Incipient Enamel Caries.

A Clinical Study of the Effect of Stannous Fluoride on the Progress of Subsurface Lesions of Dental Enamel in Adolescents.

D.J. Bradley B.D.S.

A thesis embodying an original research programme submitted as partial requirement for admission to the degree of Master of Dental Surgery within the University of Sydney.

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## CONTENTS

<table>
<thead>
<tr>
<th>Part</th>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part I</td>
<td>Introduction.</td>
<td>1</td>
</tr>
<tr>
<td>Part II</td>
<td>Review of Literature.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A. The Incipient Caries Lesion.</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>B. Studies of the Histology of Subsurface Decalcification</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>C. Studies of Enamel Reactions and the Chemistry of Subsurface Decalcification</td>
<td>60</td>
</tr>
<tr>
<td>Part III</td>
<td>Survey Material, Design and Method.</td>
<td>117</td>
</tr>
<tr>
<td>Part IV</td>
<td>Results.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A. Epidemiological Aspects.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prevalence and distribution of subsurface lesions.</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>Distribution of the various shapes of subsurface lesions.</td>
<td>178</td>
</tr>
<tr>
<td></td>
<td>Proportion of pigmentation of subsurface lesions.</td>
<td>196</td>
</tr>
<tr>
<td></td>
<td>B. Qualitative Surface Changes of Incipient Caries Lesions.</td>
<td>203</td>
</tr>
<tr>
<td>Part V</td>
<td>Summary and Conclusions.</td>
<td>222</td>
</tr>
<tr>
<td></td>
<td>Bibliography.</td>
<td>235</td>
</tr>
<tr>
<td>Part VI</td>
<td>Appendix.</td>
<td>256</td>
</tr>
</tbody>
</table>
PART I
INTRODUCTION

"Superficially, the earliest visible signs of caries are best seen on the exposed labial and buccal tooth surfaces. The initial lesion is characterised by a change in the opacity of the enamel. Instead of a glossy structure, the affected area appears as a white spot, chalky and porous, which unlike developmental faults (mottling), tends to become more clearly seen when the tooth is thoroughly dried." This quotation from Sognnaes (131) helps to illustrate the clinical appearance of the incipient dental caries lesion which is the subject of this thesis, and introduces some of the factors influencing the review of literature together with the author's clinical findings.

Firstly, mention is made of the "earliest visible signs" of the lesion and it is known that when the lesion has reached this stage of progression, the outer surface layer of the enamel has disintegrated to a point where cavitation would have occurred. Nevertheless it is considered that the lesion can be recognised prior to this early stage of cavitation, i.e., when it has reached solely the white spot stage and the outer surface layer is retained as a hard glossy structure covering
the underlying region of whiteness, or as it is termed, region of subsurface decalcification. Others would disagree or be cautious of this diagnosis on the grounds that these white spots are indistinguishable from the opaque areas of developmental hypocalcification. However the author felt justified in making a clinical distinction between the areas of subsurface decalcification and the developmental opacities on clinical experience, since it was found that the former always appeared to be in definite characteristic patterns and that these lesions became more clearly visible and increased in size when dried whereas the developmental opacities did not increase in size and could be seen only at better advantage by virtue of moisture having been removed from the tooth surface. Clinical observations were made on 2,671 subsurface lesions on 2,430 buccal(labial)/lingual surfaces recorded in 228 Metropolitan High School students whose mean age was 14 years and 5 months. The size, type, condition and position, as well as any associations (restorative, carious or developmental) were noted and then, in order to follow the clinical progress of the lesions, the same group of students was seen again six months later and the above information recorded again for all the subsurface lesions present. All of these students were participating in a Topical Fluoride Study which was being undertaken by the Department of
Preventive Dentistry, University of Sydney. From such information the possible progression or regression of the decalcification process was studied.

Secondly, Sognnaes states that "the earliest visible signs of caries are best seen on the exposed labial and buccal tooth surfaces", hence the author's survey was made on the best observable surfaces, i.e., the exposed buccal/lingual surfaces. It has been pointed out by Davies (38 and 39) that if a reduction in the incidence of caries was to be shown during longitudinal study, it was more evident on the buccal/lingual surfaces and therefore these surfaces provided the best indications of caries resistance. He was also of the opinion that when a survey is carried out for an extended period of time, there is a marked promotion of a higher standard of oral hygiene than otherwise would be customary and this could be a reason why the correlations between oral hygiene and caries are so contradictory whereas the relationship between the oral hygiene and the caries of buccal/lingual surfaces is positive because these surfaces are the most readily cleaned. In addition there is much epidemiological data which is "consistent with the hypothesis that the resistance of teeth can be modified post-eruptively by exposure to saliva, food and water and that the most sensitive epidemiological index of
resistance is the prevalence or incidence of caries on smooth buccal and lingual surfaces" (38).

Therefore it is the aim of this thesis to review and provide an understanding of the histological and chemical changes which are taking place in the enamel structure to result in visible signs of caries attack, and to present the results of an original clinical survey which was designed to reveal the possible effects that current available preventive means might have in influencing the progress or regression of the incipient caries process.
PART II

REVIEW OF LITERATURE

A. THE INCIPIENT CARIES LESION.

Introduction.

The history of the incipient caries lesion being observed as a subsurface decalcification of the enamel most probably originated from Applebaum's (1) finding in 1932 on microradiographs that a well-calcified layer of enamel was present on the surface over the decalcified region. Thewlis (145) had also reported this apparent outer hypercalcified layer as early as 1934, yet this was still repudiated in 1935 by the work of Hollander and Saper (69) and others who considered this layer to be an optical artefact known as the "Mackie Effect". To enquire further into the nature of the white line which persisted on the surface of the incipient caries lesion, Applebaum forwarded several soft x-ray negatives to Thewlis in England in 1939, who confirmed that they showed that these lines were hypercalcified layers (2) (146).

Clinical observations were made in 1956 by Neill (99) on the white spot areas and he related the position and the prevalence of the white spots on the tooth surfaces directly to the chronology of the individual's caries experience. Moreover, he suggested
that white line formation along the whole gingival margin was a good indication of likely further caries and that this indicated a cautionary approach to the carrying out of extensive restorative work; it was more reassuring to find these white spots on the tooth surfaces remote from the gingival margin.

He reported that the white spot was only an important factor in the development of caries, especially in the young dentition, and that this factor influenced the position of carious lesions on the tooth surfaces. He overlooked the major importance that here with in-vivo subsurface decalcification was the very development of caries. This phenomenon had been observed to exist in vivo as far back as 1932 and even in 1956 it had still not been related to actual caries formation by all workers.
The incipient caries lesion.

For many years experimentation has been directed to the etiology and nature of the incipient dental caries lesion and two basic theories, the acidogenic and the proteolytic, have both been evolved, but in the past, research was leveled at caries with the concept that this disease was indeed a specific one. To review the literature on dental caries with the emphasis placed on its etiology would be an immense task and although there has been accelerated progress in caries research over the last ten years due to mechanical advances, none of the former theories fully satisfies the answer as to what can be the specific cause of dental caries.

It suffices to say even from recent investigations that dental caries is not a specific disease of the teeth but a non-specific process which is brought about by a multiplicity of factors which in themselves can differ from mouth to mouth and within a single mouth (123): however a great deal is known now with regard to the limits and extents to which these multiplicity of factors can interact to result in dental caries.

Until recently the classical description of the sequence of events in the mechanism of enamel caries,
whether it be an acidic or proteolytic attack, was a direct penetration of the attacking agent (with dissolution) along the direction of the enamel rods. The appearance of the early smooth surface lesion as seen in transverse or longitudinal section was basically cone-shaped where the attack was on a broad front and which then reduced in intensity towards the dentino-enamel junction by virtue of the fact that the caries action was less marked in the deeper enamel than in the superficial enamel; and that the body of the enamel was less irregular in its formation and calcification, and therefore less accessible to attack. The initial attack seemed to rely upon either defects in the formation or the maturation of the surface, or if the initial attack was considered to be solely acidic, then it occurred by direct dissolution of the immediate inorganic components.

Alternatively, if the attack was not of this nature, it commenced in retentive areas such as the pits and fissures where the attacking agent could not be removed easily and once access had occurred, it provided the appearance in longitudinal section of an inverted-cone shape in relation to its spread from the point of access in the pit or fissure. Thus
it was established that there were two "pictures" of incipient caries depending upon the site of attack, and the validity of this hypothesis is discussed later.

This summary of the older classical concepts of caries is no doubt too brief but the review is not intended to discuss the various theories of former years: its aim is to correlate the more recently published data on the initial caries lesion of the dental enamel and provide some insight into its formation as well as an understanding of what is taking place clinically in this process when the lesion is revealed as a macroscopic white spot.

The reason for including mention of former concepts is to point out, that in considering past experimentation, there does not appear on the whole to have been any marked differentiation between advanced attack and the more general delicate process of white spot formation. It seems that what the earlier workers were describing as initial lesions in their reports and findings were, in actuality, fairly advanced lesions in contrast to what is classified now as incipient caries.

It is obvious that if the attacking agent or agents is of a high enough concentration and/or
the enamel surface markedly defective, intense attack can ensue, in other words, prompt etching of the surface can be expected; and if, in vitro, suitable conditions and a high enough concentration of the attacking agent are maintained, total destruction of the tooth enamel does not require a lengthy period. It is fortunate that conditions for such prompt surface etching, further dissolution of the enamel components and subsequent cavity formation do not exist in vivo as routine. On the contrary, conditions are usually such that if attack is to occur, it is a more lengthy and slower process where the enamel subsurface is the first discernible structure to be attacked while the outermost layer remains relatively unaffected and thus the lesion is recognisable as a clinical opaque white spot. Such a clinical reaction of opaque white spot formation is not a controlled reaction in vivo but is extremely dependent upon the individual caries susceptibility, however, in order to stress the duration of caries progress that can exist, Parfitt (106) in 1956 observed that the development of a clinical cavity from the earliest recognisable stage required an average time of over twelve months and likewise, Boyd, Wessels and Leighton (14) reported that it took approximately twenty months
for occlusal cavities in second permanent molar teeth to progress from initial lesions to definite enamel involvement and another three years for their involvement in the dentine to be physically demonstrable by the examiner's probe. Although these periods for cavity formation are not conclusive, it was shown from this and other studies that little relationship existed between the actual rate at which the clinical lesion became apparent and the rate of subsequent advance of the lesion.

Again Parfitt (106) found that between 20 per cent and 53 per cent of occlusal cavities were more than two years in the stage which was diagnosed as incipient caries, so that if lesions of the buccal/lingual surfaces are the best indicators of the caries resistance of a tooth, clinical cavity formation with definite dentine involvement should take at least two years for these surfaces.

The length of time for clinical incipient caries lesions to progress out of the incipient stage is perhaps debatable and whatever the outcome, it is reasonable to assert that the general picture of a caries lesion as seen by the dental practitioner is not too rapid a process; with the reservation that the caries activity of the individual is determined by the individual's phases of susceptibility.
Thus determination of clinical incipient caries has been up to date an estimation by the individual examiner, aided by sharp exploring tines, mouth mirrors and adequate illumination. Routine histological studies can reveal that before any breach of the enamel surface has taken place, microscopic changes are already advanced and these in turn are preceded by chemical changes which are not demonstrated by such routine histological studies; therefore what is seen as a clinical incipient cavity has already passed through the stage of initial caries and therefore what is seen and known to be an area of subsurface demineralisation of the enamel must be an intermediate stage of incipient caries and surely an indicator of a possible incipient clinical cavity. Like any other epidemiological study, intra-oral determination of caries or for that matter, an area of subsurface demineralisation, is based upon the examiner's clinical criteria and experience.

To support the interpretation of the clinical signs as an indication of actual progress of the lesion, reference is made to Darling's work in 1959 (33) where he has shown that the histopathology of the caries process has revealed its involvement already in the dentine structure while to the naked eye it was only
discernible as a white spot or on radiographs, as an initial radiolucency of solely the enamel layer. This belief was still upheld by Darling in 1965 (37) and this is shown schematically in Fig. 1.

![Histopathology Diagram]

**Fig. 1** Representation of the stages of enamel caries with special reference to stages 4a and 4b, which show that although decalcification areas are obvious here to the naked eye, it is questionable whether this lesion could be seen on bitewing radiographs before histopathological changes could be observed in the dentine (33).

In considering the formation of these white spot areas, the author has concentrated for the most part, on the assumption that carious attack on the enamel layer is a process of acid dissolution involving the mineral component. In 1890 Miller proposed his theory of the causation of caries and, today, it still can be accepted, though with some modifications; attack on enamel can still be regarded as the end result of...
bacterial fermentation of refined carbohydrates (40). However, the biggest change that has been made in these views since Miller's theory, is that greater emphasis has now been placed on the enamel structure and chemistry.

To date it has been said that bacteria have not been shown to be capable of attacking the organic components of intact enamel - "decalcification must occur first" (123). Strictly speaking, decalcification is a term applied to the mineralised hard tissue and in consideration of the recent knowledge that enamel possesses two organic components, which by virtue of their relative difference of solubility in acid (142), are classified as soluble and insoluble fractions, it is probably more correct to express concisely the acidogenic theory of attack (when applied to white spot lesions) as a dissolution of the internal enamel as a result of attack on the accessible soluble components.*

* This indefinite expression which defines neither the reagent nor the possible soluble component does allow reference to be made to the proteolysis-chelation theory of dental caries, especially in regard to the available reagent. In the case of this attack, the primary reaction involves the organic matter where proteolytic bacteria produce amino-acids as a product of enzymatic hydrolysis. Some of these amino-acids are said to behave as chelators or complexing agents and remove the calcium (i.e., cations) from the enamel, so that there is more or less simultaneous destruction of mineral apatite accompanying breakdown of the organic matter (117).
It is now well established that the outer surface enamel possesses properties which make it normally more resistant to caries attack than the adjacent subsurface region (23) so that the characteristic pattern of attack as seen by the microscope is an apparent loss of substance from the subsurface enamel structure rather than from the outermost layer which is contiguous to the source of attack (31), (32), (34), (35), (71), (85), (136). Newbrun, Brudevold and Mermagen (102) make the reservation that, when they compared the intact enamel to initial caries enamel by means of densitometric tracings of their radiographs, they found there was even slight demineralisation of the surface enamel, and this was also confirmed in the same year (1959) by Soni and Brudevold (136). This qualifies the fact that the outer surface is regarded as "relatively intact" in contrast to the subsurface; (see Fig. 2) and does not mean that it

Both Bodecker (13) and Schatz and Martin (117) present evidence to say that the organic matter is attacked by certain micro-organisms and enzymes without preliminary decalcification but it is reasonable to presuppose that the chelators are produced not from the organic matrix but rather from the food and/or salivary components (72); therefore chelation would be equivalent to acidic attack from without and conform to the acidogenic expression which is stated above.

