Afternoon Session

Chairman : R. L. HARTLES, ESQ., D.Sc., Ph.D., *School of Dental Surgery, University of Liverpool*

Theories on the mode of action of fluoride in reducing dental decay

By G. N. JENKINS, *Department of Physiology, King's College, Newcastle-upon-Tyne*

Several properties of the fluoride ion at physiological concentrations are known which may be concerned with its actions in reducing dental caries. The properties that have been most thoroughly studied as a basis of the anticaries action are: (1) the incorporation of fluoride in the hydroxyapatite of the calcified tissues, both during and after their formation, thus increasing the proportion of the less soluble fluoroapatite; (2) a tendency to favour the precipitation of mineral matter from solutions saturated with calcium phosphate, such as saliva; (3) the inhibition and, in a few instances, the slight stimulation of certain enzyme systems. These effects are not mutually exclusive and any or all of them may contribute to the reduction of caries. Other actions have been suggested but have not yet been studied sufficiently fully to be established. The possibility of undiscovered effects which may prove to be important cannot, of course, be dismissed.

Reactions of fluoride with hydroxyapatite

McCann & Bullock (1955) and Leach (1959) concluded from experiments with powdered enamel and dentine that with sodium fluoride solutions containing concentrations below 75–100 p.p.m. the most important reaction with hydroxyapatite was an exchange with hydroxyl to form fluoroapatite. With higher concentrations several other reactions were found to occur such as exchange with carbonate, the precipitation on the crystal surface of calcium and magnesium fluorides and possibly the adsorption of sodium fluoride.

Analysis of fluorosed tissues has shown an inverse relationship between the fluoride and carbonate of bone (McCann & Bullock, 1957; Weidmann, Weatherell & Whitehead, 1959; Zipkin, McClure & Lee, 1960), of incisor teeth of rats (McCann & Bullock, 1957) and of human teeth (Nikiforuk, 1961), suggesting that, even at concentrations present in tissue fluid, some replacement of carbonate can occur.

The difference between the effects on enamel of high and low concentrations of fluoride suggests that the nature of the effect on caries may depend on the means of administering the fluoride. Topical application of 2% sodium fluoride solution or dentifrices containing 0·1% fluoride produce such high concentrations that they may reduce caries in a different way from the minute concentrations of fluoride ions likely to be present in the mouth from water containing 1 p.p.m.

Effect of fluoride on solubility of enamel and dentine. Treatment of bone, tooth or calcium phosphate with fluoride solutions over a wide range of concentration, beginning at less than 1 p.p.m., can be readily shown to reduce their solubility. It has therefore long been supposed that a reduction in the solubility of enamel and

dentine is a factor (some think the main or even the only factor) in the anticaries action of fluoride. Surprisingly, until quite recently no comparisons had been made of the solubility of tooth material from people in towns with and without sufficient fluoride in the water supplies to reduce caries. Data have now been collected on intact enamel (Jenkins, Armstrong & Speirs, 1952; Jenkins, 1959*a*), enamel ground from the outer surface of teeth (Isaac, Brudevold, Smith & Gardner, 1958) and ground, whole enamel (Finn & DeMarco, 1956), which all agree in showing that the enamel formed in towns with 1 p.p.m. of fluoride or more in the water tends to be less soluble in acid than enamel from fluoride-free districts. The differences are small (not more than 11%, and the majority are much smaller), however, and some unexplained anomalies emerge. For example, the data of Isaac *et al.* (1958) refer to two enamel samples whose fluoride concentrations were almost the same (196 and 219 p.p.m.) and which differed in solubility by 5·5%; another pair with a large fluoride difference (1060 p.p.m. and 1522 p.p.m.) showed a solubility difference of only 2·9%. Also, although the fluoride content rises with increasing age the solubility of enamel from an 'over 50' group (containing 1080 p.p.m. fluoride) was greater than from an 'under 20' group with only 499 p.p.m. fluoride. My own results (Jenkins, 1959*a*) on intact enamel surfaces, although always showing a trend in favour of the solubility theory, only showed statistically significant differences in children's teeth from a town with 2 p.p.m. fluoride in the water. In permanent teeth from adults, the differences in solubility were particularly small. But it must be borne in mind that, owing to the great variations in solubility between individual teeth, large groups are needed to establish small differences. If enough teeth had been available, even the small differences observed might have proved statistically significant.

