of enamel in acid and (b) that fluoride acts as an inhibitor of the bacterial enzymes responsible for producing the acid which is believed to attack the enamel in caries. Other plausible theories are that fluoride affects the protein matrix of enamel (this suggestion has not been adequately investigated) and that it influences the shape of the tooth. It is possible, and perhaps probable, that fluoride may work in several ways. The possibility that mechanisms exist which have not so far been discovered must be borne in mind.

Reduction in solubility of enamel

It is very easy to demonstrate that if enamel is shaken with a solution of fluoride, even as dilute as 1 ppm, and then washed, its solubility in acid is

reduced. Similarly, if as little as 0.1 ppm fluoride is present in an acid solution in which the solubility of enamel is tested, the solubility is reduced (Manly & Harrington, 1959). There is no doubt, therefore, that fluoride can reduce the solubility of enamel when it is present either in the enamel or in the solvent. The practical issue is whether fluoride intakes from concentrations known to be effective in caries (1 ppm or less in water) produce sufficiently large differences in the fluoride concentration of enamel or of the oral fluids to influence solubility. Curiously enough, the question has not been extensively studied and the available evidence is indecisive. Jenkins, Armstrong & Speirs (1952) compared the solubility of the intact outer enamel of groups of teeth from towns with water supplies containing 0, 1 and 2 ppm fluoride and all the results showed a trend towards lesser solubility in the "high fluoride" teeth, but not all the differences were statistically significant. Finn & DeMarco (1956) compared the solubility of ground, whole enamel from teeth formed in an artificially fluoridated area and in a control area and found the former to be slightly less soluble. Isaac et al. (1958) made an extensive study of layers of enamel ground off teeth formed under various fluoride intakes: they, too, found the enamel with the higher fluoride concentrations was less soluble, but the differences were small (less than 5%) and there were some anomalous findings. Taken as a whole, these results give general support to the theory that fluoride acts by reducing the solubility of enamel, although they are indecisive because there is no means of knowing whether these small and erratic differences are large enough to influence caries. The presence of fluoride in the plaque,¹ whose F concentration is influenced by that in drinking water (Dawes et al., 1965), may reduce the effectiveness of the acid formed by plaque bacteria in dissolving enamel.

Another aspect of this theory arises from the *in vitro* findings of Myers, Hamilton & Becks (1952) that fluoride is more readily bound by enamel which is changed by early caries than by normal enamel. The solubility of enamel in very early carious lesions in teeth from high- and low-fluoride towns was compared by Dowse & Jenkins (1957), who found a very much higher fluoride concentration and lower solubility in the carious enamel from the "high fluoride" teeth.

These results suggest a possible mechanism by which fluoride could reduce caries in teeth already erupted when they were first exposed to fluoride. If an early cavity is formed, then the enamel presumably becomes more permeable, which facilitates the entrance and binding of fluoride in depth to the surviving enamel. A reduction in solubility at this stage would be expected to slow the rate of development of the cavity.

At first, it was thought that fluorapatite, the main fluoride-containing constituent of enamel and the substance formed when hydroxyapatite is

¹ The dental plaque is the layer of bacteria, suspended in a protein matrix which deposits on the teeth, in which the acid believed to cause caries is formed. Owing to the technical difficulties involved in studying the minute amounts of plaque which can be collected, most work on acid production has been carried out on saliva.

treated with low concentrations of fluoride, was less soluble than hydroxyapatite. In a tooth which had received fluoride, either during development or afterwards, the proportion of apatite in the form of fluorapatite would increase and so, according to this theory, the solubility would fall. Gray, Francis & Griebstein (1962) first raised doubts about this theory when they reported that the dissolution rates in acid of hydroxyapatite and fluorapatite, if compared over very short periods of time, did not differ. These workers found that the solubilities began to diverge only after several minutes' exposure to acid, and they concluded that the apparently lower solubility of fluorapatite arose as follows. As the fluorapatite dissolved, the calcium and fluoride ions released precipitated as an impermeable layer of calcium fluoride on the surface of the crystals, which remained undissolved, thus preventing solvent from reaching the crystal and dissolved ions from leaving it.

Another approach to this problem suggests that if apatite crystals form *in vivo* in the presence of concentrations of fluoride higher than usual, the carbonate in the crystals is reduced, and the solubility likewise, animal experiments having indicated that a low carbonate concentration in enamel is associated with a reduced solubility and a high caries resistance. As already mentioned, there is evidence of a fluoride-carbonate interrelationship in human enamel. More recently this concept has been replaced by the hypothesis, based on the finding that high concentrations of fluoride in a medium from which apatite is crystallizing improve the "crystallinity" of the apatite and that carbon dioxide has the opposite effect, that large, well-formed crystals, free from defects, dissolve less readily than small crystals with many defects. There is good evidence that this change in crystallinity occurs in human bone from subjects with a high fluoride intake for long periods (Zipkin, Posner & Eanes, 1962; Posner et al., 1963), but it seems, at present, to be merely a speculation that it occurs also in enamel. Bone is metabolizing and remodeling throughout life and has a much greater opportunity of being influenced by fluoride than has enamel, which, apart from the extreme outer surface, is in an environment where changes are minimal. Unless the enamel receives sufficient fluoride during its formation, it seems unlikely that the bulk of it could be influenced in the same way as bone.

Brudevold, McCann & Grøn (1965) have pointed out that many substances besides fluoride reduce the solubility of apatite (e.g., zinc, lead, tin, cadmium, copper) but only fluoride is known to reduce caries. They argue that fluoride must act through some unique property rather than through a property shared by many other substances. They have developed the idea, mentioned in section 3 of this chapter, that only in the presence of fluoride is an apatite deposited: in its absence, more soluble types of crystals such as brushite (CaHPO_4) or octacalcium phosphate are believed to be formed. This hypothesis can be considered in conjunction with the findings of Pigman, Koulourides & Newbrun (1960) that when whole teeth are treated with acid solutions, the enamel becomes soft and decalcified, but if they are

then treated with a solution containing calcium phosphate and fluoride ions, a rehardening occurs following the precipitation of calcium phosphate on the enamel surface. If this occurs *in vivo*, the caries process can be pictured as short periods of decalcification, following the rapid acid formation that occurs after carbohydrate ingestion, alternating with periods in which precipitation of calcium phosphate occurs. The effect of fluoride is not only to favour the precipitation but also, Brudevold and co-workers suggest, to ensure that the salt precipitated is of the apatite structure which is less soluble than other possible crystalline forms. Although this is sometimes regarded as an alternative to the solubility hypothesis, it can be considered as a subtle explanation of the effect of fluoride on solubility. The concentration of fluoride in the solution will determine the proportion of fluoride present in the apatite and this can influence solubility, although perhaps indirectly, as suggested by Gray, Francis & Griebstein (1962).

It is doubtful whether the very small and intermittent rise in the fluoride concentration of the plasma brought about by the ingestion of the low concentrations of fluoride that are sufficient to reduce caries would influence the crystalline form of *developing* enamel. The effect is more likely to be important in the presence of the much higher fluoride concentrations in the plaque and near the crystals dissolving and reprecipitating in a carious lesion.

It is clear that although the facts give general, but not conclusive, support to the solubility theory, the mechanism by which fluoride may reduce solubility is at present extremely controversial.

Inhibition of bacterial enzymes

The well-known inhibitory effect which fluoride exerts on certain enzymes—including some of those concerned with glycolysis—raised the possibility that reduction in acid production, or in reactions associated with it, by the bacteria of saliva or the dental plaque might be the explanation of the anti-caries action. The validity of this theory depends upon the answers obtained to two questions:

(1) What concentration of fluoride is necessary to bring about a decisive reduction of acid production?

(2) What is the concentration of fluoride ions in the plaque?