In addition it does not seem feasible to expect the large complexions of chelation to be able to diffuse out easily from the enamel structure as well as expect free calcium ions to be available (without prior decalcification of the enamel) once they are bound as chelates, and it would seem that the chelation process...

/Contd.
has remained entirely unaffected during attack.

![Graph](image)

Fig. 2 Densitometric tracing together with plotted points for polarised light intrinsic retardation values taken for the same initial carious enamel lesion in the same region. It portrays a relative decrease of mineral substance in the outer layer and a more marked removal of mineral from the underlying enamel (153).

would be a protective reaction in preference to a destructive one.

Brudevold (20) and Jenkins and Dawes (73) concluded that if the proteolysis-chelation process were to occur as originally professed, it would only be a minor adjunct to the main cause of the tissue destruction: after all, there is no experimental evidence to support the view that enamel protein is a source of chelators in caries and although some chelating agents in plaque tissue do exist, it is unlikely that their concentrations are high enough for much decalcification nor their action quantitatively comparable with that of acids. "The evidence for acids is considerable, in spite of uncertainties about the pH on the inner side of the plaque or about the critical pH necessary for decalcification in that area (73)."
For example, Coolidge and Jacobs (26) have calculated some carbonate loss from this outer surface so that lack of evidence of any alteration of this region under microscopical studies does not necessarily preclude chemical change in the fine structures. It is without doubt that the overall picture of initial caries attack on the enamel surface, whether it be naturally or artificially produced, is that the subsurface region is destroyed to a far greater extent than the outer surface; and that a similar microscopic pattern to that found in caries can be duplicated by exposing normal enamel in vitro to agents which will dissolve the mineral component (31)(102)(110)(130)(136)(137). Furthermore, dissolution of this relatively sound outer layer would allow diffusion of acid and organic material within the enamel and would alter the permeability of the surface layer.

The production of subsurface lesions in vitro is related to not only upon the pH range but to the concentration of the attacking acid. Newbrun et al (102) pointed out that subsurface lesions have been produced by exposing teeth to 0.075 per cent lactic acid, 0.5M lactate ranging in pH from 4.1 to 5.3 and to calcium phosphate buffers in the pH range of 4.0 to 5.0 (31)(137). In their experiments they used
1.0M lactate buffers in a pH range of 3.7 to 5.1 as well as a 0.01M saliva-glucose solution and the results were as would be expected: that subsurface lesions were produced consistently with incubation in the 0.01M saliva-glucose solution and only with short exposures in the case of the 1.0M lactate buffers; with longer exposure (greater than one hour) in the latter solution, etching and cavitation resulted from the strong decalcifying agent quickly overcoming the resistance of the outer surface.

Gray and Francis (56) have demonstrated that the pattern of in-vitro subsurface decalcification is an acidic reaction with resultant chemical dissolution, and presented this mechanism of white spot formation as a physico-chemical diffusion process. Wallace and Coolidge (150) examined early lesions in 436 permanent teeth and 36 deciduous teeth to conclude that the microscopic changes observed in the white spot, smooth surface lesions could be explained in physical and chemical terms.

On the other hand, the mechanism which takes place in vivo is a biochemical one in the sense that the chemical reactions in the carious enamel occur under environmental conditions which are determined by biological factors, themselves, governing the development
and in-situ existence of the enamel: what is tenable in vitro may not be entirely so in vivo.

It should be remembered that many other complexing agents have the capability of dissolving the inorganic portion of enamel but they are not available in an adequate concentration when their limited sources such as the organic component of enamel, the bacteria or the organic constituents of plaque are considered. It appears that the common and adequately available agent for enamel attack both in vivo and in vitro which can produce the same end-product of subsurface decalcification is the hydrogen ion, which Gray and Francis (56) have described as "an extremely strong complexing agent for phosphate and hydroxyl groups."
PART II

REVIEW OF LITERATURE

B. STUDIES OF THE HISTOLOGY OF SUBSURFACE DECALCIFICATION

Subsurface decalcification occurs not only upon the smooth surfaces of the teeth but also within the pit and fissure areas. Mortimer (96) in 1964 examined histologically the pattern of fissure caries and found that the variation of the fissures did not affect the incidence or what is more important, the actual pattern of caries. In both deep and shallow fissures the site of initial attack was on the walls of the fissures or if there were constrictions near the base of the fissure, the sites were occlusal to the constriction (Figs. 3 and 4). In only one case was there apparent initial attack at the base of the fissure, but on further examination it was perceived that this was brought about by the merger of two attacks on the walls of the fissure close to the base (Fig. 5). Thus the independent occurrence of these areas of subsurface decalcification on each wall and their coalescence at the base of the fissure would account for the inverted-cone shape lesion which was examined by previous workers and suggests that those lesions, so described, were not indeed as incipient as thought. In addition, it is of interest to note that prior to this description by Mortimer (96), the fact that there was a pattern of
multiple attacks occurring on the walls along the length of the fissures (when examined in horizontal section) had not been described.

Fig. 3 Microradiograph of "deep fissure" caries, showing bilateral lesions and point of entry crossing the intact surface zone. L.S. x 40 (96).

Fig. 4 Microradiograph of "shallow groove" caries, showing distinct bilateral lesions. The surface zone is intact and the attack is involving the prism structure and the striae of Retzius. L.S. x 40 (96).
Francis (46) used both silver nitrate and alizarin red S staining methods to reveal the pattern of caries development in rats on a cariogenic diet and found that it was very similar to that in humans. He concluded that "lesions developing on the walls of the deep fissures of rats' teeth were compared and likened to approximal lesions and were identical in development to deep fissure lesions in human teeth".

Fig. 5 Ground section mounted in Canada balsam and viewed by ordinary transmitted light. The zones of enamel caries can be clearly seen - (A) Translucent zone, (B) Dark zone, and (C) Body of lesion. L.S. x 50 (96).

Johansen (74) also compared pit and fissure and smooth surface lesions in low power microradiographs and noted that they were both depicted as subsurface radiolucent areas.

Nagano (98), in relating the form of the pit and fissure to the position of the initial lesion, first classified four types of pit and fissure morphologically.
These types and findings were simply:

(a) the V-type pit and fissure where the occlusal entrance was wide enough by the broad divergence of the walls occlusally to allow the base of the fissure to be seen and where the caries was observed to begin;
(b) the U-type, where the walls were more or less parallel but not approximating, and here the caries began on the walls half-way down the depth of the fissure;
(c) the I-type, where the walls were virtually in contact forming a slit in the enamel and,
(d) the IK-type, which appeared the same as the I-type except for an apparent space at the base of the fissure where the walls did not contact. In both the latter types the caries began on the walls at the occlusal entrance to these slits.

This meant that the shallower the pit or fissure, or the more the walls diverged occlusally from the base, the closer to the base was the initial lesion.

When this phenomenon is interpreted in relation to smooth surface lesions it becomes apparent that if a pit or fissure is shallow, the area resembles a smooth surface and allows full access for an attacking agent, while in the deeper types the access is poor and thus the lesion occurs closer to the occlusal surface.

This interpretation was confirmed, as were Nagano's findings, by König (79) who also pointed out that there was an "ideal" angulation of the walls of a groove which permitted the most retention of debris and plaque. Where the angle at the base was approximately 90° to 70°, the susceptibility to caries was lower than when the angle was less than 70°: however, if the
fissure was of the I-type, being very deep and narrow, the lack of ample space for retention of plaque and substrates provided a degree of protection to attack. Unfortunately, the variation in morphology along the length of the individual fissure would appear to preclude this protective property from being important in the general application of preventive dentistry, especially with regard to lack of extension of restorations in fissure areas. Broadhurst (16) was able to show, after observing 1,835 occlusal restorations of first and second permanent molars, that "ninety percent of the overall failures occurred solely at the fissures at the extremity of the restoration".

Alternatively, it could be argued that the self-cleansing property of shallow grooves is to be expected on the whole to be a major factor in a higher caries activity occurring in narrow or deep fissures rather in the shallow types, even though accessibility for attack is greater. Nevertheless the prevalence of pit and fissure caries is not under discussion but the above findings illustrate that, although the shallow pit and fissure allows the lesion to occur and be seen at its base, the other types of pit and fissure are attacked by the same process, in the same way, except that their lesions will occur
on the wall of the pit or fissure at a position which
is in relation to the occlusal access to that pit or
fissure. Comparisons of the microscopic patterns
of initial lesions which are distributed over the
entire enamel surfaces of any teeth, i.e., patterns
of smooth surface lesions to such patterns as seen
in the above pit and fissure studies, it is established
that a paradox does exist: that the carious process
occurs as the result of an identical physico-chemical
reaction. Wallace and Coolidge (150) concluded that
the features seen in the vast majority of 500 examples
of early enamel caries on the occlusal and smooth
surfaces, were identical with those of white spot
caries, "modified in some cases in respect to shape
by variations in the geometry of the enamel surface
and in some cases modified in colour by impregnation
with pigment". Therefore there does not appear to
be any physico-chemical reason for differentiating
pit and fissure and smooth surface caries.

Numerous microscopic studies have been carried
out on enamel using such methods as transmitted light,
polarised light, dark field illumination, microangiography
and microhardness tests to help interpret the relationship
between the enamel structure and the destructive
process, and in this section of the review an attempt
has been made to give a concise and graphic description
of the morphology of the initial carious lesion which is produced in vivo and looked at in vitro.

It is apparent that the most distinctive microstructural feature of dental caries is the deep demineralisation that can take place in the enamel subsurface; in other words, detectable alterations are occurring here in advance of those in the outer surface region.

It was concluded in 1956 by Darling (31) that the striae of Retzius were possible points of entry for decalcifying agents: that caries progressed intermittently with the stops or pauses occurring at each striae and this was portrayed in the characteristic outlines of both longitudinal and transverse sections of the lesion, the former having a "saw-tooth" outline and the latter having a somewhat triangular shape, with the base at the outer surface and the apex towards the dentino-enamel junction (Fig.6).

Fig.6 Diagram to show how the spreading of an initial lesion along the striae of Retzius - A, could be consistent with the apparent cessation of the lesion at each striae as seen in a transverse section - B (31).
A microradiographic study by Guzman, Brudevold and Mermagen (63) also showed that the demineralisation seemed to progress in two directional pathways, (1) inwards, following the enamel rods, but affecting the interprismatic substance to a greater extent than the rods themselves and (2) laterally, along the striae of Retzius, affecting preferentially the enamel which is adjacent to the outermost edges of the striae.

Such experiments can show that histological markings become involved in the demineralisation and that at a later stage during proteolysis the demineralisation is partly via these histological pathways. Others (129)(134) may show such a demineralisation extending 100 to 1,000 microns beneath the tooth surface which remains relatively intact. It must be understood, however, that attack does not necessarily initiate at or to this depth in the enamel and Sognnaes (129) has correlated the changes in microradiographic density of the gradients of demineralisation with previous observations on histochemistry by Sognnaes and Wislocki in 1950 (134) to show only the depth at which demineralisation below the intact surface can be observed. When the outer surface zone is considered to be at a maximum of approximately 45 microns in width (29), initial
observed demineralisation is possible anywhere beyond this depth up to 1,000 microns deep.

This point is made, not to illustrate the concept of a direct diffusion with demineralisation to this depth, but to reveal the characteristics of this type of hard tissue destruction in contrast to such conditions as erosion of teeth, resorption of bone, traumatic abrasion of teeth and even saprophytic post-mortem action on bone and dentine (131)(132); where, for example, the demineralisation gradient in erosion is at a maximum of up to 100 microns and virtually nil in resorption (less than 1 micron); that caries differs from these conditions where the preformed structural pathways do not appear to be significantly involved with the pathogenesis of the lesion. "Caries is unique in that chemicals capable of removing minerals penetrate to great depth, notwithstanding the relatively higher density of dental hard tissues." (131).

In respect to the subsurface demineralising effect of caries, it is interesting to note that there was a resultant softening of the tooth substance which was reflected in the values of the range of Knoop hardness numbers. These were recorded (129) as an average of 317 for the intact enamel at the surface and only as an average of 12 for the subsurface
region. The central and inner zones of the body of the enamel extending beyond the subsurface decalcified area and terminating at the dentino-
enamel junction, on the other hand, maintained a higher mineral content by recording Knoop numbers of 190 and 139 respectively.

The importance of such microhardness tests is further evident in considering the variation of microdensity of the actual subsurface lesion, where relatively high density zones are occasionally seen in the middle of the demineralised regions and are often attributed to a possible redeposition of dissolved minerals escaping from the deeper layers of demineralisation. These high density zones exhibit less microhardness than intact enamel, which, along with quantitative measurements of densitometric tracings of radiographs and intrinsic retardation with polarised light (136) all show that these zones, although relatively high in calcification, are not calcified to a greater extent than normal enamel.

In 1957 Gustafson (60) made thorough investigations on early enamel caries, visible at the "chalky spot" stage, and described and suggested the division of the subsurface lesion into five zones (Fig.7) which progressively from the dentino-enamel junction towards the outer surface area were: (1) a narrow translucent zone next to the yet unaffected
enamel, representing a region of increased mineralisation, (2) a larger dark zone where there was distinct solution of the minerals, (3) another narrow and intermediate zone of recalcification which separated the dark zone from (4) the body of the lesion or the zone of decalcification, and finally (5) the zone of complete destruction.

Fig. 7 Diagram to show the positions of the five zones of an early carious lesion as described by Gustafson (60).
1. The narrow translucent zone.
2. The larger dark zone.
3. The narrow and intermediate zone of recalcification.
4. The zone of decalcification or the body of the lesion.
5. The zone of complete destruction.
Mez1 (95) discussed the variations in enamel decalcification of incipient lesions by defining four zones when he examined sections to observe the patterns of the penetration of toluidin blue and silver nitrate solutions into these sections. Darling (32) merely described a surface layer, and regarded the rest of the caries zone as composed of a gradually demineralised structure without definite zones.

Looking further back in years, it has been recorded that three zones were described in the lesion by Hopewell-Smith (70) in 1918 and again, six zones by Nishimura (104) in 1926. The reasons for the various zones of initial caries and indeed their actual existence are not completely decisive but there is perhaps the advantage of dividing a lesion into zones or areas wherein particular changes can be localised and more closely studied. For the purpose of description the author has used Gustafson's terminology and this should not be interpreted as accepting such zoning as an entirely definite and consistent phenomenon (Figs. 8 and 9).