Effect of fluoride on early cavities. Myers, Hamilton & Becks (1952) showed that, if teeth are treated with ^{18}F, autoradiographs show that there is a slight uptake of fluoride by the enamel surface as a whole but a much more intensive uptake in enamel imperfections, including early cavities. This greater uptake of fluoride by enamel defects has been confirmed by several others (Ericsson, 1958; Hardwick, Fremlin & Mathieson, 1958). These results suggest that under normal physiological conditions fluoride might accumulate in early cavities and exert its effect just where it could be most beneficial. This possibility is confirmed by analyses of enamel from early cavities (Dowse & Jenkins, 1957) which was found to contain over twice the fluoride concentration of similar borings from intact enamel in the same teeth. When solubility was measured (as the amount of phosphorus dissolving in acetic acid buffer at pH 4·0) the enamel on the outside of the carious areas was found to be very much less soluble than intact enamel (confirmed by Posen, 1962). The results showed, too, that this reduced solubility of material from early cavities was even more marked in teeth from high-fluoride towns. This effect of fluoride on early cavities provides a possible explanation for the small but well-established effect which fluoride has on caries after the teeth have erupted.

The nature of the fluoride effect on solubility of enamel and dentine. There are four possible means known by which fluoride may reduce the solubility of calcified

tissues. (1) It has been tacitly assumed that fluoroapatite is a more stable crystal lattice than hydroxyapatite and can therefore resist attack by acid more effectively. (2) Gray, Francis & Griebstein (1962) state that when solubility rates of normal and fluorosed enamel are compared, by shaking the powdered enamel in acid buffers, the rates are identical during the first few minutes of an experiment; only in the later stages, is a difference detectable. Their interpretation is that, when fluoroapatite dissolves, the fluoride and calcium ions which are released into the solution combine and form a layer of calcium fluoride which covers the remaining undissolved crystal and interferes both with the diffusion of acid to the crystal and the diffusion away of the dissolved ions. (3) The third possibility is based upon two concepts both of which are controversial. The first is that, as discussed above, fluoride replaces carbonate in enamel, the other is that carbonate is preferentially dissolved from enamel by acid and that the carbonate level is therefore a factor in the solubility and caries resistance of enamel (Sobel, Shaw, Hanok & Nobel, 1960). The data on these points are conflicting (Wynn, Haldi, Bentley & Law, 1957; McClure & McCann, 1960; Nikiforuk, 1961) and until they are reconciled no conclusion can be reached. (4) The net rate of solubility may also be influenced by the precipitation mentioned in the next paragraph (Kuyper & Kutnerian, 1962).

The effect of fluoride on the precipitation of mineral from saliva

Knappwost (1951) reported experiments on eight hamsters in which he found that after 24 days' administration of 0·04 mg fluoride to half of them, followed by injections of ^{32}P into all of them, more ^{32}P was deposited on the teeth of those receiving fluoride than on those of the controls. The exact site of the deposition, whether in the muco-bacterial layer ('dental plaque') on the enamel or whether bound in the enamel itself was not determined. This work attracted little attention, but other experiments have supported the conclusion that fluoride at concentrations in the region of 1 p.p.m. favours the deposition of calcium phosphate from saturated solutions. Pigman, Koulourides & Newbrun (1960) found that teeth, decalcified and softened by the slow drip of acid for 2–10 h in their device known as the 'artificial mouth', were remineralized and rehardened when the medium contained 1–6 p.p.m. of fluoride. The solids obtained by centrifuging saliva become calcified if they are incubated with solutions saturated with calcium phosphate, and Brudevold, Amdur & Messer (1961) found that the calcification could be accelerated by fluoride in concentration even as low as 0·2 p.p.m.