Bibby & Van Kesteren (1940) investigated the effect of a range of fluoride concentrations on pure cultures of salivary bacteria and found that while 2 ppm F had a small but detectable effect on acid production, much higher concentrations were needed to affect bacterial growth. The concentration of fluoride in saliva is 0.1-0.2 ppm,¹ which is much below that which Bibby & Van Kesteren found was necessary even for the smallest detectable effect. In view of the striking evidence that fluoride could reduce the solubility of teeth *in vitro* (which, as already noted, preceded any evidence about the

¹ Recent studies with the fluoride electrode suggest that only some 20 % of the fluoride in saliva is present in a free ionic form (Grøn et al., 1968).

effect of fluoride on the solubility of teeth *in vivo*), the anti-enzyme theory received little attention.

Borei (1945) reviewed the evidence on the factors which influenced the inhibitory action of fluoride on enzymes and pointed out that if fluoride was added to an organism at an acid pH, its effect was greatly enhanced. This point was tested on salivary organisms and it was found that if fluoride was added to salivary bacteria at a pH of 5.0, as little as 6-10 ppm stopped acid production completely for some hours and even promoted a slight rise in pH, caused by alkali production, which was not only unopposed by acid production but even slightly stimulated (Jenkins, 1959, 1960). Of other workers in this field, Capozzi et al. (1967) studied the anti-enzymatic activity of the following fluorine compounds, not least with a view to their utilization in toothpastes: NaF, SnF₂, Na₂SiF₆, MgSiF₆, Na₂PO₃F and an amine fluoride indicated as GA297. In 4-mM concentration all these compounds inhibited lactic acid formation by lactobacilli, though Na₂PO₃F did so only after 24 hours. In tests with crystalline enzymes, enolase and phosphoglyceromutase were inhibited to the greatest extent.

Fluoride in plaque and its possible significance. Hardwick & Leach (1963) investigated the fluoride concentration in plaque from adults and found it surprisingly high, even in a town without fluoride in its water supply, averaging 66.9 ppm, with a range of 6 to nearly 180 ppm. It has been shown that the plaque fluoride is related to the fluoride of the drinking water (Dawes et al., 1965). Plaques collected from children in towns without fluoride and with 2 ppm in their water supplies averaged 26 ppm and 49 ppm respectively. It is not entirely clear why these figures were both *lower* than those obtained in the first investigation, but it is probably because there were several differences in procedure in the two surveys. These concentrations are several hundred times greater than those in saliva and could only remain in the plaque if the fluoride is present in some bound form, which has not yet been identified. Studies with the fluoride electrode show that the concentration of free ionic fluoride is below 1 ppm in plaque from a low-fluoride town and between 1 and 2 ppm in plaque from a town with 2 ppm in the water; in other words, about 95 % of the plaque fluoride is bound (Jenkins, Edgar & Ferguson, 1969). It has nevertheless been found that when samples of plaque from high (2 ppm) and low fluoride areas are allowed to stand with sucrose and the pH changes are measured, there is a significantly more rapid pH fall in the plaque from the low-fluoride town. These apparently contradictory results can perhaps be reconciled by the finding that if pure cultures of bacteria isolated from plaque are grown in fluoride-containing media they store fluoride and produce acid from sugar more slowly than fluoride-free controls. These results suggest that much of the fluoride of plaque may be inside the bacteria where it exerts an inhibitory effect.

Although the plaque fluoride has usually been considered in relation to anti-enzymatic effects, it also may influence caries by reducing the effectiveness of acid in dissolving enamel.

In addition to acid production, another type of activity by the plaque bacteria has recently been discovered, and although its importance in caries has not yet been proved, it seems likely. This activity is the synthesis of at least two types of polysaccharide from ingested sugar. It is very probable that the course of events in the plaque following the ingestion of sugar is as follows. Acid-producing bacteria rapidly convert some of the sugar to acid and the pH of the plaque falls and, more slowly, some sugar is built up into polysaccharide. When the sugar is all metabolized, acid production may continue from the stored polysaccharide and this may have the effect of lowering the pH still further or of prolonging the time during which the pH is low. It is believed that the enamel can only dissolve if the pH is below some critical figure, which varies from plaque to plaque but is in the neighbourhood of 5.5. The lower the pH reached, or the longer the time it is below the critical figure, the greater the decalcification will be expected to be. Two groups of workers (Kleinberg & Sandham, 1964; Weiss, Schnetzer & King, 1964) have shown that the synthesis of carbohydrate from glucose by oral bacteria, as well as acid production, is inhibited by concentrations of fluoride (2 and 4 ppm) well within those likely to be released when the plaque is acid.

Enzymatic site of inhibitory action. The enzyme of the Emden-Myerhof scheme which is most sensitive to fluoride is enolase (Warburg & Christian, 1942, confirmed by unpublished data of Jenkins). If enolase is the site of inhibition of glycolysis in the saliva or dental plaque, it would be expected that phosphoglyceric acid would accumulate instead of lactic acid. Repeated attempts by a variety of methods to demonstrate the presence of phosphoglyceric acid in saliva incubated with sugar and fluoride have failed (Jenkins, Ferguson & Edgar, 1967) and it seems unlikely that enolase, in spite of its great sensitivity, is the site of enzyme inhibition in saliva. Another piece of evidence suggesting that inhibition occurs at some stage much earlier than enolase is that if phosphoglyceric acid (which, according to Fosdick & Wes-singer (1940), is more highly ionized than lactic acid) accumulated in lieu of lactic acid, the pH would fall to lower values in the presence of inhibitory concentrations of fluoride. In fact, fluoride greatly reduces the pH change, which implies that it inhibits some stage before that at which acid forms. It has been found that glucose utilization is reduced by fluoride, corresponding with diminished acid production, and it is concluded that a very early stage of glycolysis is inhibited—either hexokinase or the passage of glucose through the cell wall (Kleinberg & Sandham, 1964, and unpublished data from the writer's laboratory). There is evidence that hexokinase is not sensitive to low concentrations of fluoride (Berger et al., 1946); this, while

it has not been thoroughly tested under a variety of conditions, increases the probability that glucose uptake by the cell is affected rather than any specific enzyme.

Bramstedt, Kröncke & Naujoks (1960) found that concentrations of fluoride of less than 1 ppm *increased* the rate of metabolism of sugar by certain organisms taken from the mouth. They suggested that this action might occur *in vivo* and result in a greater rate of removal of sugar, thereby reducing the time during which sugar is available for acid production. Although the acceleration—rather than the inhibition—of some enzyme action by low concentrations of fluoride has been well established by other workers, these paradoxical effects have not been demonstrated *in vivo*. The relation of the work of Bramstedt and associates to the action of fluoride in caries remains uncertain.

Source of fluoride in plaque. There are three possible sources from which the plaque can obtain fluoride: (1) saliva, (2) food and drink, and (3) the enamel surface. A few simple calculations show that most of it must come from either saliva or ingesta and not from the enamel surface. Although the fluoride concentration in plaque is high, the absolute amounts contained by the plaque in one mouth are minute owing to the small bulk of the plaque. If plaque is allowed to accumulate for a few days the average weight is only in the neighbourhood of 10 mg. If this contained 50 ppm fluoride, the weight of fluoride would be 0.5 μg , which is equal to the fluoride in 10 ml of saliva (assuming a concentration of 0.05 ppm) or in as little as 0.5 ml of drinking water containing 1 ppm. It is likely that the plaque fluoride is derived from the slow trickle of saliva or from the much larger but intermittent washes with drinking water. The work of Carlson, Armstrong & Singer (1960b) with ^{18}F had given grounds for expecting that the fluoride concentration in saliva would be related to that of plasma. This has been established by means of the fluoride electrode (Grøn et al., 1968). The increased fluoride of plaque in high-fluoride areas is probably largely derived from the slightly raised concentration of fluoride in the saliva.