Gustafson has already stated (60) that "all lesions do not develop in the same way and at the same speed" and thus the translucent zone, which has shown an increased negative birefringence over that of normal
Fig. 8 Ground section of early caries embedded in Canada-balsam and viewed by polarised light. The lesion is surrounded by a blue line which is the translucent zone - 1. The dark zone - 2, is shown as a red area, the centre of which is yellow revealing more isotropy and hence further demineralisation. The body of the lesion - 4, has a strong blue colour, the intensity of which diminishes gradually and gives the impression of being a heavily mineralised area. The second zone ends just under the surface which is also blue in colour. (60).

enamel, is not always present around all carious lesions: in point of fact, his division into zones is based upon a fully developed lesion and does not indicate this zone to be necessarily present or obvious in an initiating lesion. Inconsistency in the presence of the more-highly calcified zone of recalcification was questioned by various workers because Gustafson's investigations were carried out without microradiography. Therefore the
Fig. 9 This is same section as in Fig. 8 but has been embedded in alcohol medium. The body of the lesion - 4, is now seen as a yellow or red area except for that part near the dark zone - 2; this part has remained blue and represents the zone of calcification - 3. (60).

problem was then re-examined by Gustafson and Gustafson (61) where it was found that this zone of calcification did not exist in all lesions and was something of an exception. As was pointed out in 1957 (60): "the carious lesion does not, of course, develop at the same rate in all teeth. Where a carious lesion develops very rapidly, the different zones are not formed in the same way just described. Sudden cavitation can take place without visible remineralisation in zones three and one and without demineralisation of the surface."
Crabb and Mortimer (30) have also found more recently (1966) that even the positively birefringent dark zone is not always present along the advancing front of the lesion: this dark zone is usually apparent in the outer third of the enamel in peripheral sections of advanced lesions but in sections cut close to the centre of the lesion, there is often absence of the dark zone. This absence is more usual in the inner two thirds of the enamel as the lesion approaches the dentino-enamel junction, however, in more incipient lesions of the outer third of the enamel, it has still been shown (28)(30) that the positive dark zone is often missing along the deepest part of the advancing front.

Nevertheless by the assessment of intrinsic birefringence and microradiography it can be shown that the earliest evidence of decalcification is found in the dark zone and there is increasing decalcification towards the centre of the lesion. No evidence of decalcification has been found in the translucent zone (7)(35)(36)(60)(61).

When an area appears as a translucent zone in transmitted light but not polarised light, there is also no indication of decalcification, however decalcification becomes evident when the area darkens (Figs. 10 and 11). If similar lesions are examined by polarised light in various imbibition media, the earliest manifestation of
damage to the enamel is the formation of 1 per cent of what is called "spaces" (32)(33) in the enamel. The "spaces" are distributed chiefly in the prism interfaces and cross-striations and are found running through the otherwise intact surface along the striae of Retzius and if the same lesion is viewed by transmitted light in balsam it appears as a wholly translucent area, i.e., a dark spot is not yet visible in transmitted light (Figs. 11 and 12).

Darling (32) found that the earliest lesions containing 1 per cent to 5 per cent of "spaces" (calculated from observation with polarised light) did not show decalcification on microradiographs. Either microradiography was not sensitive enough or the first "spaces" were produced by another process than decalcification. Loss of the relatively soluble fraction of the enamel protein was suggested and this has been criticised (131) on the grounds that this conclusion is based upon changes in birefringence alone and that removal of the entire organic content of enamel would itself only lead to an approximate loss of 2 per cent by volume; so that the changes seen by polarised light around the 5 per cent level of "spaces" detect a mineral loss associated with a possible loss of organic material and which would not be great enough at this initial stage to be demonstrated by microradiography.
Fig. 10 Ground section of a very early lesion of enamel caries in quinoline seen by transmitted light, showing dark zone which has now contracted to a continuous area surrounded by a translucent zone (34).

Fig. 11 Very early lesion of enamel caries seen by transmitted light in quinoline, showing a translucent zone only (34).
Fig. 12 Ground section of early lesion of enamel caries seen by polarised light in air. This is the same section as that seen in Fig. 4 (34).

Obviously both Gustafson and Darling have placed importance on the appearance of "spaces" in the progress of the lesion and it is fitting that their meaning of these should be defined and understood more clearly.

Gustafson (58)(60)(61) considered that enamel at the submicroscopic level was composed of crystals embedded in organic material which consisted mainly of fibrils and between these were the "spaces" into which the crystals were deposited during mineralisation. To obtain enamel of varying degrees of mineralisation, these "spaces" were either filled with crystals or they were not, and if so, they were filled with other substances which he did not describe and stated as unknown. The
"spaces" were thought to have the same shape as the crystals (i.e., they were longitudinal with their long axes almost parallel to that of the prism).

Darling further suggested that the intact enamel contained 0.1 per cent of "spaces" and these he believed to be due to the normal structural markings (131) which themselves are of an organic nature and this belief would conform to those considerations of Gustafson, explained above, of "spaces" in the organic material. He further stated (33) that the amount of "spaces" produced by the destructive process of the lesion could be assessed by the use of various mounting media of different refractive indices in polarised light examinations and that these "spaces" corresponded fairly accurately to the degree of decalcification which could be shown, with the exception that the value of "spaces" was always slightly greater than the degree of decalcification. In the case of the translucent zone of caries there was no demonstrable decalcification yet calculations revealed 1 per cent of "spaces" all of which were large enough to admit the large molecules of mounting media. He asserted that they did not appear to grow by enlargement of those "spaces" found in the adjacent normal enamel but rather replaced them abruptly (35). However
Sognnaes's criticism (131) must be remembered; that such a statement is based entirely on changes in birefringence in polarised light.

More recent examinations of the enamel structure have revealed the existence of a pore system (18)(111) in contrast to the previous concept of "spaces" i.e., "submicroscopic rods separated by pores or channels which are freely penetrated by gases and by both organic liquids and aqueous solutions of inorganic salts" (111), so that in mature enamel this system is relatively limited both in total volume and accessibility to all kinds of liquids. Alternatively, other pores of much larger sizes also exist undoubtedly in the enamel, the entire system being reasonably extensive yet almost impermeable. On the whole this infers that the "spaces" are a consequence of the inorganic content and determined by the distribution of the crystallites rather than by the organic matrix. The apparent behaviour of the pore system under the microscope could result from many pores, all with similar narrow diameters or a smaller number of larger pores connected by very narrow channels. This latter arrangement would then be more in accordance to the earliest stages of enamel breakdown by caries or lactic acid decalcification where Darling (35)(36) envisages a sudden obliteration of the minute "spaces" by the appearance of large pores to the extent of
1 per cent by volume in that region of the lesion. The reason for this arrangement is still not known. This condition of a 1 per cent of "spaces" could be duplicated (33) by in-vitro treatment of normal enamel with an organic solvent (cold ethylenediamine), which presumably removes the relatively less-calcified soluble matrix of the enamel; but surely it should not be interpreted as a 1 per cent loss of volume of enamel substance but simply a 1 per cent alteration in birefringence value.

The enamel may show a positive or negative birefringence depending upon the extent of the pore system. The intrinsic birefringence of the apatite is always negative in opposition to a positive form birefringence produced by the pores of differing refractive index. To date, the positive intrinsic birefringence of the organic fraction of the enamel has been disregarded because of the very low positive values produced; however, by using electron microscopy Frank et al (52) have revealed that not only are the apatite crystals dissolved at the same time in the prism as in the interprismatic substance, but also the progressive broadening of the intercrystalline spaces are filled by an amorphous organic material and it appears that this amorphous material is different
in ultrastructure from the normal organic matrix (50). It is of interest to add at this point that the former findings of Frank et al (52) and Frank and Brendel (50) confirmed Gustafson's investigations (61) where it was found that during decalcification some prisms had their centres unaffected, with demineralisation at their peripheries, while others revealed greater mineralisation of the peripheries than in the centre. Thus it appears that either action can occur in decalcification.

The investigations of Frank et al (52) and Gustafson and Gustafson (61) were carried out on small carious cavities and with regard to the intercrystalline spaces, it is unfortunate that it is not known what percentage of volume they form before they do contain organic matter, however there seems no doubt that this filling process does occur eventually and at what level is a problem for future research. Darling has maintained (36) that the more negative birefringence above normal enamel shown in this zone of translucence "is consistent in amount with its being caused by the elimination of the minute spaces of normal enamel along with their positive form birefringence". Perhaps it can only be surmised that should the 1 per cent level of "spaces" become filled with amorphous organic material (at this initial stage
of caries attack), the small increase of positive intrinsic birefringence produced would not be detected while at the same time, the opposing positive form birefringence could have been reduced by occupation of the "spaces" with the organic material and hence imbibition with mounting media prevented.

The solution to what happens at this low level of enamel alteration is the answer to the enigma of caries and remains unsolved, but after incipient attack other available and environmental factors can operate so that if the lesion is to progress, it finally becomes clinically visible when the enamel shows a 5 - 25 per cent (or more) by volume of structural "spaces". There is a change in the enamel opacity and the subsurface decalcification is clearly evident, the relatively intact surface layer only portraying a 2 per cent alteration (131). Beyond this point of destruction it is obvious that while continual attack occurs, the zone of decalcification progresses to complete dissolution of the minerals and complete destruction of the organic matrix. The hardness value of the region decreases to almost zero and finally cavitation takes place usually when the lesion has reached or nears the dentino-enamel junction. The final decomposition would make this region appear dark in all types of light examinations and show
a true isotropy from the lack of birefringent substances.

It can be readily seen that with the intermittent character of the process of attack, demineralisation will lead ultimately to cavity formation and while it does so, it alternates with possible remineralisation. Indications that remineralisation can take place within the carious lesion depend on the fact that types of crystallites not found in normal enamel are found in the lesion (129); and that these crystallites are only found in a process of remineralisation. Mézié (95) had divided demineralisation and remineralisation of carious lesions as seen by the electron microscope and he had observed in reflected light and using staining techniques that in certain areas of some lesions it was difficult to say whether a normally-appearing area was not indeed an area consisting of stage-one demineralisation followed immediately by stage-one remineralisation. Such reasoning would not be completely in the sphere of reality without co-ordinating other methods of studying the same sections of enamel and therefore, for example, could apply to any enamel surface which appeared clinically sound in an oral cavity wherein caries had occurred: these sound surfaces could be said to possess a natural or acquired caries resistance as compared to those
that had been affected or they had been simply not attacked by caries at all.

Microscopic study alone cannot show the origin of the mineral salts for remineralisation and it is possible that some of the local remineralisation results from shifting of calcium salts from other areas of the carious lesion and/or the availability of ions for recrystallisation from the oral fluids by diffusion. Schmidt-Nielson (118) has demonstrated beyond doubt that slightly decalcified surface enamel can remineralise and it is only natural to expect this occurrence when attack is taking place in a fluid saturated with calcium and phosphate ions such as saliva.

All that is known to date by microscopy is that, during caries, there is a change in the crystallite composition and shape (75)(76) and it could, alternatively, be the result of a selective demineralisation based on the assumption of regional differences in the chemical composition of the sound structure. Johansen (75)(76) combined determinations of chemical composition of his samples with the observations seen by electron microscopy and felt that the lack of change in the Ca/P ratio of carious enamel was consistent with the concept of a recrystallisation of apatite, even though he admitted an intermediate product of dicalcium phosphate
(CaHPO₄) could be involved.

It is worthy to note here that chemical studies of Brudevold, McCann and Grøn (23) confirmed the concept of apatite recrystallisation. They have shown with solubility studies in saliva that, over a considerable range of pH values, solutions of calcium and phosphate are induced uniquely by the presence of fluoride ion to precipitate out as hydroxyapatite in preference to octacalcium phosphate (Ca₈H₂(PO₄)₆·5H₂O) or to whitlockite (β-Ca₃(PO₄)₂). The importance of the role of fluoride in caries prevention is illustrated here by its ability to preserve the apatite structure in solutions of calcium and phosphate at low pH values. It appears that the solubilities of both hydroxyapatite and fluorapatite are controlled by the same activity product of dicalcium phosphate (CaHPO₄), which means that the presence of small amounts of fluoride will have the effect of favouring apatite reprecipitation.

Although Frank et al (51) make no mention of crystals of remineralisation in their studies on enamel caries, they were able to show inorganic recrystallisation within the lumina of the dentinal tubules. "In the early stages of dentine caries rhombohedral crystals of β-Ca₃(PO₄)₂ - whitlockite may be seen in sclerosed tubules, sometimes at the boundary of the peritubular zone and the occluded
tubule, and sometimes within the tubule (51)"; so that their presence in enamel as seen by the electron microscope might eventuate in further study.

Gustafson (60) considered earlier (1957) that the translucent zone contained a greater amount of minerals than did the enamel before caries attack and the only explanation given was that the minerals were first dissolved out in the lesion and then precipitated in the surrounding enamel, and he also believed that dissolved minerals were reprecipitated, (the particular form is not stated) in an intermediate zone of recalcification, as a result of the action of some of the components from the disintegrated organic material from the main body of the lesion. On the other hand, Bergman et al (7) were unable to demonstrate microradiographically any difference between the translucent zone and the adjacent normal enamel in respect to the mineral salt content.

Nevertheless, the previous discussion of this section has been concerned with the progression of the lesion by means of dissolution and although recent observations have been unable to determine the very nature of the reprecipitated crystals, the point is made that both processes of demineralisation and
remineralisation have always been considered as phenomena of the progress of the lesion.

At this stage brief reference is made to the expression of "points of entry" for two reasons: firstly, that this expression is repeatedly used in the literature in association with the initiation of caries and secondly, that "points of entry" are possibly involved in the subsurface decalcification process, either concomitantly or afterwards.

Awazawa (4) investigated the different stages of initial caries at the enamel surface with the replica technique and electron microscopy and it was seen that the caries begins at many little spots, which gradually extend upon the incipient demineralisation. These spots portray multiple "points of entry" for the demineralising action at the submicroscopic level but do not necessarily determine the level of incipiency of attack. It is correct to assume that attack could commence at one point on the surface and increase laterally, or that it would be of a homogenous broad nature increasing in intensity; yet it is apparent that it is neither and is partly the result of its being confronted by a variable layer, the variability being established essentially by the shape and direction of the enamel prisms (15)(59)(93)(121).
Basically, the surface may be more or less smooth in different teeth and also in different places of the same tooth, and naturally all the variations influence the appearance of the carious lesion.