The application of these results to the carious process is still speculative but two possibilities arise. It has been shown in animals that the resistance of a tooth to caries is increased after eruption (Finn, Klapper & Volker, 1955), presumably because the final stages of calcification involve an uptake from the minerals of the saliva (Fanning, Shaw & Sognnaes, 1953). If this occurs in man, it might be speculated that one effect of fluoride is to accelerate or increase these posteruptive changes. In addition, if fluoride favours calcium phosphate deposition from saliva in vivo then it may encourage some remineralization of carious cavities during the intervals between the

demineralization which is thought to occur immediately after meals as a result of acid production. The net effect would be to retard the development of the cavity.

Anti-enzyme effects of fluoride

The well-established inhibitory effect of fluoride on glycolysis suggested that its action in caries might be to prevent or reduce acid production by salivary bacteria. The first studies on the effect of various concentrations of fluoride on bacterial acid production were made by Bibby & Van Kesteren (1940). They found that 1 p.p.m. had a detectable effect on acid production by pure cultures of oral bacteria (as measured by titratable acidity) but much higher concentrations (over 250 p.p.m. fluoride) were needed to inhibit bacterial growth. Wright & Jenkins (1954) carried out similar experiments on mixed salivary organisms and confirmed that 1 p.p.m. was effective and found that even 0·5 p.p.m. of fluoride produced a small but statistically significant inhibition of acid formation.

Borei (1945) has reviewed the factors that have been shown to influence the fluoride inhibition of yeast metabolism. These factors include the state of nutrition of the organisms, the pH and the concentration of other ions (especially magnesium and phosphate).

Lilienthal (1956) was the first worker to consider the importance of these points on the fluoride inhibition of salivary organisms. He studied the effect under various conditions of a range of concentrations of fluoride on acid production by salivary sediment suspended in bicarbonate buffer at pH 6·8. Acid production was estimated by measuring the CO_2 release from the bicarbonate buffer. This method precluded the possibility of testing the effect of variations in pH on fluoride inhibition; also the magnesium and phosphate ions present in saliva would be mostly removed by this procedure. Lilienthal found that, in general, 19 p.p.m. was the minimum concentration of fluoride that inhibited salivary bacteria under his conditions and concluded that previous workers were in error in suggesting that much lower concentrations could inhibit.

I have reinvestigated the effect of low levels of fluoride on acid production by saliva–glucose mixtures with particular emphasis on the influence of the pH at which the organisms were exposed to fluoride (the factor which Lilienthal was unable to test). The results (Jenkins, 1959*b*) show that, if saliva is adjusted to pH 5·0 by the addition of lactic or hydrochloric acid and then incubated with sugar solutions containing concentrations of fluoride exceeding 6 p.p.m., the pH rises during the following few hours. In the control mixture, containing no fluoride, the usual fall in pH occurs.

The chief mechanism for the rise in pH in the presence of more than 6 p.p.m. of fluoride is thought to be that, under these conditions, acid production is completely inhibited leaving unopposed the various alkali-producing mechanisms of salivary bacteria, such as ammonia production by urease and deamination, and amine production by decarboxylation of amino acids. I have found, however, that in addition to inhibiting acid production 10 p.p.m. of fluoride when added to saliva increase slightly the rate of alkali production and lactate removal by saliva (Jenkins, 1960),

but these effects could not account for more than a small part of the observed rise in pH. It is not known whether this is an effect on individual enzyme systems or whether it arises by the inhibition of some species of bacteria making more nutrients available for competing species. Bramstedt, Kröncke & Naujoks (1960) have found that the respiration of some organisms in pure culture is increased by low concentrations of fluoride, which favours the first possibility.