It has been shown that the uptake of fluoride by the apatite of enamel is virtually irreversible, so it is most unlikely that enamel fluoride could diffuse into the plaque to provide a significant proportion of the plaque fluoride. If fluoride from the enamel enters the plaque at all, it could do so only by dissolving the apatite, thus releasing fluoride along with other constituents of enamel. It is clear that this process must be extremely limited in extent, otherwise the fluoride of the enamel surface would gradually fall with increasing age, when in fact it rises. Although only a tiny proportion of the plaque fluoride can be derived from the enamel, it is possible that it is of importance in caries because the release will occur into the innermost layer of plaque (which presumably influences the carious process more than the plaque as a whole), and at a time when

the plaque is acid and the plaque bacteria are apparently most sensitive to fluoride.

Before the concentration in plaque had been measured, it had sometimes been suggested that the enamel surface, with its high concentration of fluoride, might exert an inhibitory effect on the plaque bacteria. The idea was supported by Briner & Francis (1962), who found that if a pure culture of *Lactobacillus casei* was incubated in a culture medium in contact with the ground surface of enamel either from a tooth with a naturally high fluoride content or from one which had received fluoride by "topical application" of a sodium or stannous fluoride solution, the bacteria produced less acid than when in contact with a control tooth. The conclusion that the fluoride in the enamel had inhibited the bacteria does not prove that an undamaged, natural tooth surface would exert a similar effect on the plaque bacteria, however, for the following reasons. The intact surface of the tooth is much less soluble than the ground surface used in the experiments of Briner & Francis and all enamel surfaces are much more soluble in the culture medium than in plaque or saliva, which contains concentrations of calcium and phosphate ions sufficient to saturate it over a wide range of pH and thereby prevent any enamel dissolving at all. In other words, such experiments are extremely prone to produce a "false positive" result indicating that the fluoride of enamel could exert inhibition.

Relative Importance of Systemic and Local Effects of Fluoride in Caries

If the mechanisms so far mentioned really are the means by which fluoride reduces caries, it is possible to discuss which of the actions are exerted systemically and which are local effects in the mouth responsible for the change which occurs after eruption. Epidemiological studies on caries rates in subjects who first received fluoride either before or after the eruption of their teeth have shown that the main effect requires the intake of fluoride during tooth formation: a smaller effect has usually been found, however, if fluoride is received after the teeth have erupted. For example, in Grand Rapids, Mich., after ten years of fluoridation, the 16-year-old group (who were 6-7 years old when fluoridation was introduced) showed a 26% lower DMF (decayed, missing, filled) rate than the controls, as compared with an approximately 60% reduction in the DMF rate of permanent teeth for the children born after fluoridation (Arnold et al., 1956). A similar post-eruptive protection was observed in Evanston, Ill., in the permanent, but not in the deciduous teeth (Hill, Blayney & Wolf, 1957). The fluoridation study carried out in Great Britain by the Ministry of Health (1962) reported reductions of 26% and 14% of caries in the deciduous teeth of 6- and 7-year-old children five years after fluoridation began (the figures for the 3-, 4- and 5-year-old children, whose teeth had formed under the influence of fluoride,

were 66%, 57% and 50% respectively). However, Russell & White (1961) did not find such an effect after seven years' fluoridation in Maryland.

It is not known whether caries rates are affected in teeth which first receive fluoride many years after eruption, because no surveys appear to have been carried out among mature adults in areas with artificial fluoridation.

The action on the solubility of enamel is partly dependent on fluoride deposited in the enamel during formation, but is probably supplemented by the uptake known to occur on the enamel surface after eruption. The entry of fluoride into early cavities obviously occurs as a local effect after eruption and may play an important part in the post-eruptive reduction in caries.

If fluoride exerts an anti-enzymatic effect, it must be mostly local, following the uptake of fluoride from saliva or drinking water. The controversial question of whether any plaque fluoride can be derived from the enamel (i.e., from a largely pre-eruptive source) has already been discussed.

Effect of Fluoride on Size and Morphology of Teeth

Several observations on human subjects and experimental animals have shown that the size and morphology of teeth can be influenced by fluoride intake. It might be expected that smaller teeth and shallower fissures would reduce the contact points and other food-trapping areas.

Forrest (1956) and Ockerse (1949) commented on the well-rounded cusps and shallow fissures of human teeth from fluoride areas in Great Britain and South Africa, respectively, but presented no statistical data. Wallenius (1957) reported that teeth formed in a fluoride area were, on the average, 1.7% wider than those from a control area, contrary to the trend of other findings. In New Zealand, Cooper & Ludwig (1965) measured the mesio-distal and bucco-lingual diameters and the cusp depths of the lower first permanent molars of children in fluoridated and control areas and found the diameters about 2% smaller and the cusp depths 5% smaller in the fluoride area (Table 5). These differences were statistically significant, but it is debatable whether they are large enough to have a clinical effect on caries. Moreover, the effect on size and shape is apparently not specifically due to fluoride because some of these differences were observed also in Napier, a town where the intake of molybdenum was high because the vegetables had a high molybdenum content. Paynter & Grainger (1956) found that, in rats fed 12 ppm sodium fluoride (i.e., 6 ppm fluoride) in the diet, the size of the molar teeth was reduced and the proportion of molars with rounded fissures was higher than in the controls. Kruger (1962) studied the size and shape of the fissures in rats' molars after the injection of fluoride. A dose was employed which was much higher than would ever be received from food or

TABLE 5
EFFECT OF FLUORIDE INGESTION ON MORPHOLOGY OF HUMAN TEETH^a

	Control	Fluoride	Significance of difference (P)
<i>Lower 1st permanent molars</i>			
Mesio-distal diameter (mm)	10.30 (86) ^b	10.11 (100) ^b	0.01
Bucco-lingual diameter (mm)	10.31 (99)	10.18 (100)	0.05
<i>Upper 1st permanent molars</i>			
Cusp height (mm)	2.68 (66)	2.54 (63)	0.01
Buccal convexity (mm)	0.35 (98)	0.31 (98)	0.01

^a After Cooper & Ludwig, 1965.

^b The figures in parentheses indicate the numbers in each group.

water: 0.108 mg of fluoride injected daily into each rat up to the age of 14 days—an amount which is approximately equivalent to 54 mg in a human baby. However, the rat needs about 10 ppm of fluoride in drinking water (i.e., ten times the human dosage) to reduce caries, and this species may be less sensitive than man to other effects of fluoride. Some of the morphological differences found by Kruger were statistically significant and some were sufficiently marked to be clearly visible in photographs without elaborate measurements. Other trace elements (boron and molybdenum) were observed to have similar effects on the shape of fissures—a finding which agrees with that of Cooper & Ludwig in the children's teeth in Napier, New Zealand.

Further points on the effect of fluoride on the morphology of human teeth are dealt with by Adler in Chapter 9.

There are several mechanisms by which fluoride (and other trace elements) could modify the morphology of the tooth, but it is not yet known which of them is effective. Some investigators have speculated that the fluorapatite crystals differ slightly in size from hydroxyapatite crystals. Others have pictured an inhibitory action on some of the enamel-forming cells which prevents growth in part of the enamel. It is believed that cusp formation depends on such a differential rate of enamel production on the part of the ameloblast (Kronfeld, 1935). It is possibly influenced at an even earlier stage in enamel development by local changes in the mitosis rate of the cells of the dental lamina which alter the intensity of its folding. The histology of developing teeth in animals treated with fluoride was examined by Kruger (1962), and although some damage to groups of ameloblasts was noted, it was inadequate to explain the eventual changes observed. Moreover, no histological changes were observed after the administration of boron, although this element produced an effect on tooth morphology similar to that of fluoride.

Fluoride and Non-specific Hypoplasia

Zimmerman (1954) pointed out that there was a type of enamel defect which could easily be confused with fluoride mottling but nevertheless differed from it in some respects (e.g., it was asymmetrical in distribution). This defect, usually referred to as "idiopathic mottling", occurs in areas without fluoride in their water supplies.