Concerning the suggestion that caries did not occur at one point, it should be realised that the carious attack on the normal enamel surface is being considered, not on a surface affected by a structural hypoplastic, hypocalcified or traumatic defect. It is of interest to note, in addition, that in comparing two sections of enamel, one of initial caries and the other of a lamella, both sections being of equal magnification, it can be perceived that the lamellae would not be existent in the number necessary to be responsible for the points of attack shown in the initial caries section (4). Gustafson and Gustafson (62) also state it has never been proved that there is a relation between the number and character of lamellae and the caries susceptibility.

If there were straightforward entry of an attacking agent through the "undamaged" enamel surface, it is believed to be chiefly along the striae of Retzius and thereafter there is no preferential dissolution of the enamel components, i.e., the prism interfaces, cross-striations and the underlying striae of Retzius.
Decalcification is not inevitably a reversal of the pattern of calcification: it can only be said that the steps in the enamel destruction are in reverse order to those in enamel formation.

Any visible change around the "points of entry" is slow in comparison with the rest of the lesion and by the time the underlying lesion has reached the dentine, or nearly so, the striae of Retzius in the surface zone can be seen on microradiographs as slightly decalcified lines (34), whereas in polarised light, form birefringence reveals that the "points of entry" have only reached the stage where they occupy approximately 5.0 per cent of the enamel volume. In all cases the "points of entry" or breaches occurred along the striae of Retzius as small wedges which passed through the surface zone as fine threads and then opened out into the striae of Retzius in the body of the lesion (32). (Fig.13)

It might be said that the striae are merely involved with the rest of the enamel in the carious process, becoming exaggerated when viewed in the morphological picture: when there is preferential dissolution of the striae at the outer surface at the same time as the subsurface lesion is taking place. In keeping with recent interpretation of the enamel
structure (15)(18)(76)(93)(111), Frank felt (49) that if incremental lines became more prominent on microradiographs of carious enamel this fact was related not to the dissolution of the actual lines but to dissolution of those apatite crystals on the borders of the striae and prisms and in the inter-rod substance.

It is difficult to accept the fact that once the carious process has penetrated the surface enamel, it should attack the underlying striae more rapidly (Darling) (34) before there is any evident change in the "points of entry". Darling defended such criticism (33) by explaining that he did not consider the striae of Retzius to be the only important feature, but that striae were constantly related to lesions of early attack which he had examined; in his work he had found that prismatic substance was involved only after the lesion entered the tooth, and that entry seemed to occur via the striae of Retzius.

This author interprets the fact that with such an answer Darling was perhaps considering caries as a penetration attack at a more advanced level rather than an ionic diffusion reaction in the subsurface enamel. Darling substantiated his statement by correlating the above examined lesions with in-vitro
ones produced by an organic solvent (ethylene diamine), which is not a true imitation of the physico-chemical process of subsurface decalcification: again, in his earlier studies (31) he has also shown on occasion that areas of decalcification were similar to initial caries even in the absence of organic dissolution.

Mortimer's observations (96) of fissure caries revealed that they had appearances comparable to those interstitial lesions described by Darling (32) but in more advanced lesions, modifications occurred in relation to the prism direction and the incremental lines seemed to be less common around a fissure (from normal enamel studies); this would explain apparent lack of their involvement.

Crabb (28) also considered that, apart from the possibility of large portals of entry occurring in certain teeth where the striae of Retzius are marked and where they run out to the enamel surface, there appears to be no clear cut evidence at present for specific "points of entry". In artificial lesions produced via a window on the enamel surface which has been marked off by varnish, well-defined borders can be seen at either end of the lesions which are parallel to the prisms and not to the striae of Retzius. Although it can be argued that artificial caries is not produced under the same conditions as the natural lesion, electron microscopic studies by McMillan et al (92)
and Fosdick and Hutchinson (47) have shown that the pathways of entry at the level of structure which can be determined by light microscopy are more likely to follow the prism direction than the striae of Retzius. If the general direction of attack is parallel to the long axes of the prisms, lateral spread may follow where the attack is partially stopped by the resistant planes of the striae of Retzius (Figs. 6 and 13) and hence the striae become visibly prominent leading to the false conclusion that they have been the pathways along which the attack has initially entered.

![Diagram](image)

**Fig. 13** Diagram to illustrate the possible mode of spread of demineralisation in the outer enamel in which the striae of Retzius are prominently marked. In A, a stage has been reached in which the attack has bypassed areas of subsurface-resistant enamel and the surface "wedges" are showing involvement. The primary attack appears to be along the long axis of the direction of the prisms but is channelled laterally and along the direction of the striae by the resistant layers of the "striae of Retzius complex" (in directions arrowed). In B, the area shaded black indicates the spread of further demineralisation, which appears to start from the base of one stria and moves out towards the stria above it (28).

Thus "it seems probable that the Retzius lines do not play any major role in the development of caries, and that they only become specially prominent when disintegration of the organic matrix commences" (60).
So far it suffices to say that the pattern of caries is the resultant of the demineralisation and the remineralisation which takes place in the enamel substance beneath an intact outer surface layer.

With regard to the pre-eruptive morphological development of the enamel, final calcification is completed at the surface zone after the matrix has reached its full width from the dentino-enamel junction to the outer surface (3)(29), and it shows a higher mineralisation than the immediately subadjacent enamel. The width of the surface zone is reported as 5 to 15 microns and the subadjacent zone of delayed mineralisation as 15 to 30 microns wide (29) so that the intact surface of an initial caries lesion is approximately 30 to 45 microns wide. By the term "delayed mineralisation", it is explained in the work of Crabb and Darling (1962) (29) where they have shown by the pattern of mineralisation, that there is firstly a zone of high mineralisation at the dentino-enamel junction which extends peripherally towards the enamel surface and as it does so, its gradient of mineralisation diminishes. Although the process of mineralisation does not follow the incremental pattern of the matrix as originally believed by some workers, the surface zone when affected in its turn, is seen to mineralise considerably more so and in advance of the underlying zone which appears to be the final stage in the mineralisation process.
Soni and Brudevold (135)(136) claimed that the hardness of enamel was not necessarily related to the degree of mineralisation. This is certainly true when it is considered that increased hardness can occur by exposure of the enamel to fluoride and is the result of change of crystal structure of the mineral rather than an increase in its concentration. It appears that not a great deal is known with regard to the importance of the degree of mineralisation in relation to the initiation of caries as the evidence is conflicting: a probable solution to this question could be obtained from studies comparing the surfaces of unerupted and erupted teeth and is another problem for future research. On the other hand there is sufficient evidence to show that, although the character of the attack does not change, the newly-erupted tooth is most vulnerable to demineralising agents and that this vulnerability decreases with the intra-oral age of the tooth when it acquires more resistance to such attack by the acquisition and/or loss of organic and inorganic elements (19)(71)(85)(144). The non-caries surfaces on carious teeth do not differ from surfaces of non-caries teeth in solubility (19), and the longer a tooth (either carious or non-caries) is present in the oral cavity, the more resistance it acquires to decalcification (27). This age factor was shown by
Cotache (27) who subjected 92 whole teeth, extracted from patients whose ages ranged from 12 years to 95 years, to electrolytic decalcifying solutions over periods of up to 12 hours; he found also that the carious teeth from young persons were less resistant to decalcification than carious teeth from old persons and that the age dependency of resistance of either carious or non-carious teeth became more pronounced with increasing time in the decalcifying solution.

Nevertheless, if the age factor of resistance and hence the differing compositions between the subsurface region and the outer layer are disregarded; where it has been revealed that "white spot" formation (i.e., subsurface decalcification) can still be produced on teeth whose outer layers have been polished away (55)(138), such "white spot" formation still depends upon the mechanics of the demineralisation process. Sperber and Buonocore (138) suggested that certain acids, by concentrating at or near the surface of the enamel, either by forming a type of ionic shell or possibly surface-bound complexes, might act to slow down the diffusion processes involved in dissolution. It would be logical to bear this evidence in mind as an additional chemico-mechanical resistance effect in natural lesions where the composition of the outer layer is being considered.
Experiments have shown that the enamel surface of teeth at all ages is largely covered by a thin organic membrane or cuticle, which lies between the enamel prisms and the cariogenic agents. From the developmental aspect the cuticle can be divided into three types (122): (a) the primary cuticle, a thin continuous pellicle which covers the entire surface of the enamel and consists of the remainder of the ameloblasts after they have completed the formation of the matrix, (b) the secondary cuticle which is comprised of the remaining cells of the enamel organ when they produce a stratified epithelial covering called the reduced enamel epithelium and finally (c), an exogenous cuticle which is derived from organic matter in the oral environment being deposited upon the teeth where the primary and secondary cuticles have been worn away, so that it is, in a sense, "self-regenerating".

The protein of the enamel cuticle, whether it be developmental or exogenous, is resistant to concentrations of acid and alkali greater than any encountered in the mouth and therefore is resistant in general to acids, alkalis and proteolytic enzymes but it is permeable to such substances as lactic acid, hydrochloric acid, sodium chloride, glycerine, aspartic acid, glucose, methylene blue and calcium and phosphate ions (119)(120).
Hence it seems that lactic acid which is important in the context of the acid theory, the amino acids associated with the chelation theory, and the ions of remineralisation can all permeate the intact cuticle. If these demineralising agents are able to diffuse through the enamel cuticle, it is unlikely from this information that the cuticle itself has much preventive effect against caries, as revealed from its remaining intact in the "chalky spot" and "brown spot" types and stages of caries (120). Likewise it follows that it permits a post-eruptive mineralisation from the saliva, facilitated by changes in the organic matrix (58). However when the cariogenic agents pass through the cuticle to attack the tooth it could be that there is a protective effect (rather than preventive) of a "binding" nature to the immediate adjacent enamel structure so that acquisition of any organic material affords a degree of protection to the outer surface.

Soni and Brudevold (137) carried out microradiographic and polarised light studies on artificially-produced lesions and were able to show that the greater resistance to acids afforded by the surface than the subsurface zone persisted even after all organic material had been removed and therefore the solubility protective mechanism was definitely involved with the inorganic portion.
This finding, of course, does not exclude the fact that the organic portion also plays a part in resistance to acid attack when both the inorganic and organic portions are naturally existent in the tooth in its in-vivo environment.

The relatively higher resistance of the narrow surface zone to demineralisation can be explained partly by the higher fluoride content present in this zone and partly by a protective interaction of organic surface films deposited on the inorganic phase of the tooth substance from the adjacent liquid phase of saliva (56)(71)(131).

Both these explanations would infer that the surface layer acquires a composition which enables it to withstand acid or proteolytic attack; that chemical conditions are such that dissolution of the inner parts simply precedes dissolution of the outer layer. In addition there is the theory of remineralisation of the surface from the minerals present in the saliva where they are deposited in the intervals between attack. Current investigations still reveal that each process has its involvement and none is entirely independent of the other, unless independent conditions for attack exist and this is not so feasible in the light of present knowledge. Just as there exists synergistic causes for the carious process, experimental evidence
has shown that there also exists a synergistic effect for its prevention; one example being the fluoride ion becoming the "catalyst" and accelerating the rate of remineralisation of enamel in fluids containing calcium and phosphate ions (80).
PART II

REVIEW OF LITERATURE

C. STUDIES OF ENAMEL REACTIONS AND THE CHEMISTRY OF
SUBSURFACE DECALCIFICATION

Introduction.

Two important fundamental characteristics of the apatite crystal of enamel which determine its chemical structure are an ability for ionic exchange reactions and an availability of crystal surface area for these reactions. The latter characteristic is dependant upon the crystal size and both x-ray diffraction measurements and electron micrographs (105)(113) have revealed that the average diameter of the apatite crystal, whether it be synthetic, of bone, dentine or of enamel is generally in the order of 300 Ångstrom units whereas the crystals of enamel alone are the largest and best developed, and are reported by Sognnaes and Stern (133) as averaging 3,000 to 5,000 Ångstrom units in length and 500 to 1,200 Ångstrom units in width.

Apart from measurements of length and breadth, calculations can show that it is the thickness of the crystals which primarily determines the specific surface (101), since a decrease in length will not increase the surface area in the same proportion as will a decrease in the thickness. Nevertheless,
such crystals are very small and because of the size of the individual crystals, exchanging ions are presented with an enormous surface area which increases possibilities for their reactions.

With regard to the above former characteristic of ability for ionic exchange, these reactions may be either isoionic or heteroionic (100)(101); the former is the exchange of the normal apatite ions, calcium, phosphate, and hydroxyl from the solution phase with similar ions in the surface of the solid phase with no net change in composition of the two phases while the latter reaction is exchange when different ions are involved so that various cations displace calcium and/or various anions displace the phosphate or hydroxyl groups. It is obvious that for the liquid-solid relationship to be in equilibrium there is perpetual transfer of the normal apatite ions, to an frc, across the crystal-solution interface. If there were net gain or transfer of ions from solution to the solid phase, there would be mineral acquisition or crystal growth, and naturally dissolution would result from net transfer in the opposite direction, i.e., a crystal undergoing dissolution is accepting and losing ions at the same time but the rate of loss exceeds the rate of gain.
At present the exact nature of this interface has not been clarified but it is accepted that there exists a strong electric field on the crystal surface which attracts charged ions so that an \textquoteleft\textquoteleft immoveable solvent layer\textquoteright\ is bound to the crystal, i.e., the hydration layer or shell, and after exhaustive study, Neuman and Neuman (100)(101) have stated that the hydration shell must be considered as part of the crystal due to its involvement in exchange of ions. These workers represent the hydroxyapatite crystal suspended in water with four regions: the crystal interior, the crystal surface, the hydration shell beyond the surface and in which three, exchange takes place, and fourthly, a weakly-held orientated boundary layer which separates the \textquoteleft\textquoteleft crystal unit\textquoteright\ from the bulk solution (Fig.14).

![Diagram](image)

\textbf{Fig.14} Representation of a cross-section view of hydroxyapatite crystal in aqueous suspension. According to this construction, S1 and S2 have the same composition (101).

The hydration shell, to a limited extent reflects the composition of the bulk solution in the actual hydroxyapatite crystals because a wide variety of
ion substitutions (heteroionic exchange) from the bulk solution can take place in this bound concentration of multicharged polarised anions and the hydrated cations of calcium.