The fluoride concentration of plaque

The crucial test of the anti-enzyme theory is the concentration of fluoride ions in plaque from people in towns with water supplies differing in fluoride concentration. Since the fluoride concentration of saliva is less than 0·25 p.p.m. it has been assumed that plaque concentrations would be similar and that the detection of such low concentrations by conventional methods, in the few milligrams of plaque that it is feasible to collect from one individual, would be impossible. Hardwick *et al.* (1958) therefore devised extremely sensitive radioactive methods of fluoride estimation and preliminary results on random samples suggested much higher concentrations in plaque than had been expected, namely up to 30 p.p.m. with half the samples above 6 p.p.m. (Hardwick, 1961). A series of estimations of the fluoride concentration of plaque from children in North Shields (water supply free from fluoride) and West Hartlepool (where the fluoride concentration is 2 p.p.m.) have shown average concentrations of 26 and 47 p.p.m. respectively (Hardwick, Jenkins & Dawes, unpublished). This difference is statistically significant, in spite of great individual variation, and indicates that the plaque fluoride is affected by the level of fluoride in the water. Plaque is known to contain calcium and phosphate in much higher concentrations than does saliva (Allen & Moore, 1957; Dawes & Jenkins, 1962) and, although much of the calcium is probably bound to protein at neutral pH values, it is freely extractable by buffers at pH 5·0. The fluoride might, therefore, be present in some bound form, perhaps as fluoroapatite, and be ineffective as an enzyme inhibitor, although this seems less likely when the plaque is acid. Work is now in progress to determine the proportion of plaque fluoride that is free under various conditions, and until it is known the significance of the anti-enzyme theory remains speculative. It has been found that very low concentrations of fluoride in a solvent greatly lower the solubility rate of enamel (Manly & Harrington, 1959). If the plaque fluoride is released as the pH falls after a meal, this fluoride might be expected to reduce the effect of the acid in dissolving enamel in addition to inhibiting enzymes.

Although it seems probable that most of the fluoride of plaque is derived from drinking water and tea (which contains about 1 p.p.m.) there is another possible source of part of it. The fluoride concentration of the surface enamel has been found to be between five and ten times greater than that of the inner enamel (Jenkins & Speirs, 1953; Brudevold, Gardner & Smith, 1956) and, although fluoride would not diffuse into the plaque from such a tightly bound form as fluoroapatite, it is conceivable that, when acid is produced after carbohydrate ingestion, some of the outer crystals of the enamel may dissolve and so release some fluoride into solution. If this occurred the bacteria of the inner layer of plaque (presumably those most concerned

with caries) would be exposed to fluoride under the acid conditions which increase their sensitivity to it. As the plaque pH rises with the using up of the carbohydrate and the neutralization of the acid by saliva some of this fluoride might recombine with the enamel or be replaced by fresh fluoride from drinking water or even from the low concentrations (below 0·25 p.p.m.) in saliva. If this release occurs at all, such a replacement must alternate with uptake because the concentration of surface fluoride of enamel tends to rise with age (Brudevold *et al.* 1956).

Other possible fluoride actions of importance in caries

Two other suggestions have recently been made of fluoride action which may be relevant to its role in caries. Paynter & Grainger (1956) found that the teeth of young rats from mothers receiving 12 p.p.m. of sodium fluoride in their drinking water were significantly smaller than in controls, but that there were no differences between the depths and angles of the fissures. Kruger (1962), on the other hand, has stated that the injection of approximately 100 μg fluoride into rats aged 7–10 days resulted in the mesial fissures of the first mandibular molars being wider and shallower. Both these results, although not consistent with each other, could be related to caries-resistance and it is desirable that observations on the relation between size, morphological form and fluoride intake be made in man.