Forrest (1956) carried out a survey of the incidence of mottling in 324 children in areas of England where the water-borne fluoride varied between 0.1 and 5.8 ppm, and found that there was a higher incidence of idiopathic mottling in areas with virtually no fluoride than in those with 1 ppm. A similar finding was reported by Ast et al. (1956) in the Newburgh-Kingston fluoridation study. In Newburgh (with fluoride), 36 cases of non-fluoride mottling were observed among 438 subjects studied (8%), as compared with 19% among the 608 control subjects. The two reports cited above—one from an area with natural fluoride and the other from a locality where experimental fluoridation had been introduced—strongly suggest that a fluoride intake which is about the optimal for caries prevention *reduces* the incidence of idiopathic mottling. There is no adequate explanation of this reduction, nor is the cause of the non-fluoride mottling definitely known. Jackson (1961) reported that such mottling is more frequent on the upper permanent central incisor than on other teeth and suggested trauma of the deciduous predecessors as a main cause. Abscesses in the predecessors, which could damage the developing permanent teeth or lead to premature extraction, and thus trauma, seem an unlikely cause since they are most frequent on the molar teeth and would therefore lead to a high incidence of idiopathic mottling in their successors (the permanent premolars) which Jackson found did not occur.

Jackson (*op. cit.*) also suggested that a temporary increase in fluoride intake could not be dismissed as a cause of this type of mottling. While there can be no doubt that fluoride intake varies widely and could be exceptionally high for short periods (e.g., for periods during which larger amounts of foods high in fluoride such as tinned fish or strong tea were ingested), it is more difficult to explain the asymmetrical distribution on the teeth on a dietary basis. Jackson suggests that there may be periods of asynchronous development of homologous teeth, and if a period of high fluoride intake coincided with active growth of enamel in one tooth only, then a very limited asymmetrical defect might occur. However, the chances of these factors occurring simultaneously seem too small to account for the quite frequent occurrence of "idiopathic mottling".

A recent report (Richards et al., 1967) confirms the higher prevalence of non-fluoride enamel hypoplasia in low-fluoride areas only for regions with mean maximum temperatures below 80°F (27°C).

Mechanism of Other Possible Dental Benefits of Fluoride

As discussed in detail in Chapter 9, there is evidence that both periodontal disease and malocclusion are slightly less prevalent in fluoride areas. These benefits are probably secondary to the effect of F in reducing caries.

5. SUMMARY (I. Zipkin)

At least two efficient mechanisms act to maintain the concentration of fluoride in the body-fluids and soft tissues at low levels of concentration. These are the rapid and efficient excretion of fluoride by the kidney—discussed at length in Chapter 5—and the high avidity of the calcified structures for fluoride. Thus, fluoride is either eliminated in the urine or innocuously “sequestered” in the skeletal and dental tissues after consumption by the human at low levels of intake for long periods of time. Human body-fluids such as blood, milk and saliva contain 0.1-0.2 ppm F and these concentrations are little influenced by intakes of fluoride as high as 4 ppm F in the drinking water. Soft tissues such as heart, liver, lung and spleen contain less than 1 ppm F (wetweight). Kidney and aorta contain higher concentrations of fluoride, probably due in the former to the presence of concentrated filtered urine in the collecting tubules and in the latter to calcified areas, an age-related phenomenon. No evidence has yet been provided that fluoride ingested at 1 ppm in the drinking water affects intermediary metabolism of foodstuffs, vitamin utilization or either hormonal or enzymatic activity.

Levels of fluoride in the drinking water up to 4 ppm, which produced an eightfold increase in bone fluoride, did not alter the chemistry of the major constituents of bone, but reduced the concentration of carbonate and citrate by 10% and 30% respectively. The changes in the carbonate and citrate levels may play a part in the enhanced resolution of the X-ray diffraction patterns of bone, leading to a better crystalline texture which renders the apatite or mineral phase of bone more stable and perhaps more resistant to resorption. The deposition of fluoride reaches a plateau with age in human bone and only a small portion is very slowly released following removal of the fluoride source. It has been postulated that fluoride is necessary for the formation of apatite and hence for production of the mineral matrix of bone. Histologically, no changes were seen in human bone which could be related to its fluoride content.

Several clinical studies in the USA and in Europe have shown that a maximum in caries reduction (about 60%) is coincident with a minimum of mottling or dental fluorosis when the drinking water contains 1 ppm F. Non-fluoride opacities and malocclusion appear to be reduced in fluoride areas and somewhat less periodontal disease has also been reported. Concentrations of fluoride in saliva (0.1-0.2 ppm) are insufficient to produce

cariostasis by inhibiting the growth or enzymatic activity of the oral bacteria. The very much higher levels of fluoride in plaque (20 ppm is a typical figure) are thought to be largely present inside bacteria, and there is evidence that they inhibit acid production. Fluoride is incorporated into the enamel of teeth to form fluorapatite, a less soluble apatite than hydroxyapatite, and the outer layer of "high fluoride" teeth shows a small but fairly consistent tendency to be less soluble *in vitro* than that of "low fluoride" teeth. If fluoride enhances the "crystallinity" of the enamel mineral as it does in bone, this perhaps introduces another factor to help explain the cariostatic effect of fluoride.

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CHAPTER 7

Toxic effects of larger doses of fluoride

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1. INTRODUCTION (A. Singh & S. S. Jolly)

Investigations of toxic effects of fluoride in humans have evoked a lively interest throughout the world because public health programmes of fluoridation for the prevention of dental caries have always been considered to involve the risk of remote cumulative intoxication. However, the indices of early intoxication are poorly defined and this has resulted in an element of speculation and confusion about the toxic potentialities of the fluoride ion. At the very onset, a clear distinction must be made between acute toxic effects, which result from a single massive dose, and the chronic toxic effect of large doses spread over a number of years. The latter may be confined to a minor physiological alteration or may produce a major crippling disease.

A number of biological effects have been ascribed to fluorides. Although many reports of such effects are unsubstantiated, several have been studied sufficiently to deserve careful summarization, including the effects on bones, teeth, kidney, thyroid, neurological functions and growth in general. Smith & Hodge (1959) have related the concentrations or doses of fluoride to the biological effects indicated in the tabulation below:

<i>Concentration or dose of fluoride</i>	<i>Medium</i>	<i>Effect</i>
2 parts per 1 000 million	Air	Injury to vegetation
1 ppm	Water	Dental caries reduction

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<i>Concentration or dose of fluoride</i>	<i>Medium</i>	<i>Effect</i>
2 ppm or more	Water	Mottled enamel
5 ppm	Urine	No osteosclerosis
8 ppm	Water	10 % osteosclerosis
20-80 mg/day or more	Water or air	Crippling fluorosis
50 ppm	Food or water	Thyroid changes
100 ppm	Food or water	Growth retardation
More than 125 ppm	Food or water	Kidney changes
2.5-5.0 g	Acute dose	Death

Clinical Observations of Acute Fluoride Intoxication

Current knowledge about the doses of fluoride that produce acute intoxication is derived principally from suicidal or accidental poisoning (Lidbeck, Hill & Beeman, 1943; Sharkey & Simpson, 1933). With the more widespread use of fluoride in industry, in agriculture and in the home, there is need for additional evaluation of acute toxic effects.

The acute lethal dose of fluoride for man is probably about 5 g as NaF (Goodman & Gilman, 1965). Although the precise doses of different fluorides are not known, the probable range is 2-10 g for soluble compounds such as hydrofluoric acid, hydrofluosilicic acid, potassium fluoride, sodium fluoride, sodium fluosilicate, and ammonium fluoride. The form of F used and the method and length of administration, as well as individual susceptibilities, have varied the toxic effects to such an extent that a comparison of the data obtained is scarcely warranted. However, there is evidence that fluosilicates are more toxic than either NaF or CaF_2 and that NaF is more toxic than CaF_2 . The minimum fatal dose is determined by the carrying vehicle, by the promptness and completeness of vomiting and by the speed of initiation of therapy.