Neuman and Neuman (100)(101) have found it "possible to differentiate four main classes of heteroionic interaction" which are fundamentally:

I. A reaction in which the ions diffuse into the hydration shell but do not concentrate there. It is a steady state after a few hours but readily reversible, the concentration being directly proportional to the concentration of the ions in the bulk solution. Examples of such ions which are of this Class I reaction, being dependent upon their concentration in solution are the monovalent ions of potassium, sodium, chloride and fluoride.

II. A reaction in which the ions enter the shell and participate in the neutralisation of the crystal surface-charge asymmetry. Again a steady state is attained in a few hours, but readily reversible and usually involves multivalent ions which are easily polarisable due to their own structure or presence of water of hydration; e.g., magnesium, strontium, radium, uranyl, carbonate and possibly citrate.
III. A reaction in which ions pass through the hydration layer and replace ions on the crystal surface with possible neutralisation of the surface-charge asymmetry. Again a steady state is attained in a few hours and the system is reversible. Ions of strontium, radium and carbonate are of this Class as well as Class II and in addition, ions of sodium and fluoride are in this group.

IV. A reaction where ions can displace lattice ions both in the surface and the crystal interior. The reaction takes months to reach equilibrium and appears to be irreversible. A few ions such as strontium, radium, lead and fluoride are capable of intracrystalline exchange.

In analysing the kinetics of the radioactive phosphate exchange in a model system to determine the mechanisms involved in isoionic exchange, Neuman and Neuman (101) set out three steps of reaction on a chronological basis. Any exchange of phosphorus which occurs "between roughly 1 and 10 hours is numbered as Step II. All that precedes this is noted as Step I."

Step I represents a rapid exchange and could not be subjected to a kinetic study. The possible mechanism is a process which allows simple diffusion of ions between the hydration shell and the bulk solution.
It is not always easy to differentiate between Class II and Class III heteroionic interactions and the mechanism involved in the Step II isoionic reaction is also less obvious than Step I; however, it appears that the electrical potential barrier is the most important variable affecting the rate of exchange. This is to say (in terms of model experiments) that the rate of loss of an ion into the hydration shell should decrease with an increase of the opposing electric potential in the bound hydration shell of polarizable ions. Hence it follows that the greater the electropositivity of the crystal surface, the greater the amount of bound, polarizable phosphate ions required in the hydration shell to neutralise the charge asymmetry. Alternatively, if the ionic strength of the bulk solution ($S_1 + S_2$ in Fig. 14) is increased, the phosphate in the hydration shell is also increased and produces an increased potential barrier and thus, a reduced rate of loss of phosphate ions from the crystal surface (101). Consequently a Step II isoionic reaction is dependent upon the ionic strength of the bulk solution and the mechanism which is consistent with this fact is the escape or self-diffusion of surface ions to the hydration shell.
In Step III, the exchange is between non-hydrated surface positions and the successive intracrystalline layer positions, the reaction originating within the crystal. Although this step is independent of ionic strength, it is dependent upon temperature, increasing markedly in rate with small temperature increases. Neuman and Neuman (101) have explained the position and although this step is often referred to as "recrystallisation" and/or "thermal aging", it does not involve a dissolution and redeposition of crystals because its rate is independent of the ionic strength of the solution and, although the intracrystalline exchange is strongly temperature-dependent, it occurs at an ever decreasing rate.

Basically, the deeper the diffusion into the crystal interior, the slower the rate of diffusion and this reaction is determined by the size of the crystallite: on the other hand, if the lattice is dense and the diffusing ion large and multi-charged (phosphate) then it would be more probable to assume the ion migration is possible by the presence of unoccupied lattice positions or defects, known to be present in the hydroxylapatite crystals (112)(114) (115)(116).
Both iso- and heteroionic exchanges are consistent with lattice substitutions whereas crystals that are defective in one ion can be expected to have a "net electric charge" that will become balanced by the surface adsorption of a layer of ions of opposite charge (101). If there were a "hole in the lattice structure", it would represent an absence of both cation and anion, but a crystal being defective in only the cation or anion alone would require (115) its charge to be balanced by the addition or surface adsorption of the necessary anion or cation, whichever is the case. In either of these above situations, it would seem unlikely that a net electric charge should exist on a crystal surface rather than a series or variation of electric charges which are being balanced continually by ions of opposite charge from point to point on the crystal surface.

On the whole, the exchange between ions in the hydration shell and the crystal surface is a fairly rapid reaction depending on the rate at which the ions diffuse out from their lattice positions, whereas exchange within the crystal is slow and is dependent upon "thermal migration of defects and vacant lattice positions" and only a few ions are sufficiently similar to the normal lattice ions to permit their entry. Presumably any ion present in solution can
and will penetrate into the hydration shell but only specific ions tend to concentrate there (101).

Posner, Fabry and Dallemagne (115) term the apatite phase of the inorganic portion of bone and teeth, the pseudoapatite class of the defect hydroxyapatite series, where the term is used to describe all "finely divided calcium phosphates which are not calcined at high temperature upon formation and differ from hydroxyapatite in every property but x-ray diffraction pattern". On account of "statistical calcium defects" in the hydroxyapatite structure, a series of calcium phosphates (pseudoapatites) with a Ca/P weight-ratio range of 1.94 to 2.26 can all give an hydroxyapatite diffraction pattern.

It is generally accepted (115) that the major phase of the inorganic portion of bone and teeth is a low Ca/P hydroxyapatite, whose Ca/P weight-ratio ranges from 1.94 to 2.26 so that many calcium phosphates are precipitated from solution which will give the x-ray diffraction pattern of hydroxyapatite but depart from the ideal stoichiometry, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, of this substance (Ca/P weight ratio being 2.15).

When calcium ions are missing at random from certain structural positions in the lattice, the electric neutrality is maintained by the presence of hydrogen bonds, in proper proportions, between the oxygens of the adjacent orthophosphate groups (116).
If hydroxyapatite were precipitated in the presence of excess calcium ions, then a resultant deficiency in phosphate ions could also lead to the adsorption of hydroxyl ions (101). However, indications are that the apatite portion of tooth mineral, derived from aqueous physiological solution, will always consist of a calcium-deficient apatite. "It is further suggested that these biological apatites can take up calcium (and other ions that can fill the calcium positions) from the body fluids and release hydrogen ions previously held by hydrogen bonding in the solid. The difference in stoichiometry found in various specimens of bone and tooth salt may possibly be related to changes effected by exposure to serum-containing ions (Ca\textsuperscript{+++}, Mg\textsuperscript{+++}, Sr\textsuperscript{+++}, etc.) that react with calcium-deficient apatites (112).

Hence it appears that defects can account for the variable stoichiometry of the apatite series - when they become balanced by either internal substitution or surface adsorption of ions.
The enamel reactions.

It would appear from the histology of the incipient caries lesion and from the evidence concerning ionic exchange reactions that the possible enamel reactions during incipient caries formation are those of dissolution and recrystallisation. If there is agreement on a differential decalcification of certain structures, then it is not unreasonable to accept that several causes play some part; these being the variations in degree of mineralisation, the variability of the actual chemistry of the structures, the accessibility of the structures to the pathways of attack, and the differences in the structure and distribution of the organic matrix of the enamel. The marked difference in susceptibility between the surface zone and the deeper enamel makes it unlikely that this difference will depend to any great extent on the degree of mineralisation or the accessibility of the structure to the pathways of attack and that it is more likely to depend on fundamental chemical or structural differences (35).

Mature enamel consists of approximately 96 per cent of hydroxyapatite crystallites in an organic matrix and although references are made to the concentration of the organic material in surface enamel, little is known about it. This matrix consists
largely of protein and according to nitrogen analysis it must amount to roughly 0.5 per cent of dry weight of the enamel. Since the enamel is a product of ectodermal cells, it was generally believed that the organic part was of a keratinous nature.

Investigations on enamel protein have shown that there are two fractions, one soluble and one insoluble (107)(140)(142). The larger soluble fraction consists of citric acid, peptide, collagen and glycoprotein, while the smaller insoluble fraction is formed by keratin and collagen. This data was compiled by Stack (140)(142) and to quote, "the amount of this insoluble protein in a full dentition appears to be no greater than the keratin represented by a full-grown human hair". The insoluble protein differs from skin or hair keratin however, and contains a small amount of cystine and a notable percentage hydroxyproline (25), which is an amino acid characteristic for collagen, yet, which still remains after extraction (in experimental work) of the associated collagenous material.

Alternatively, Piez (109) considered the protein to have some structural feature of β- keratins and to be related to collagen in that it contained hydroxyline, large amounts of proline and "perhaps hydroxyproline".
X-ray diffraction study by Perdock and Gustafson (107) in 1961 revealed that the insoluble tooth enamel protein (keratin) did not belong to the collagenous group of proteins so that it was classified as a "keratin" on its own ("eukeratin") and this individuality would probably be a factor in the retention of the hydroxyproline after removal of the collagen: moreover, Fearnhead (45) concluded in 1964 from his experiments that there was insufficient evidence to justify the assumption that the insoluble component was a keratin.

With regard to the soluble fraction, it is of interest to note that there is ten times more carbohydrate in the surface than in the body enamel, suggesting that the major portion of the soluble protein in the surface is composed of glycoprotein (20).

As Stack (142) disclosed that the organic content of normal intact enamel did not depend on the age, area on the tooth, or type of tooth; and that sound enamel from carious teeth had the same organic content as that from intact teeth, then such organic increase must be associated with physical defects produced in the enamel from "wear and tear". In any case, without these "cracks" it would be unlikely that large molecules of organic matter could penetrate the spaces and pathways of intact enamel. An histological study of
brown pigmentation in the enamel surfaces on sections made from 82 teeth (11) showed that the brown discoloration was due to changes in the organic matter of the surface enamel in so far as it was either lodged bacteria and/or debris consolidating, or deposits of constituents of saliva, and as the age increased the pigmentation intensified. Bhussry and Bibby (11) also demonstrated that the pigmented areas were highly resistant to the action of acids in decalcification so that it can be assumed that any acquisition of organic material is a protective measure, whether it be pigmented or not, and where in initial caries, the lesions has its beginning in the subsurface enamel and not on the surface.

In further study on the "brown spot" according to nitrogen analysis, Bhussry (9) supported the idea put forward by such workers as Hardwick and Manley (64), Hodson (68), and Bibby (12), that these spots were formed by organic replacement of inorganic material lost through decalcification occurring in the mouth. The organic content of these areas was found by Bhussry to be higher than that of sound enamel and they had a decreased hardness and lower density which suggested a loss of inorganic material.

The author considers that both processes would occur together after repeated acid attacks on the
enamel which must occur naturally throughout life so long as suitable substrates for the production of acids are present in the oral cavity. The "voids" created by initial acid dissolution, whether they be a result of removal of the soluble protein or preferentially-dissolved crystallites, are bound to be replaced by extraneous organic material or inorganic ions depending not only upon their availability but also upon the diffusibility and the size of the replacing molecules.

A significant increase in nitrogen with age has been demonstrated by Bhussry in 1958 (8) in the outer portion of enamel by relating nitrogen to volume, rather than to weight. Sound enamel from non-carious teeth did have a higher nitrogen content than sound enamel from carious teeth, therefore, the higher organic content cannot be correlated strictly with greater susceptibility to caries. In any event it must be recognised that local environmental factors play a significant role in determining the susceptibility of caries.

In the same experiments, buccolingual surfaces had a higher density and a lower nitrogen content than enamel from the mesiodistal surfaces of teeth from similar age groups, yet it is a fact that the
mesiodistal surfaces show a greater incidence of caries attack than the buccolingual surfaces. One hypothesis is that the higher organic content quoted is derived from the retention of the enamel cuticle in protected mesiodistal areas rather than its being removed by abrasion on the exposed buccolingual surfaces: alternatively, the proportion of the percentage by weight of the cuticle in relation to overall weight of the protein is so small that it would seem that the presence or absence of the cuticle could scarcely account for differences of the magnitude shown in Bhussry's data (8). If this is so, then it supports the fact that there is organic replacement of the substances dissolved out of the enamel after acid attack, which itself is more prevalent on the mesiodistal surfaces.

Enright and Friesell (42) had concluded as early as 1932 that "the cessation that is sometimes observed in carious processes of the enamel is due to the presence of acid insoluble protective organic coatings."

Bhussry's further findings on white opaque enamel (10) upheld the view that there was not only an alteration in structure, but an increase in the organic material in both types of white opaque areas, i.e., in either subsurface decalcification spots or
hypocalcified developmentally defective areas. The "white spot" areas showed a lower density than the developmentally hypocalcified areas and at the same time emitted bright fluorescence under ultra-violet light, which was indicative of an increased organic content in these areas, however, this latter alteration which corresponded to the lowered density of defective areas would probably be the contrasting effect of a reduction in mineral content of these areas.

To reiterate at this point on the decalcification of the structures of the dental enamel, it is known that there are different degrees of solubility, so that the soluble structures are the interprismatic substance, the cross striations, the prism core and the striae of Retzius, while the insoluble structures are the prism cortex, the surface zone of the enamel and the zone immediately beneath the striae of Retzius. If the cause for this difference is a difference in their respective inorganic components, then such a difference has not been found up to date: but a difference in the organic components has been shown and this is one explanation of the small amount of spaces without apparent decalcification in the earliest carious lesion as has been observed by Darling in 1962 (34) as a translucent zone by transmitted light
in balsam and quinoline media (Fig.11) and he assumes initial attack is principally a solution of the soluble protein. This assumption is furthered by the evidence that lesions identical with the very early stage of enamel caries can be produced by an organic solvent which does not dissolve the inorganic components and most certainly does dissolve the soluble protein fraction: this solvent is ethylenediamine (33).

Such a theory would not disagree with acid as the attacking agent as precisely similar lesions can be produced by dilute acid, but it is because of the reason that caries is produced in vivo by dilute acids rather than ethylenediamine that one can be critical of this presupposition: reference is made by Gray and Francis (56) to the fact that organic material is ever present at the time of enamel attack so that it is "very doubtful whether the organic component can ever be completely omitted in an in vitro system for reproducing the typical natural lesion". In addition, a study of the initial lesion at the one stage of progression, such as that of Darling quoted above, has never been correlated directly with chemical analyses performed on lesions of the precise same stage of progression.
Moreover, there is also the evidence of Isaac et al (71) to show that the difference in solubility between surface and subsurface layers was not to be related to the organic content of enamel; where solubility increased from the outermost to the innermost layer (about one-half way into the enamel) in all his experimental teeth at each level of pH used (4.0, 4.5, 5.0), the difference being most marked from the surface layer to the adjacent subsurface layer. The organic material had been previously extracted with ethylenediamine, demonstrating that the organic material in the enamel was of little significance in the solubility effect.