Little (1962) has suggested, from the appearance of electron micrographs, that the matrix of the prism cores of caries-resistant enamel (including that made caries-resistant as a result of high fluoride intake) is less readily disrupted in Brain's solution (formic acid saturated with calcium phosphate) than that of caries-prone enamel. As an independent check of the concept that fluoride raises the caries-resistance of enamel by altering the solubility of the enamel protein I have in progress an investigation of the substances dissolved by various solvents from powdered enamel from teeth differing in caries-resistance and fluoride exposure.

Is fluorine a dietary essential?

Two further questions may be of nutritional interest. Is fluorine an essential constituent of the diet and can dental caries be regarded as a fluorine deficiency disease?

Attempts to find the answer to the first question by the use of fluorine-free diets have been hampered by the ubiquity of fluorine in foods and the virtual impossibility of removing it without damaging the diet in other ways. The early work, carried out in the 1930s, will not be described because neither the fluoride concentration of the diet nor that of the animals' tissues could be accurately estimated by the methods then available (for references see Muhler, 1960). Two recent attempts have been made by this method (Muhler, 1954, 1960; Maurer & Day, 1957). Rats receiving the most highly purified diet (containing 0·1 p.p.m.) in Muhler's experiments did show reduced fertility and difficulty in rearing their young, but as fluoride supplements brought about little improvement this effect was produced by deficiencies other than of fluoride. Maurer & Day, giving diets too low in fluoride for direct estimation (but calculated from the fluoride content of the animals receiving them to contain not more

than 0·007 p.p.m.) also concluded that fluoride was not essential for the rat, as their animals appeared normal compared with controls receiving the same diet and a fluoride supplement. The teeth, though not examined microscopically, showed no caries or other gross defect.

In another approach, a diet made up from plant foods grown on rain-water solutions of specially purified salts, when fed to two rats resulted in their death from starvation after 48 and 70 days owing to total destruction of their teeth by caries (McClendon, 1944). This great susceptibility to caries of rats on this diet was confirmed in two larger experiments with the fluoride-free basal diet on thirty-one rats of which about half received fluoride supplements (McClendon & Gershon-Cohen, 1953, 1954). In one of the experiments, 84% of the molars of the rats on the fluoride-free diet were carious compared with only 6 and 25% of the molars of the groups on fluoride supplements. Growth, reproductive capacity and length of life were also impaired on this fluoride-free diet.

There is no agreed explanation for these contradictory results and more work is needed before the question of the essentiality of fluorine can be answered.

There is increasing evidence that the low caries incidence found in certain areas of the world is associated with a high fluoride concentration in the teeth, and it is reasonable to suppose that the high concentration of fluoride is at least one of the causes of the caries-resistance. This has been found in analyses of teeth from the inhabitants of India, Iran and the Canadian arctic (Shaw, Gupta & Meyer, 1956; Shaw, Resnick & Sweeney, 1959), of Malaya, India and Pakistan (McCombie, 1959) and of Greece (Hadjimarkos & Bonhorst, 1962). The reason for the high fluoride concentration is not known. It may be that the diets of these areas contain foods rich in fluoride, and sea salt has been suggested as one possible source (Shaw *et al.* 1956). Alternatively, it is possible that other constituents of the diet may be influencing fluoride utilization. A low-calcium diet, known to favour fluoride absorption from the gastro-intestinal tract in animals, is suspected of enhancing its effect on mottled enamel in man (Massler & Schour, 1952). The low level of calcium in many diets in Asian countries could perhaps explain the high fluoride concentration of the teeth without necessarily involving fluoride sources absent from Western diets. It has also been reported that 0·1 p.p.m. of molybdenum in drinking water increases the effectiveness of fluoride in reducing caries (Adler, 1957) and molybdenum has been stated (although at the much higher concentration of 50 p.p.m.) to increase in the rat fluoride absorption from the intestine but not from the stomach (Crane, 1960).

This work gives ground for suggesting that a low fluoride concentration in the teeth of Europeans and Americans may be a factor in their high caries rates and thus supports the description of dental caries as a fluorine deficiency disease, although obviously many other additional factors influence it.

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