Acute fluoride intoxication, whether caused by ingestion or inhalation of relatively large amounts of fluoride-containing compounds, has not been so well described as chronic fluoride intoxication. This is due, in part at least, to the fact that acute fluoride intoxication is rare. In Roholm's (1937) world-wide survey of the published literature on the subject, 112 cases were recorded. Greenwood (1940) extended this survey and recorded 18 additional cases. From 1939 to 1957, 305 additional cases were recorded in the medical literature. The latter figure suggests a wider prevalence than really exists, because 263 cases of poisoning occurred in a single episode at the Oregon State Hospital (Lidbeck, Hill & Beeman, 1943). Thus, in the medical reports covering a period of 85 years, there were only 132 scattered cases of acute fluoride poisoning, with 303 additional cases related to two epidemic-type accidents.

The acute effects of the ingestion of massive doses of fluoride are first those of an irritant poison, and later become apparent in enzyme systems such as those engaged in metabolism, energetics, and cellular respiration and in endocrine functions. However, no system of the body can be

considered exempt. Thus, in cases of acute poisoning, early involvement of the alimentary, cardiovascular, respiratory and central nervous systems, with corresponding symptoms, is a characteristic feature and such cases commonly have a fatal outcome in two to three days.

The frequency of the symptoms reported in connexion with 34 fatal cases of acute fluoride poisoning as described by Roholm (1937) are shown in Table 1. The best available description of massive, non-fatal intoxication by NaF is the case given by Peters (1948).

TABLE 1
FREQUENCY WITH WHICH SYMPTOMS WERE OBSERVED IN 34 FATAL CASES
OF ACUTE FLUORIDE POISONING ^a

Symptoms	No. of cases
Vomiting	31
Pain in abdomen	17
Diarrhoea	13
Convulsions, spasms	11
General weakness and muscular weakness and collapse	8
Pain or paraesthesia in extremities	7
Paresis, paralysis	5
Difficulty in speech and articulation	5
Thirst	5
Perspiration	5
Weak pulse	5
Change in facial colour	5
Nausea	4
Unconsciousness	4
Salivation	3
Impaired swallowing	3
Motorial restlessness	2
High temperature	2

^a After Roholm (1937).

The acute toxicity of fluoride manifests itself chiefly by local corrosive action, besides action due to absorption. After ingestion of fluorine compounds in high doses, there is diffuse abdominal pain, diarrhoea and vomiting. There is excessive salivation, with thirst, perspiration and painful spasms in the limbs.

It is obvious that rapid measures to empty the stomach and reduce fluoride absorption are most effective for preventing death or damage from massive fluoride ingestion. Provoked vomiting, followed by the ingestion of a large volume of milk, will generally be the immediately available emergency treatment. The same precautionary measures may be taken should a child ingest large quantities of caries-preventive fluoride tablets or fluoride tooth-paste, regardless of the fact that the risks are very small in such cases according to calculations and limited experience.

Robinowitch (1945) described an interesting variant in his patient, who apparently took a large amount of NaF and died of the effects of altered

calcium metabolism but without the appearance of severe gastroenteritis, which has been mentioned in almost all the other reports. Tetaniform spasms due to lowered serum calcium have been described in some of the cases of acute fluoride poisoning.

Similarly, the inhalation of gaseous fluorine leads first to irritation of the mucous membrane of the eyes and air passages and subsequently to symptoms due to absorption.

The irritant effects of fluoride, sometimes referred to as local effects, chiefly concern occupational injuries to the skin. More specifically, these local effects concern the corrosive action of:

- (a) solutions of fluoride-containing acids on the skin;
- (b) fluoride-containing acid vapours or gases on the eyes, nasal mucosa and face;
- (c) such vapours or gases on the respiratory tract.

The pathological changes in acute intoxication are haemorrhagic gastroenteritis with a tendency to necrosis, acute toxic nephritis and varying degrees of parenchymatous damage in other organs—for example, liver and heart

TABLE 2
FREQUENCY OF PATHOLOGICAL CHANGES OBSERVED
IN 32 FLUORIDE-INDUCED FATALITIES ^a

Pathological changes	No. of cases
Corrosive phenomena in mouth, throat or oesophagus	8
Inflammatory or corrosive phenomena in stomach	30
Haemorrhagic stomach contents	10
Changes in duodenum	11
Changes in small intestine	16
Changes in large intestine	2
Corrosion of organs neighbouring stomach	2
Hyperaemia of abdominal organs	6
Acute nephritis	8
Degenerative changes in liver	3
Haemorrhage or oedema of lungs	5
Subendocardial haemorrhage	2
Discoloration of skin or mucus membrane	2
Sublevel haemorrhages, brain hyperaemia or oedema	1
No definite change	1

^a After Roholm (1937).

muscle. The frequency of the gross pathological changes reported in connexion with 32 fatal cases of acute poisoning described by Roholm (1937) is shown in Table 2.

Acute Experimental Fluoride Intoxication

No description of acute fluoride intoxication would be complete without a reference to the experimental work, which has been more closely

studied and whose results are better appreciated than the scantily studied clinical cases of intoxication. Tappeiner (1889) used dogs, rabbits, guinea-pigs and cats as experimental animals. As much as 0.5 g NaF per 100 g of body-weight was given internally and 0.15 g was given by injection subcutaneously and intravenously. The following characteristic symptoms were observed:

- (1) A condition of drowsiness and weakness resulting from paralysis of the vasomotor centres;
- (2) Cramps which attacked a single organ or the entire body and were epileptic in character;
- (3) Paralysis of the vasomotor centres;
- (4) Acceleration and deepening of the breathing with paralysis;
- (5) Vomiting;
- (6) Secretion of salivary and tear glands which was not controlled by atropine;
- (7) Early rigor mortis following death.

Leone, Geever & Moran (1956), in their experimental work on dogs and mice on the acute and subacute toxicity of sodium fluoride, concluded that the mean acute lethal dose of sodium fluoride in unanaesthetized dogs infused to death by continuous intravenous infusion at the rate of 5.4 mg of fluoride ion per minute was 36.0 ± 0.5 mg/kg. The principal effects were progressive depression of blood pressure, heart rate and central nervous system with vomiting and defecation, all occurring with administration of approximately 20 mg/kg. At a mean dose of 30.6 mg/kg, there was a depression of the respiratory rate and a conversion to atrioventricular, nodal or ventricular rhythm with terminal ventricular fibrillation or asystole.

In a group of dogs infused intravenously with selected fractions of the acute lethal dose, an approximate LD_{50} was estimated to be 20 mg/kg. The major effects observed in this group were vomiting, defecation and central-nervous-system depression. In dogs given fluoride by mouth, single doses up to 3100 mg/kg produced only vomiting and diarrhoea and transient moderate depression. A slight drop in serum calcium followed the infusion of fluoride in another group of dogs in which serum Ca was determined. The pathological findings were chiefly those of generalized hyperaemia and acute focal haemorrhages.

The gross pathological changes in acute poisoning provide evidence of the potential toxic properties of fluoride and are an indication of the possible hazards and dangers inherent in a single exposure to a large concentration of F. But it must be emphasized that the response of tissues to the relatively minute concentration derived from natural sources or absorbed from industrial contamination over long periods of time is quite different and does not simulate acute toxic effects.

2. CHRONIC TOXIC EFFECTS ON ENAMEL ORGAN

(B. R. Bhussry)

The influence of chronic fluorine intoxication on the structure of enamel organ during its formation results in the development of an endemic hypoplasia known as "mottled enamel". Reference to these hypoplastic lesions of enamel dates back to Eager (1901). On the basis of the clinical appearance of the teeth, Black and McKay originally introduced the term "mottled enamel" and defined mottled teeth as "characterized by minute white flecks, yellow or brown spot areas scattered irregularly over the tooth surface". The permanent teeth are particularly affected, although occasional mottling of the primary teeth may be observed.

Regarding the nature of the hypoplastic lesions, McKay & Black (1916) considered the possibility that mottled enamel resulted from the influence of some factors in the water supply of endemic areas. Further evidence concerning this hypothesis was provided by Kempf & McKay (1930) and McKay (1933) following clinical studies at Bauxite, Arkansas, and Oakley, Idaho, respectively.