Nevertheless, dental caries differs from other types of hard tissue destruction because it is only in dental caries that subsurface demineralisation and the preformed pathways appear to play a significant role (123), and it is the composition of the outermost layer(s) that has an important influence on the caries resistance or susceptibility of the tooth to decay.

When the previously mentioned principles of ionic exchange are applied to crystallisation of the enamel matrix, a higher degree of calcification in the superficial enamel can be expected because of its direct exposure to the available calcium and
phosphate ions in tissue fluids before eruption and in saliva after eruption (24). It follows also that the crystallisation can occur only as long as there is free passage of ions. When the spaces between the crystals approach molecular dimensions, such free passage of ions becomes increasingly restricted by charges on the crystal surfaces (101). Although the body of the enamel would be calcified to a "mature" state, the superficial outermost region would have attained "maximal" calcification in comparison, and would physically prevent further "maximal" calcification of the underlying body of enamel.

Investigations have shown that certain elements, including fluorine, zinc, lead, and to a lesser extent iron, silver, manganese, silicon and tin, occur normally in greater concentrations in the surface than in the subsurface enamel, whereas carbonate, sodium and magnesium have an increased distribution in the interior enamel rather than in the surface. The presence of carbonate and citrate greatly increases the solubility of hydroxyapatite (101) and although the concentration of citrate, unlike carbonate, is higher in the outer surface than in the body enamel, the citrate is not present in a concentration large enough to influence enamel
solubility when compared with carbonate. Nikiforuk and Grainger (103) in 1964, were unable to find any significant difference in the citrate concentration of carious and non-carious teeth when correlating the fluoride-carbonate-citrate content of enamel. On the other hand, the fluoride ion does inhibit enamel dissolution markedly and if it is accepted that this property is a mechanism for caries inhibition, then it has been demonstrated repeatedly in the past by the effectiveness of topical applications of fluoride ion to the teeth (46). Also, if the fluoride content of the enamel is increased by exposure in an optimum fluoride environment, there is a decrease in the caries susceptibility while conversely, it is the decrease or loss of inorganic carbon dioxide with age from the enamel surface by preferential dissolution (85) that could be regarded as the acquisition of a certain amount of caries resistance.

It has been stated by McCann and Bullock (88) that "the reactions of enamel, and particularly of dentine, have been shown to be much more complicated than those of synthetic hydroxyapatite in the presence of fluoride ions." At low fluoride concentrations the synthetic hydroxyapatite appears to form fluorapatite and then more and more calcium fluoride as the concentration of fluoride ion increases.
However, the fluoride ion reacts with enamel apatite in several different ways and McCann and Bullock (88) have summarised five different reactions which can take place in vitro between enamel and the fluoride ion, namely, "(a) possible exchange with CO₂ on the crystal surfaces, (b) direct adsorption, (c) precipitation as magnesium fluoride of the magnesium released from crystal surfaces, (d) double decomposition at high fluoride levels to form calcium fluoride \((\text{CaF}_2)\) and sodium orthophosphate \((\text{Na}_2\text{HPO}_4)\), and (e) formation of fluorapatite by exchange with hydroxyl ion."

The reactions with enamel in vivo would differ qualitatively because of the presence of saliva and other substances. McCann and Bullock (88) did not expect magnesium to be dissolved from the tooth in most cases for precipitation of \(\text{MgF}_2\) to occur and with the additional knowledge that in carious lesions there is a decrease of magnesium ion as well as an increase of fluoride ion (75), it is unlikely that there is much \(\text{MgF}_2\) precipitated.

The possible exchange of fluoride ion for the \(\text{CO}_2\) to form fluorapatite would be the result of a Class II or Class III heteroionic exchange reaction (Pages 63 and 64) and most dependent upon the individual concentration of carbonate ion available, thus it may take place to a limited extent.
On the other hand, direct fluoride adsorption by a balancing heteroionic interaction on the crystal surfaces can always take place as can the formation of calcium fluoride by double decomposition of the fluoride salt and the apatite, provided the fluoride concentration is sufficiently great for the latter reaction to occur. Like adsorbed fluoride, calcium fluoride is gradually converted to fluorapatite during the course of time according to a Class IV heteroionic interaction (Page 64). McCann (87) has shown that the fluoride reaction with synthetic hydroxyapatite depended on two factors, the Ca/P ratio of the apatite and the fluoride concentration. He found that fluorapatite was formed at all Ca/P ratios (Ca/P weight-ratios 2.05 to 2.16) at a few parts per million of fluoride; calcium fluoride or fluorapatite was formed with fluoride solutions up to 0.2 per cent concentration and depending upon the Ca/P ratio; and above this concentration of fluoride, calcium fluoride was formed with all ratios of calcium and phosphorus.

However, the fluoride reaction on a tooth surface in saliva is in the presence of excess calcium ions and it is sufficient to have only a concentration of eight parts per million of fluoride for the solubility product of calcium fluoride to be exceeded
and for calcium fluoride to be formed in large amounts. With respect to the high concentrations of fluoride used in topical fluoride solutions, CaF$_2$ is always precipitated and is unlikely that there is any fluorapatite formed in such a reaction. It is possible that some of the adsorbed fluoride and some of the calcium fluoride is converted gradually to fluorapatite in time. It is important to emphasise the fact that in-vivo formation of fluorapatite is determined by the fluoride concentration in relation to the solubility product of calcium fluoride.

With regard to the formation of fluorapatite, it is of interest to note that Posner and Eanes (114) describe how the smaller, more symmetrical fluoride ion forms a stronger ionic bond than does the hydroxyl ion with calcium ions in the apatite structure, and suggest that this stabilizes the fluoride-containing hard tissue against chemical dissolution reactions. Diffraction studies on the crystal size and chemistry of bone apatite by Zipkin et al (155) and Eanes et al (41) have shown that a rise in the level of available fluoride was accompanied by an "increase in crystal size and/or a decrease in crystal strain" which was consistent with the substitution of fluoride ion for hydroxyl ion in the apatite structure.
It is suggested that both these factors produced
a more stable bone apatite by virtue of the improved
"crystallinity" and to date it is presumed by
analogy that fluoride may have the same effect on
dental tissues. It is clear, however, that such
a change in crystal dimension has its effect on the
solubility, since a crystal, improved in size and/or
strain, will react more slowly in a given solution
than a smaller and/or less perfect crystal.

Fluoride is incorporated in the enamel at the
time of calcification or prior to eruption, from
the tissue fluids; but after eruption and as long
as the tooth is present in the oral cavity, fluoride
is taken up by the enamel surface at a rate which
is rapid for the first few years following enamel
formation and then gradually tapers off to reach a
level of uptake which is proportional to the
concentration of environmental fluoride, the
deposition of fluoride in the surface enamel being
always more pronounced than in the subsurface
enamel (22)(24).

Some recent analyses made by Brudevold, McCann
and Grøn (23) of the outer layer of enamel of teeth
from areas with 1.0, 1.5 and 2.5 ppm. of fluoride
in the water, have shown that although the fluoride
content of this layer increases with the increase of fluoride level in the drinking water, the magnesium content of the outer layer does not. They found from their analyses that the fluoride uptake by the enamel was independent of such components as CO₂, citrate and K⁺: "hence the inhibition of caries observed in fluoride areas is attributable to the presence of fluoride, rather than to secondary changes in the concentration of other enamel constituents" (23). Naturally this finding does not exclude the possibility that there is a correlation between caries resistance and "secondary changes" of other enamel constituents. Davies (38) has evaluated the significance of epidemiological studies in relation to caries resistance and he concluded: "that the evidence is consistent with the hypothesis that trace elements are the most important factors in determining the resistance of the teeth themselves but that further work is required to determine the effect of the interaction between different trace elements, the effect of techniques of refining and processing on the micro-nutrient content of foods and of the variations in the uptake of specific trace elements by specific surfaces of teeth with age and under different environmental conditions."
Although many trace elements accumulated in the surface enamel may have their respective importance, it is known (85) that the concentration of the carbonate in the external enamel decreases with age whereas there are no alterations of carbonate concentration in the body of the enamel. The level of the carbonate in the saliva is dependent on the rate of secretion and in resting saliva it is low, increasing in concentration as the rate of flow of saliva increases. Since phosphate concentration in the saliva remains relatively stable and is always at a higher level than that of serum, the phosphate to carbonate ratio is considerably greater in resting saliva than in serum and the oral conditions are such that they are likely to favour an exchange in surface enamel of carbonate with phosphate. Brudevold (20) considered "loss of carbonate from the surface must be an important factor in enamel maturation and acquisition of a certain amount of resistance to caries with age." However, it is difficult to estimate how significant a role the carbonate content could play in acquiring caries resistance without prior knowledge of the individual enamel carbonate concentration.
Little and Brudevold (85) state that prior to eruption a slight increase in the surface CO$_2$ (outermost and subsurface) enamel is apparent as the enamel matures, but after eruption the trend is reversed and the surface CO$_2$ content is reduced progressively up to the age of 40 to 50 years. They studied differences in the CO$_2$ content of the superficial and inner portions of intact human enamel of unerupted and erupted permanent teeth in various age groups and reported that the CO$_2$ concentration consistently increased from the surface towards the dentine and that this distribution seemed developmental. Brudevold et al (24) have also shown in teeth from different geographical areas that, apart from the age factor, the concentration of carbonate in the surface enamel was much less than that in the body enamel, the concentrations being 350.0 - 440.0 micromoles per gm. in outer enamel and 525.0 - 654.0 micromoles per gm. in the body enamel.

Caries susceptibility has been related to high carbonate teeth in the cotton rat (125)(127)(128) and the possibility that this susceptibility is related to high composition of the human enamel is further indicated in the working hypothesis that, (a) acids dissolve carbonate preferentially from
bone and tooth mineral, i.e., that carbonate
dissolves more rapidly from high carbonate enamel
and dentine, and (b), that \( \text{Ca}_3(\text{PO}_4)_2 \) is more soluble
in the presence of carbonate due to formation of
unionised carbonate-phosphate complexes (35)(125).

It has been discussed previously that other
components such as carbonate, citrate and fluoride,
as well as other anions and cations are primarily
deposited in varying amounts into the adsorption
layer. Therefore it would seem that this composition
of the adsorption layer determines the initial
solubility of the tooth mineral so that, if carbonate
were present in large amounts then the tooth solubility
would be increased. Sobel et al (127) calculated
that carbonate could occupy 3-16 per cent of the
volume occupied by the hydroxyapatite crystal and
that the hydration shell would be approximately 5.4
per cent of the volume occupied by the hydroxyapatite
crystal. Thus, for example, if all the carbonate
were present in the hydration shell alone, and
occupying 3 per cent of the volume of the crystal,
then it would occupy as much as approximately 60
per cent of the volume of the hydration shell, so
that if this concept is valid, caries attack depends
on those crystal surfaces which possess a higher
content of soluble components, i.e., carbonate.
Interrelationship of tooth composition, diet, body fluids and caries susceptibility led Sobel (126) to believe that increased phosphate in blood serum and saliva could alter the composition of the tooth to render it less vulnerable to attack by virtue of the decreased relative carbonate content. However, not much experimentation was carried out on this belief and the results were formed from rat studies alone, being presented very briefly by Sobel in 1952 (124); a decreased caries score was recorded in 1949-52 in rats with low carbonate teeth (as compared to high carbonate teeth) and again this trend was paralleled in 1953-54 (127), but it would be more convincing if more recent and detailed work followed up this hypothesis.

Coolidge and Jacobs (26) calculated on a volume basis that 40 per cent of the calcium, 41 per cent of the phosphorus, and 66 per cent of the CO₂ originally present in the normal enamel, had been lost when the lesion had reached the "white spot" stage. (While only one calculation of this kind was made, the uniformity of composition and hardness of enamel at this stage encouraged these workers to believe that their calculation of the fraction of each constituent lost, probably would lie fairly
close to the general value for this type of lesion.) In contrast to this calculation, it is to be noted that Stack (141) found "chalky" enamel to contain consistently three to four times as much organic matter as the adjacent sound enamel. This would be the logical result of persistence of the organic matrix as indicated by the occurrence of rod sheaths (75), even though rods and inter-rod areas frequently lacked the fine fibrils observed in the sound tissue; and any apparent increase in organic material, the result of acquisition from the oral fluids into the spaces produced by such inorganic loss as quoted by Coolidge (26).

Johansen's chemical studies (74)(75) of carious lesions have shown an increase in water and organic material and a decrease in ash, but in analysis of the ash portion, although relatively minor changes were revealed in the calcium and phosphorus contents, there was a marked decrease in magnesium and carbonate.

McClure (91) also concluded that ash, calcium, and phosphorus content of human enamel did not vary with caries experience or age and that even an eightfold increase in enamel fluorine was not related to any change in enamel ash, calcium or phosphorus. This finding agreed with Little and Brudevold (85)
who found that there were no marked differences in the calcium or inorganic phosphorus distribution with age or enamel depth but they did find that the carbon dioxide to phosphorus ratio varied and ranged from a low 0.05 at the outer surface to a high of 0.115 near the dentino-enamel junction; like Sobel (124)(125), they considered a low CO$_3$\cdot$PO$_4$ ratio of the outer layer of enamel to contribute to the caries resistance of the teeth.

With regard to the fluoride content of carious enamel, it was shown by Brudevold et al (22)(24) and by Johansen and Nordback (77) to be five times higher than in sound tissue. Notwithstanding, attention is drawn to McClure's work (90) on the fluoride content of enamel of sound and carious teeth in 1948 when he demonstrated that a difference in fluorine content of enamel from sound and carious teeth was not a general rule among most individual dentitions. In other words, the caries history of one tooth as compared with another tooth in the same dentition was not related to its fluorine content. In these experiments by McClure however, both the sound and carious teeth were cleaned prior to mineral analyses so that all carious matter had been removed from the carious teeth with burs. It is interesting to compare this finding with the work of Johansen and Nordback (77) where carious lesions showed a higher
concentration of fluoride than corresponding sound tissues, especially in the light of a possible recrystallisation theory.

The fact that there is a marked increase in fluoride and a decrease in carbonate and magnesium observed in carious enamel is certainly suggestive of selective demineralisation, and the fact that many crystallites in all regions appeared well-preserved, implies that these crystallites at least in part are the products of a recrystallisation process; it follows that persistence of crystallites themselves in advanced caries could be explained on the basis of crystallite composition change resulting in a reduced solubility.