The association of mottled enamel with the presence of fluoride in drinking water was suggested by Smith, Lantz & Smith (1931), Churchill (1931) and Velu (1931). Subsequent investigations revealed a quantitative relationship between the fluoride concentration of the water supply and the severity of mottled enamel (Dean & Elvove, 1935, 1936, 1937).

Classification of Mottled Enamel in Human Teeth

Dean (1933, 1934) noted a qualitative variation in the distribution of mottled enamel among persons using a common water supply containing fluoride and a quantitative diversity in the incidence among children from different endemic areas. He classified the degree of clinically observed mottling into seven categories, ranging from normal to severe. Since the degree of dental fluorosis may in part be related to the fluoride content of the water supply, the influence of regional climatic conditions on water consumption and therefore on total fluoride ingestion should be recognized (Arnold, 1943; Galagan & Lamson, 1953; see also Chapter 2, section 2, Chapter 8, section 4, and Chapter 9 of this monograph). The basis for each classification of mottling was as follows:

1. Normal—The enamel is translucent, smooth and presents a glossy appearance.
2. Questionable—Seen in areas of relatively high endemicity. Occasional cases are borderline and one would hesitate to classify them as apparently normal or very mild.
3. Very mild—Small, opaque, paper-white areas are seen scattered irregularly over the labial and buccal tooth surfaces.

4. Mild—The white opaque areas involve at least half of the tooth surface, and faint brown stains are sometimes apparent.

5. Moderate—Generally all tooth surfaces are involved, and minute pitting is often present on the labial and buccal surfaces. Brown stains are frequently a disfiguring complication.

6. Moderately severe—Pitting is marked, more frequent and generally observed on all tooth surfaces. Brown stains, if present, are generally of greater intensity.

7. Severe—The severe hypoplasia affects the form of the tooth. Stains are widespread and vary in intensity from deep brown to black. This condition may sometimes be referred to as "corrosion" type of mottled enamel.

On the basis of this classification, Dean et al. (1935) attempted to determine a mottled enamel index of the community. This was arbitrarily defined in terms of the "degree of severity of mottled enamel" observed clinically. Similar hypoplastic changes of a non-fluorotic nature have since been recognized and found to be more frequent in low-fluoride areas (cf. Chapter 6, section 4). Such changes probably account for the "fluorosis index" line that appears in the 0.1-1.0 ppm region in Fig. 4 of Chapter 9. The differential diagnosis between mild fluorosis and non-specific mottling may be difficult in individuals whose history of early fluoride ingestion is unknown; some morphological characteristics have been given by Nevitt, Frankel & Witter (1963).

Microscopic Appearance of Mottled Enamel in Human Teeth

The literature concerning the microscopic appearance and nature of fluorosed enamel is sparse. The series of mechanisms leading to the mottling of enamel during its development and mineralization are as yet not well understood. McKay & Black (1916) reported varying degrees of discoloration of the enamel surface in ground sections of mottled teeth. They observed a lack of interprismatic substance between the regular, well-formed enamel rods, and the presence of brown pigmentation in the outer third of enamel. Williams (1923) demonstrated that areas of fluorosed enamel were more easily permeable to dyes and silver nitrate than normal enamel, possibly owing to the developmentally defective nature of the outer enamel.

Ainsworth (1933) reported the presence of irregular white and brown patches in the enamel of teeth in schoolchildren from Maldon, England. On examination of ground sections of these teeth under ultraviolet light, Ainsworth found that while sound enamel normally fluoresces bright blue, the brown stain in the mottled enamel did not fluoresce. He suggested that the stains may be of extraneous origin, and the lack of interprismatic substance of enamel may provide a pathway for the stains, thus confirming the earlier observations of Williams (1923).

Erausquin (1934) considered the permeability of the external zone of mottled enamel as being similar to that of the "immature unerupted enamel". Applebaum (1936) used soft X-rays to examine sections of mottled teeth and reported a decreased X-ray density in the outer portion of fluorosed enamel. He observed that the X-ray radiolucency of enamel was directly related to the severity of mottling in teeth.

Newbrun (1957) employed the microradiographic technique and confirmed Applebaum's findings of decreased X-ray density in the outer third of mottled enamel. The microdensitometric tracings of fluorosed teeth showed distinct hypocalcification in that region.

On the basis of histological and controlled decalcification studies of mottled enamel, Bhussry (1959a) demonstrated the presence of brown pigmentation in the outer third of enamel which was resistant to solubility in acids (Fig. 1, I & II). His observation that pigmented areas emitted a high intensity of fluorescence (Fig. 1, III) when examined under ultraviolet light was in contradiction to that of Ainsworth (1933).

Microradiographic observations of fluorosed enamel (Bhussry, unpublished data, 1965) demonstrate areas of subsurface hypomineralization (Fig. 1, IV). Although diffuse radiolucent patches are always apparent, the variation in the X-ray density along the enamel rods and cross striations is obvious. This pattern is limited to the outer zone of mottled enamel.

Gustafson (1961) utilized the polarized light and microradiographic techniques to demonstrate variations in the radiodensity and birefringence in irregular hypomineralized areas of fluorosed enamel, which were more pronounced along the striae of Retzius. An arcade appearance at the dentino-enamel junction was observed in mottled enamel.

Gerould (1945), using electron microscopy, demonstrated that sections of mottled enamel etched with HCl exhibited much finer detail of background structure than did similarly treated normal enamel. He suggested that structural differences may be due to the reduced acid solubility of the fluorapatite of mottled enamel as compared with the hydroxyapatite of normal enamel.

Awazawa (1962) prepared replicas for electron microscopic examination of teeth showing varying degrees of mottling. He reported that the enamel was relatively rich in organic material while there was a deficiency of the inter-rod substance. The enamel rods on the tooth surface were poorly mineralized with abnormal crystal size.

Investigations in Experimental Animals

Fluorosis in the white rat is manifested as a pronounced alteration in the development and appearance of the incisor teeth. The influence of fluorine on the developing teeth of experimental animals always appears to be identical, regardless of the mode of fluoride administration (McClure, 1939).

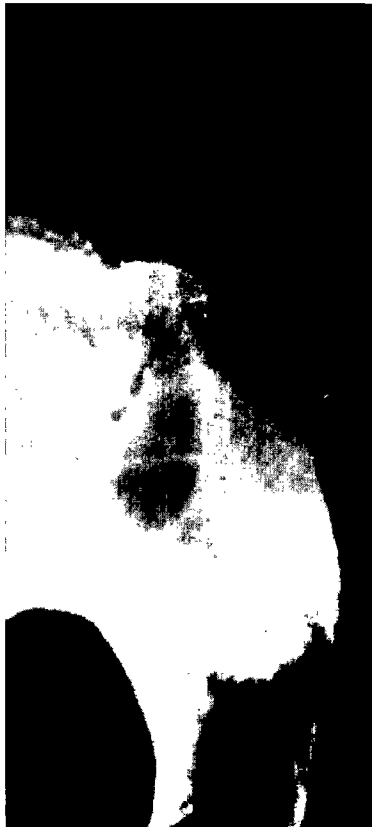
FIG. 1
MOTTLED ENAMEL IN HUMAN TEETH

- I. Unstained ground section through a mottled tooth. The smooth surface contour and pigmentation in the outer third of enamel are obvious. Scattered irregular hypocalcified areas (h) in the body of enamel are common. (Magnification: $\times 25$)



- II. Detail view of the subsurface pigmented region of mottled enamel shown in I. The structure of the enamel rods and cross striations suggests disturbance (hypocalcification) in this area. (Magnification: $\times 250$)