However several problems arise in a study of the solubility of enamel and it is pertinent at this point to return to the basic principles of solubility with special reference to hydroxylapatite (67)(101)(113). Although the space lattice of hydroxylapatite has been calculated so that the location of each calcium, phosphate and hydroxyl group is known, each molecule must contain at least 18 atoms or ions which apart from having a complicated arrangement, varies minutely but significantly from sample to sample. It is well known that only a pure synthetic hydroxylapatite could conform with the ideal stoichiometry of the
formula, \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \), and it is only for convenience that this formula is used to describe the enamel apatite.

Mention has been made already of a defect series of biological apatites of identical X-ray diffraction pattern which differ chemically from this formula. Posner (112) considered it more in keeping to name such a series of apatites as "divalent cation-deficient apatites" rather than non-stoichiometric or defect hydroxyapatites: hydrogen ions achieve electric neutrality of the low-cation apatites by their inclusion as hydrogen bonds (116) in the correct proportions between the crystallites and if the empty cation position is eventually occupied, it is possible that the hydrogen will leave the apatite by forming hydronium ion \( (\text{H}_3\text{O}^+) \) with a resultant drop in conductivity. Hydronium ions may replace up to 2 calcium ions in the calcium-deficient lattice when the formula could be expressed as \( \text{Ca}_{10-n}(\text{H}_3\text{O})_{2n}(\text{PO}_4)_6(\text{OH})_2 \), where "n" is less than 2 and represents the number of replaced calcium ions (101)(143).

Nevertheless crystallographers do not agree on the locations of many of the substituting ions and it is quite apparent that the biological apatites are complex. They are precipitates from solutions which
contain, besides calcium and phosphate ions, also carbonate, chloride, citrate, fluoride, magnesium and sodium ions in appreciable concentrations (147). It is acceptable that other substances will be precipitated simultaneously with the apatite and studies by x-ray diffraction analysis (154) have shown that some of these substances or ions upon substitution into the apatite crystal, are generally capable of distorting the crystallites and changing the unit cell dimensions.

In addition to the complexity of the biological apatites, the solubility of a calcium-phosphate-aqueous system is also confusing at near-neutral or alkaline values of the pH. At very low pH value of less than 4.0, the system is governed by the normal solubility product constant of primary calcium phosphate or $\text{Ca(H}_2\text{PO}_4\text{).H}_2\text{O}$. At higher pH value up to 6.0, the system is governed by the $K_{sp}$ of secondary calcium phosphate or $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, but above pH 6.2, secondary calcium phosphate is unstable and undergoes hydrolysis spontaneously. The product of this reaction always exhibits the crystal structure of hydroxylapatite. "Because the physiological pH is more alkaline than 6.2, hydroxyapatite is the only solid phase of calcium and phosphate that can form under physiological conditions." (101)
Accepting the fact that the solubility product is a constant for a given substance at a given temperature, there are two factors which can influence the "escaping tendency" of a solid to go into solution. The first factor is the interionic attraction of ions in the crystalline lattice and the second factor is, that if the crystal is extremely small, there is a larger surface area and the surface ions are not so attracted to the underlying ions as they would be in the case of a larger crystal; consequently, greater activity, "escaping tendency" and resultant solubility is possible. If however, foreign ions are introduced into the equilibrium and there is subsequent substitution into the crystal lattice, the forces of ionic attraction are altered and the solubility of the solid is either increased or decreased. Secondly, since ionic interaction from foreign ions i.e., the substitution, is so varied in vivo, the effect of the infinitely small hydroxyapatite crystal is not to increase its solubility but to enhance the varied effects of interionic attraction within the crystal leading to general decrease in solubility if fluoride were the predominant foreign ion introduced. In this instance the smaller particle size does not increase the solubility but allows greater fluoride substitution and the small crystallites of the hydroxyapatite which are converted to fluorapatite
are now less soluble than large particles, and the solubility of the hydroxyapatite is not as would otherwise be expected.

With bone apatite it has been demonstrated by Posner and Eanes (114) that the introduction of fluoride ion stabilizes the apatite structure by a resultant increase in crystal size. Although this change of crystal size has not been shown with enamel apatite, these experiments on bone and the fact that the solubility of enamel is decreased with the introduction of fluoride both suggest that a change in enamel crystal size is an additional factor for decreased enamel solubility from fluoride action.

Gray, Francis and Griebstein (57) have explained that the "hydroxylapatite is one of several solid calcium phosphates that can coexist with aqueous solutions of calcium phosphate ions depending on the relative concentrations of calcium, phosphate, and hydrogen ions (hydronium ions) in the solution."

If the apatite were attacked by a buffer (pH range 4.0 to 6.0) so much solid will go into solution until there is an equilibrium concentration of calcium and phosphate ions in solution as well as in the solid phase, however, if the saturation of the solution is exceeded, precipitation of secondary calcium phosphate occurs rather than precipitation
of hydroxylapatite (101), which means that the calcium phosphate that is dissolving and reprecipitating at the solid surface is not the hydroxylapatite but dicalcium phosphate dihydrate (CaHPO₄·2H₂O). Hence it is meaningless to describe an equilibrium solubility point for hydroxyapatite in acid solutions below pH 6.0 when it acquires a "surface-coating" of another phase, i.e., another slightly more soluble form of calcium phosphate.

It has been discussed previously that ionic interaction is the reason for the variable composition of the hydroxylapatite crystal and so long as such action is occurring, it can be considered as an indefinite series of nearly perfect isomorphous substitutions. Consequently no solubility product has ever been demonstrated for hydroxyapatite and as Hodge (67) points out, no solubility product can be calculated when the crystals exhibit such variable composition as the result of indefinite isomorphous substitutions. The inability to express the solubility in terms of a solubility product does not imply that anything but an equilibrium condition between the ions of calcium and phosphate exists; it is obvious from the nature of the hydroxylapatite crystal that such equilibrium of the mineral phase in the adjacent oral fluid is possible by a moment-to-moment interchange
of ions from the crystal surfaces into solution and from the solution back on to the crystal surfaces. Thus the hard tissues of the tooth are in an exchange equilibrium with the saliva and there is two-way movement in this biological system whereby ions move from the tooth to the adjacent solution and from this solution to the tooth. In terms of incipient caries, the most significant portion of the tooth composition would be the crystals at the surface of the enamel where Brudevold and his co-workers (24) (85) showed that the composition can differ from the composition of the deep layers. (In gross analysis of enamel, composition differences may not be revealed although they exist in the surface layer.) Accordingly, the first recognisable stage of a clinical carious lesion is the so-called "white spot" which is characterised by an apparently sound enamel surface overlying a region of reduced enamel density where the mineral phase of the tooth substance has been partially removed by decalcification.

However, some loss of enamel mineral substance from even this relatively sound outer layer has been demonstrated by comparison with the outer layer of normal enamel by Soni and Brudevold (136) using intrinsic retardation measurements of polarised light as well as densitometric interpretations of microradiographs.
In the formation of an incipient carious lesion, the reactant must, for the most part, by-pass the surface to diffuse into the enamel and react with the subsurface enamel.

Wachtel (148) exposed tooth enamel in vitro to lactic acid over an observed period of two months and hydrofluoric acid for periods up to twenty one months; in both instances phosphorus was lost at a constant rate with time but, in the lactic acid buffer, the enamel became visibly white whereas there was no visual change under hydrofluoric acid attack. The difference in reaction products was explained on the assumption that fluoride ions were removed fairly rapidly from solution by the calcium ions on the etched surface of the enamel, resulting in further dissociation of the hydrofluoric acid which produced a local increased hydrogen ion concentration and more rapid dissolution of the enamel to primary calcium phosphate ($\text{Ca(H}_2\text{PO}_4)_2$). This would not occur with the lactic acid and a slower conversion of the apatite crystals to secondary calcium phosphate ($\text{CaHPO}_4$) would occur. The increased amount of phosphorus removed by the hydrofluoric acid from an equivalent area, whitened by lactic acid, could be attributed, as pointed out by Brudevold (19), to the "greater dissolving power of acid" in the conversion of $\text{CaHPO}_4$ to $\text{Ca(H}_2\text{PO}_4)_2$. 
The in-vitro variables which are listed by Gray et al (57) and Wachtel (148) as important for enamel dissolution are (i) the area of the exposed enamel, (ii) the stirring rate, (iii) the temperature, (iv) the hydrogen ion concentration and (v), the concentration of the undissociated weak acid in the buffer system. Although variables (i) and (iv) (the area of the exposed enamel and the hydrogen ion concentration) are self explanatory in influencing the rate of the dissolution reaction, Gray (53) found the reaction between the acid and the enamel to be diffusion-controlled. The solubility rate was only slightly dependent on temperature (20 per cent increase for a 10° rise from 27° to 37° C) whereas it was most dependent on agitation since the rate of dissolution was controlled by diffusion processes in the solution phase which was bathing the enamel surfaces. "It was not possible to reach an agitation speed that, when exceeded, did not further increase the solubility rate." (53) In unbuffered solutions the solubility rate was directly proportional to the hydrogen ion concentration, however, in buffered solutions, the rate was proportional to the concentration of the undissociated acid which supplies hydrogen ions to the reaction since in the latter case, a molecule of undissociated acid which has reached the enamel surface can then dissociate to yield an hydrogen ion
for reaction with the enamel. "Both pH and buffer concentration are important and must be known before any prediction can be made about the rate of progression of decalcification. Measurement of the pH of dental plaque, therefore, cannot be used as an indicator of caries activity without also considering the identity and concentration of the organic acid anion or anions present in the plaque." (57) Moreover Gray (54)(55) has established that the rate of enamel dissolution was not only dependent on all three factors, the hydrogen ion concentration, the acidic buffer concentration and the acidic buffer strength (dissociation constant), but that the effect of these three factors was interrelated.

Thus the rate of enamel dissolution was attributed to the diffusion of hydrogen ions and undissociated organic acids to the tooth surfaces, and also to the diffusion of reaction products away from the surface.

Increase of the buffer capacity and buffer strength would vastly increase caries activity and therefore the type of acidic anion produced by bacteria would be an important consideration in the dissolution process. "The complexing of calcium by the acidic buffer anion serves to remove the reaction product, calcium ion, from the vicinity of the reaction
site where calcium ion can inhibit the progress of the reaction. The inhibition effect by calcium ion can under some conditions be due to a common ion effect, but under the present acidic conditions, it definitely is not a common ion effect at equilibrium and probably not during the approach to equilibrium." (55)

Gray and Francis (56) have shown by solubility product measurements that when enamel, dentine, bone or synthetic apatite are in acidic solution and the resulting dissolution reaction is in equilibrium, the composition of this solution is in equilibrium with dicalcium phosphate rather than hydroxyapatite. This means that the enamel crystal surfaces have been, converted to dicalcium phosphate. Furthermore the rate of subsurface dissolution was also retarded by the presence of other cations such as magnesium, tetramethylammonium, ammonium, potassium, sodium, stannous tin, zinc, lithium and indium; all cations not being equally effective. Obviously such retardation of dissolution is not a common ion effect with hydroxyapatite and it is considered that surface deposits are formed from precipitation of these cations with the reaction products of the enamel. On the other hand, anions in general, with the exception of phosphate and fluoride ions, had no effect on the rate
of dissolution. Although the compositional differences of the enamel can modify the dissolution rate as the equilibrium solubility is approached, it is most important to realise that it was also revealed by Gray (53) that enamel of teeth from high fluoride areas dissolved exactly at the same initial rate as enamel of the teeth from low fluoride areas. However accumulation in solution of such substances as soluble calcium salts, phosphate and fluoride ions, released from enamel during acid dissolution eventually lead to suppression of further acid attack. Thus the initial solubility rate of enamel was found to be decreased by the presence of reaction products (calcium and phosphate ions), of most if not all cations, and of fluoride.

Spiers, Spinnelli and Brudevold (139) found that fluorides, zinc, lead, stannous tin, molybdate, and cadmium all had an appreciable effect in reducing the rate of dissolution of synthetic hydroxyapatite in the presence of low concentrations of acidic buffers. Fluoride had the greatest influence, and the overall effect of retarding dissolution by these elements, was attributed to a surface-coating action on the hydroxyapatite crystals. These findings were only suggestive of certain ions in the oral fluids decreasing dissolution of tooth mineral, and with the exception
of fluoride, none of these elements have been shown
to have a topical effect in reducing caries.

Leach considered (84) it was unlikely that
insoluble surface complexes were formed by anions
and suggested that possible presence of multi-charged
ions in the hydrations shell of apatite crystals
retarded the diffusion and dissolution. He tested
the interaction effects of acids (alanine, aspartic,
citric, glutamic, lactic, malic, malonic, succinic
and valine) on powdered enamel and dentine, giving
evidence that, with the exception of citric acid,
the final pH and the amount of decalcification
correlated with the buffering capacity of each solution.
The fact that at equilibrium the amount of enamel
dissolved was proportional to the amount of titratable
acid present, whether or not multi-charged anions
were also present, induced Brudevold et al (23) to
report from these experiments that during acid exposure,
a pH gradient must develop in the enamel; if the pH
is lowest at the surface then the ability of anions
to depress dissolution would be greatest in this
region and less effective in the subsurface region
of higher pH; this would favour subsurface decalcification
lesions.
Studies (53) on enamel dissolution have shown that its rate was primarily dependent on and proportional to the undissociated acid concentration. On such a basis, a mechanism was proposed by Gray et al (57) for incipient carious lesion formation founded on the diffusion of undissociated acid into the enamel to cause subsurface decalcification. "If attack of acid at the surface of the enamel is prevented by certain external conditions (enamel cuticle or soluble organic polymers), the hydrogen ions and undissociated acid molecules of the buffer solution will continue to diffuse past the enamel surface. The structure of dental enamel, with the enamel rods orientated almost perpendicular to the tooth surfaces, serves to channel this ionic diffusion along specific pathways into the enamel. Once past the protected surface the hydrogen ions are free to react with and dissolve tooth substance. However as soon as local concentration of dissolved calcium and phosphate become appreciable, the acid attack will stop – not to resume until the acidic ions and molecules have diffused further into the enamel structure, or until the calcium and phosphate ions have diffused out of the enamel. Cyclic repetition of these diffusion-controlled processes will lead to decalcification of the tooth structure in depth, e.g. subsurface decalcification and white spot formation."
To allow the liquid to solid ratio in the mouth to be maintained in equilibrium, the bathing solution must be of a metastable nature so that its concentration of calcium and phosphate ions are neither too high for precipitation nor too low for dissolution of the tooth to eventuate. Any condition that can vary this metastability of the saliva for the concentration of the calcium and phosphate ions to fall to a level where the saliva is no longer supersaturated, is another causative factor for decalcification.