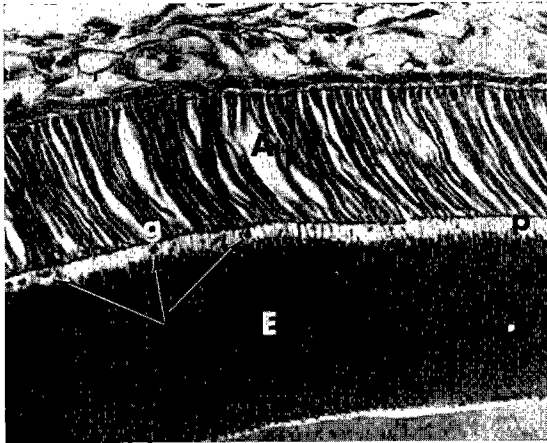
- III. Fluorophotomicrograph of the ground section shown in I. Note the increased intensity of fluorescence pattern corresponding to the pigmented area observed in the outer third of enamel. (Magnification: $\times 25$)



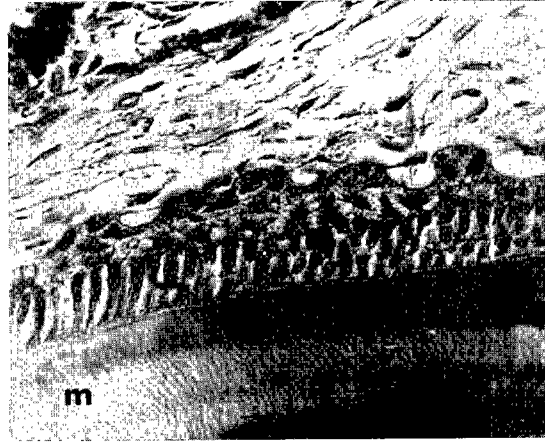
- IV. Microradiograph of the section shown in I. The subsurface radiolucency (r) is limited to the area of pigmentation. Hypomineralization along the enamel rods and cross striations is obvious (h). (Magnification: $\times 250$)



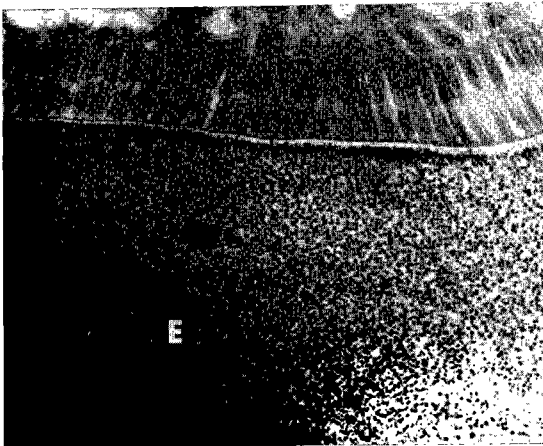
FIG. 2
EFFECT OF FLUORIDE ON THE DEVELOPING TEETH OF RATS



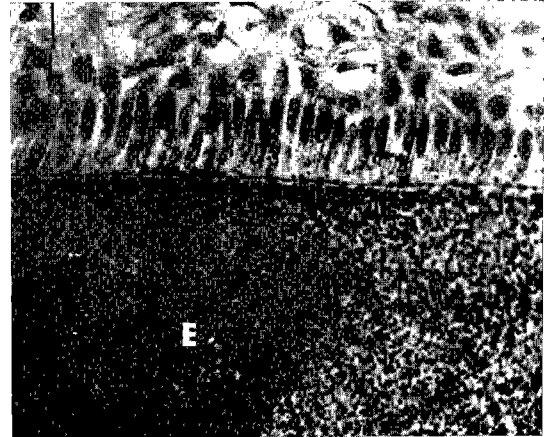
- I. Developing molar tooth of 10-day-old rat given 7 injections of sodium fluoride (150 ppm daily). The zone of pre-enamel matrix (p) is wider than that observed in the controls. Small acidophilic globules (g) are usually present in the area of Tomes' processes of the ameloblasts (A). The newly formed enamel matrix (E) appears normal in texture and staining reaction. (Masson trichrome stain; magnification: $\times 250$)



- II. Developing molar tooth of 15-day-old rat given 7 injections of sodium fluoride (150 ppm daily). The incremental line (l) is accentuated, and the enamel maturation (m) (secondary mineralization) is retarded and shows an irregular pattern. (Masson trichrome stain; magnification: $\times 250$)



- III. Developing molar tooth of 15-day-old control rat. The black silver precipitate in the cuspal region demonstrates the amount and pattern of enamel mineralization. (von Kossa staining reaction; magnification: $\times 400$)

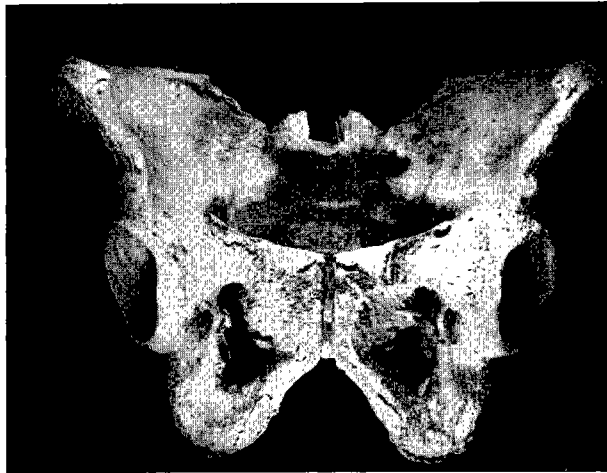


- IV. Developing molar tooth of 15-day-old fluoride-treated rat (same animal as in II). The decreased aggregation and intensity of silver precipitate in the enamel matrix (l) suggests delayed mineralization. The black band at the junction of the ameloblasts (A) and enamel matrix indicating mineral deposition is absent. (von Kossa staining reaction; magnification: $\times 400$)

FIG. 3
MOTTLED ENAMEL IN A CHILD FROM AN ENDEMIC FLUOROSIS
AREA IN PUNJAB



FIG. 4
PELVIS FROM AN ADVANCED CASE OF FLUOROSIS, SHOWING THE
ABNORMAL APPEARANCE OF BONES AND THE LAYING-DOWN OF IRREGULAR BONE



I. Front view.



II. Side view.

FIG. 5
MANIFESTATIONS OF ADVANCED FLUOROSIS IN FOREARM BONES AND FEMUR



I. Forearm bones, showing gross degree of calcification of interosseous membrane.



II. Femur, viewed from each side, showing irregular bone formation.

FIG. 6
MANIFESTATIONS OF ADVANCED FLUOROSIS IN VERTEBRAE



I. Fused dorsal vertebrae, showing narrowed intervertebral foramina and irregular osteophytes.



II. Third cervical vertebra, showing a huge exostosis projecting into the spinal canal.

FIG. 7
MANIFESTATIONS OF ADVANCED FLUOROSIS IN SKULL ^a



Base of skull, showing irregularity of the foramen magnum.

^a Reproduced, with permission, from Singh et al. (1963). Copyright 1963, The Williams & Wilkins Co., Baltimore.

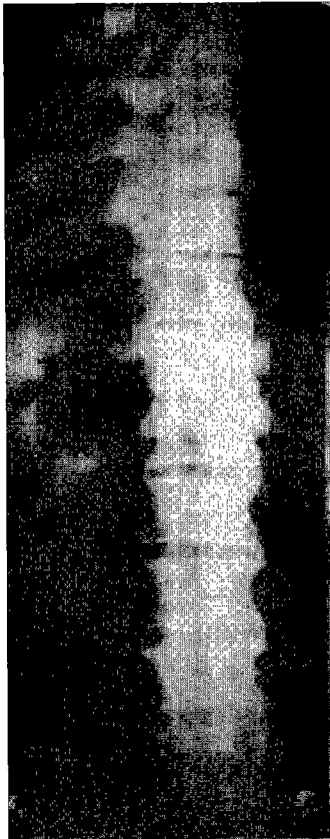
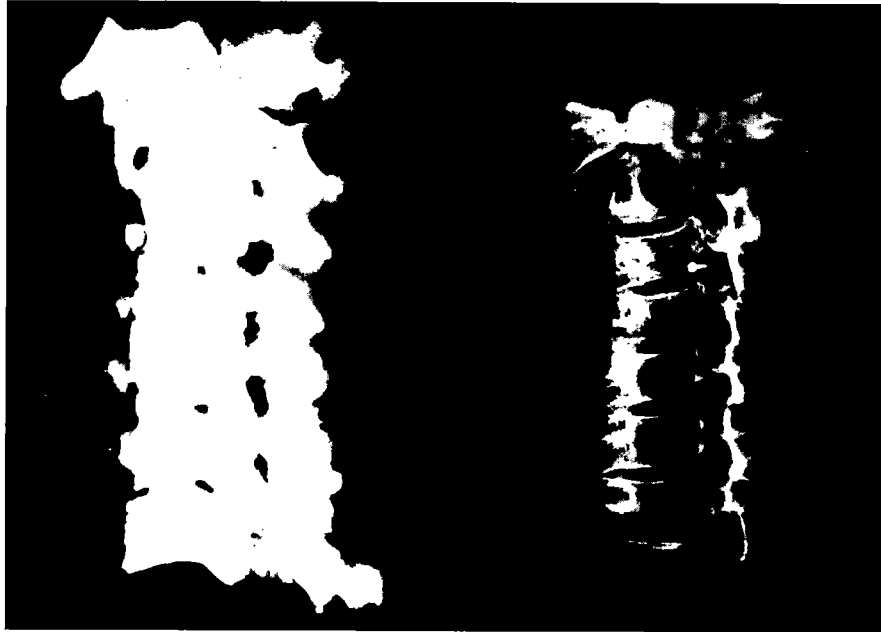


FIG. 8
SKIAGRAM OF LUMBODORSAL SPINE SHOWING
OSTEOSCLEROSIS AND MARKED OSTEOPHYTOSIS



FIG. 9
SKIAGRAM OF CERVICAL SPINE
SHOWING MARKED OSTEO-
SCLEROSIS

FIG. 10^a
POST-MORTEM SKIAGRAM OF THE CERVICAL SPINE FROM A CASE
OF FLUOROSIS (LEFT) AND OF A NORMAL CERVICAL SPINE (RIGHT)



The chalky white appearance of fluorotic bones and the markedly narrowed intervertebral foramina are quite obvious.

^a Reproduced, with permission, from Singh & Jolly (1961).

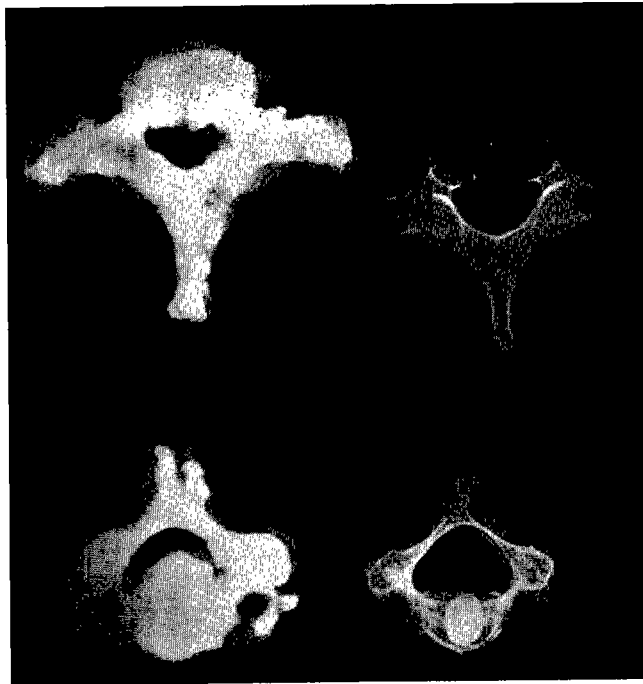


FIG. 11 ^a
POST-MORTEM SKIAGRAMS
OF FLUOROTIC DORSAL AND
CERVICAL VERTEBRAE (LEFT)
AND OF CORRESPONDING
NORMAL VERTEBRAE (RIGHT)

Marked narrowing of the spinal canal
is apparent.

^a Reproduced, with permission, from
Singh & Jolly (1961).

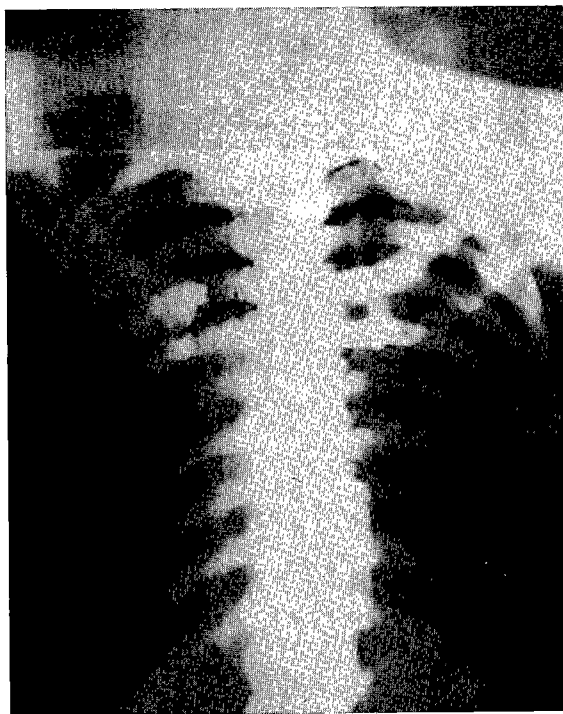


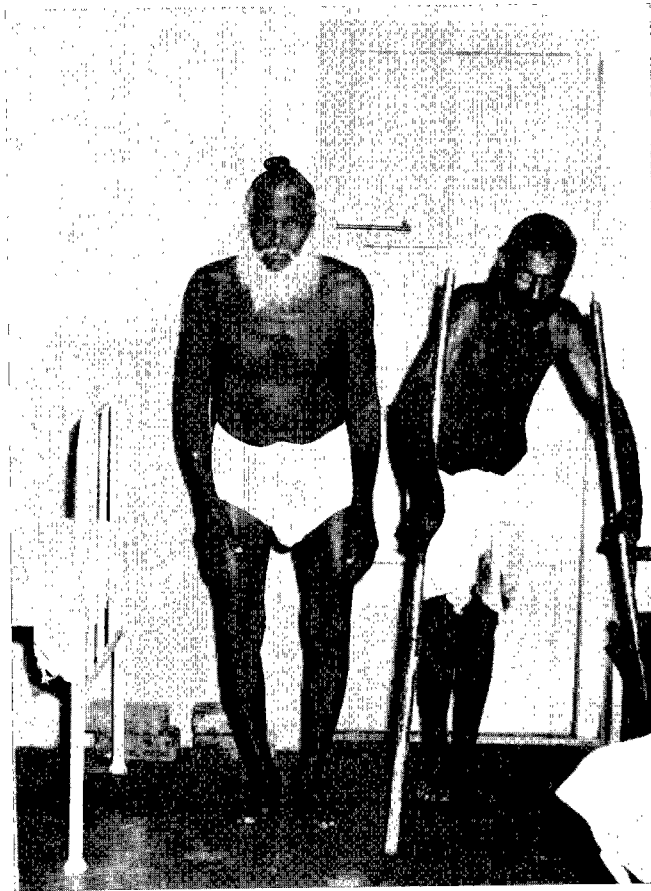
FIG. 12
X-RAY OF THE CHEST SHOWING
THE CONTRAST OF CHALKY WHITE
BONY CAGE WITH RADIOLUCENT LUNGS

FIG. 13^a
HISTOPATHOLOGICAL PICTURE OF BONE BIOPSY
SHOWING DISORDERED LAMELLAR PATTERN



^a Reproduced, with permission, from Singh & Jolly (1961).

FIG. 14
PATIENTS SUFFERING FROM ADVANCED ENDEMIC FLUOROSIS



The gross degree of invalidism and the kyphotic deformity of crippling fluorosis are quite obvious.