Schmidt-Nielsen (118) has shown that saliva is nearly always more or less supersaturated with hydroxyapatite and that this supersaturation is evident prior to secretion. The saliva can become unsaturated if acid is present in sufficient quantity to decrease the pH below the limit for saturation and this quantity of acid depends upon the degree of saturation and the buffer capacity of the saliva.

Ericsson (43) calculated a critical pH point for saliva, below which the tooth begins to decalcify, as being generally between 5.5 and 6.5; the rest saliva value being in the lower range and the stimulated saliva value, the upper range. Brudevold (20) pointed out that these calculations were made regrettably with the assumption of a definite solubility product constant for apatite as "the point of departure" which
is now untenable as it tends to ignore the many variables affecting the tooth solubility, and he felt that it was more important to recognise the observation that although levels of calcium, phosphate and pH differ in the parotid and submandibular saliva, the two types were supersaturated to the same degree in regard to apatite. This deduction was made from the work performed by Schmidt-Nielsen (113) who showed in the saliva from a number of subjects that mandibular saliva was markedly supersaturated and parotid saliva considerably less supersaturated.

Later discovery that the solubility of calcium phosphate in saliva is increased at the physiological pH level, more so than one would have imagined, immediately makes it clear that the saliva is not so supersaturated to the high degree as hitherto thought. This increased solubility is a result of influence by the carbonic acid buffer system in saliva and applies to the same degree to the hydroxylapatite of the dental enamel (43). However, if the earlier assumption of enamel protection provided by a high supersaturation is to be reduced, it can be counterbalanced by the fact that a decrease in saturation, by introduction of acid, has proved to be slower than expected. In addition, teeth can
dissolve slowly at neutrality or even in alkaline solutions which are free from calcium and phosphate ions. Hence Ericsson (43) considers "that through its special solubility conditions the saliva exercises a new kind of buffer effect: a resistance to changes in the solubility for calcium phosphate".

Wah Leung (149) has reviewed saliva and dental caries and affirms the general tendency for the buffering capacity of stimulated saliva to be higher in caries-immune persons than in caries-susceptible ones; but this does not define a positive correlation between buffering power and caries since he considers there has been a lack of definite results in measurements, the unique action of the most important carbonic acid; bicarbonate buffer system at oral pH, not having been taken into account. This system is concerned with the elimination of carbon dioxide from the liquid phase and such loss occurs rapidly, as soon as saliva is exposed to the atmosphere of the mouth and reduces the buffering capacity. However, bicarbonate concentration rises with the rate of flow, producing a greater salivary buffering power as well as a higher pH (72).

Ericsson (44) is in accord with the negative correlation between buffering power and caries incidence even though no other salivary factor has a better established relationship to the caries rate, since
the buffering power depends on the amount of weak acids present whose pH values are similar to the oral pH values.

Wah Leung (149) substantiates this lack of correlation between salivary properties and caries with the fact that saliva exerts its primary influence on the plaques and only secondarily affects the tooth surface; yet he conceives the remineralisation of a decalcified area from the precipitation of ions from less stable saliva solutions in caries-free individuals.

Leach (83) has pointed out that the extent of the decalcification that can take place or is about to take place is not indicated by the pH of the buffering solution, the reason being that the solubility in the buffer solution can vary with the amount and nature of the solid material present, e.g., the amount of fluoride ion present in the sample. It is obvious that dissolution is indirectly proportional to the pH of the liquid phase but because of the variability of the enamel and saliva, it is not possible to know to just what extent: however, the pH is the one variable, that by itself, can give an indication as to whether any demineralisation might occur.
Accepting all these variables associated with the enamel solubility, such as the environmental pH value, the amount of undissociated acid present, the "quality" of the saliva and the enamel; it follows that the actual extent of enamel destruction is just as variable. If dissolution factors are maintained at length in vivo, rapid breakdown and cavitation of the enamel surface will occur. However, there is a gradual process of dissolution in vivo with the "cyclic repetition of these diffusion-controlled processes" (57) brought about by either slight variations of the natural state of equilibrium or extreme variations at shorter periods whereby the outer surface remains relatively intact while the subsurface enamel is attacked. This process is a result of the bacterial plaque-tooth system found in nature, when carbohydrate is broken down to produce the necessary acidic buffer solution. Protection against surface attack is afforded by the combination of the mucin, and the calcium and phosphate ions available in the saliva as well as the already organically-acquired resistance of the outer enamel layer itself. It has been shown from the evidence considered previously that the greater resistance of the outer surface is also acquired from the accumulation of fluoride therein and/or the presence of smaller concentrations of carbonate; for if
there is not a general decrease in solubility produced by the effect of fluoride ions, then, following any carbonate exchange with fluoride and phosphate in saliva (20) there is the status of less carbonate available for dissolution and resultant "porosity". The alteration of the tooth's chemical structure following eruption into the oral cavity can be regarded as a process of maturation (101) and is believed to be associated with the action of the saliva which contributes significantly to the ionic exchange and permeability of the enamel.

Gray, Francis and Griebstein (57) have explained that in experimental "white spot" formation, the acid solution always contained at least one natural polymeric substance such as agar, protein or salivary mucin, and in the mouth, the bacterial plaque-tooth system follows this pattern. If surface protection is not entirely brought about by chemical means of the inorganic portion, then it is known that polymeric saccharides and proteins can adsorb on the surface, producing a protective coating effect. Hence interaction with the immediate enamel surface is restricted and the hydrogen ions will tend to diffuse into the immediate subsurface region of lower acidity and Gray and Francis (56) believe this coating effect is essential for the production of the "white spot" seen in early caries.
Sognnaes (131) has stated that salivary organic matter can settle on the tooth surfaces or large organic molecules enter partially-demineralised enamel of carious teeth: the finding of hydroxyproline in the enamel of erupted, and not in unerupted, teeth supports this proposition that there is a post-eruptive influx of organic material from the mouth into the enamel.

On the other hand, Schmidt-Nielsen (118) has demonstrated beyond doubt in 1946 in his experiments on tooth solubility in relation to the composition of saliva, that slightly decalcified surface enamel can remineralise from the inorganic constituents in saliva. Furthermore, Wolf and Neuwirt (151)(152) in 1948, attributed the arrest and repair of caries to the deposition of calcium salts into the decalcified regions; and the fact that remineralisation of decalcified enamel resulted in the deposition of calcium phosphate salts, has also been shown more recently in 1965 by Pickel et al (108). In the latter experiments, enamel which had been softened by exposure to buffered acetic acid, was rehardened by exposure to saliva and the rehardening was enhanced by the addition to the saliva of a variety of calcium and phosphate compounds.
In similar studies by Koulourides, Feigin and Pignan (82) it was found that the addition to saliva of such salts as Ca, $\text{HPO}_4$, NaCl and F, in various strengths and combinations, had the effect of improving the initial rates of the rehardening process but that the final recovery was never greater than for unaltered saliva itself (Fig.15).

![Diagram](image_url)

Fig. 15 Typical rehardening of buffer-softened teeth in saliva (in vitro). Volume of whole saliva = 40 ml.; temperature = 37°C. After two hours the teeth were re-tested for hardness and then re-exposed to freshly collected saliva, the procedure being carried out three times. The curve shows an average for 12 teeth (82).

Koulourides et al (82) considered the extent of rehardening in their experiments was "greater than that reported by Pickel et al (108) who chose milder conditions in order
to increase the possibilities of detecting differences in tested agents." Previous in-vitro work in 1961 by Koulourides, Cueto and Pigman (81) showed that softened teeth regained their initial hardness after four hours in solutions with a Ca/P molar ratio of 1.67 which also contained 1 p.p.m. of fluoride; however, it was noted more recently (82) that when the rehardening solution used was saliva, a concentration of above 20 p.p.m. of fluoride was needed before a marked increase in rehardening activity was revealed.

Heuser (65)(66) considered the possibility that the remineralisation process will occur more intensely in saliva from caries-resistant than from caries-susceptible individuals, could be due to the variety of ions available in the saliva. Moreover Heuser (65)(66) showed that topical application of fluoride ion resulted in a much quicker repair of "enamel defects" than was the case if the repair was left to the action of saliva alone.

Brudevold et al (21) found that salivary sediments invariably calcified when exposed to calcifying solutions since the sediment could act as a matrix for calcification and when this did occur, not only could apatite crystals catalyse the crystallisation but traces of fluoride in the calcifying solution greatly accelerated the mineral formation. Alternatively the crystallisation was affected by the variety of ions in the saliva where the presence of magnesium or carbonate could inhibit its rate.
In 1958 McCann and Fath (89) had used $^{32}$P labeled phosphate to study the effect of various amounts of fluoride on the phosphate exchange of different mineralised tissues and synthetic hydroxyapatite, and found that the fluoride ion increased the rate of exchange of $^{32}$P for up to 140 days with the synthetic apatite, human enamel and dentine, and rat dentine and bone. The rate was also increased but only during the first 24 hours, when the enamel had been previously treated with fluoride, either in vivo or in vitro although in this case, the rate of exchange was less than the untreated control, as the effect of fluoride ion already in the enamel was to decrease the solubility of the enamel.*

Previously it has been assumed that the reduced solubility of fluorapatite as opposed to that of hydroxyapatite is the mechanism of fluoride protection, however, "solubility studies in saliva over a considerable pH range have shown that the solubility of both is controlled by the same activity product, that of CaHPO$_4$."(153) Furthermore, Brudevold et al (23) have reported that unique ability of the presence of fluoride in suspensions of calcium and phosphate ions to induce apatite formation, even at a low pH value of 2.0.

* The initial increase rate of phosphate exchange was probably due to a release of fluoride ions into solution from the enamel rather than the effect of the introduced sodium fluoride as used in the experiments.
This finding and the fact that the $K_{sp}$ of Ca$_5$F$_2$PO$_4$ controlled the solubility of both fluorapatite and hydroxyapatite lead them to conclude that "maintenance of the status quo of the mineral phase in the alternating process of demineralisation and reprecipitation" is the major role of fluoride in caries prevention.

Considering the relatively small and intermittent amount of acid in the dental plaque, it must be assumed that the deviation from equilibrium conditions in the formation of initial caries lesions must also be slight, and that this alternating process of demineralisation and remineralisation takes place. Hence it could be said that the rate of enamel solubility is not as important in protecting teeth against caries as those factors which favour the formation of apatite.
PART III

SURVEY MATERIAL, DESIGN AND METHOD.

Purpose of Study.

This study was undertaken to investigate both the incidence and the clinical appearance of the condition of incipient enamel caries, to record the macroscopic areas and to report on the extent of these areas in caries-susceptible individuals. It was proposed to test the efficacy of inhibiting incipient caries formation by available preventive measures.
Description of the material studied.

The subjects used were 14 to 15 year old students of both sexes who were examined prior to the author's examination by one of two standardised examiners of the Department of Preventive Dentistry in a longitudinal field survey of 2,500 students in 17 Sydney Metropolitan High Schools: in this caries field survey other conditions were recorded such as, staining, opacities, hypoplasia, fluorosis, trauma, hypodontia, or any other clinical aberrations (with the exception of malocclusions) and in addition, both Oral Hygiene and Russell Periodontal indices were scored.

The overall number of 2,500 students had been distributed equally with respect to school, class, sex and age, into four study groups for the original caries survey. These four groups were designated as 1B, 2B, 1C and 2C, and each group received a combined treatment in accordance with the following code:

1. Topical application of a 0.85 per cent saline solution for a 30 seconds duration.
2. Topical application of a 10 per cent stannous fluoride solution for a 30 seconds duration.
B. The associated use of a dentifrice containing no fluoride ion and which was supplied liberally for the subject's own use.
C. The associated use of a dentifrice containing 0.4 per cent stannous fluoride ion and which was also supplied.

This provided four groups in which there were two major variables, one receiving no topical fluoride application
and the other receiving topical fluoride. To each of these variables was added the use or non-use of a fluoride-containing dentifrice.

For further examination of the subsurface decalcification lesions present, 228 subjects were selected within each school; no distinction being made for male or female, nor to whatever fluoride-treatment group the selected 228 subjects belonged. The only requisite for selection was the presence of subsurface decalcification. These subjects were re-examined six months later with regard to their subsurface lesions and of the 228 subjects, 18 subjects were absent and one subject had had orthodontic bands placed on the teeth, so that only 209 subjects were seen at the second examination. The original Topical Fluoride Study at the High Schools had been in progress for one year at the time of selection of subjects for the study of subsurface lesions. This meant that at the author's first examination the group of 228 students had received two "topical fluoride" treatments over one year (at six-monthly intervals) and at the second examination, they had received three treatments over an eighteen months period, i.e.,

Examination 1 = 2 "topical fluoride" treatments over twelve months.

Examination 2 = 3 "topical fluoride" treatments over eighteen months.
The group was subsequently divided into respective fluoride-treatment groups for evaluations to be made. Where prevalences or distributions were summarised at both examinations for comparison, the scoring for the 19 subjects who were not scored at Exam.2 was not included in the calculations for Exam.1, so that resultant figures are given for the identical group of subjects, observed at both examinations.
Details of the clinical examination.

The clinical examinations of the permanent teeth were conducted with the aid of portable equipment which permitted a prophylaxis before the obvious lesions of subsurface decalcification were recorded. A right-angled Doriot type handpiece with a Pascal "prophy" attachment and "prophy" rubber cups were used for the prophylaxis to remove all oral debris and plaque from the tooth surfaces: the respective surfaces were dried by compressed air and then examined in the light of a Planet "Nearlite" Lamp using an Ash No.5 mouth mirror and an Ash No.49 single-ended probe. The lamp had to be moved frequently to obtain maximum effective lighting on the surfaces and indeed there appeared to be an optimum position for each surface whereby the lesion could be recognised. It was necessary to dry these surfaces repeatedly as the subsurface lesion is not easily discernible on a wet surface. The time taken for each examination was from 2 to 13 minutes with an average time of approximately 7 minutes. The areas of subsurface decalcification with any relevant factors were recorded in code by a dental assistant. An example of the simple chart used for this recording is shown as Fig.16; the figure also illustrates an actual chart completed on this programme.
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Age: 13/11  
Date of Birth: 7-4-1851  
Sex: M

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<td>AWD</td>
<td>S4 1g 12 AWD</td>
<td>S4 1g 12 AWE</td>
<td>S4 1g</td>
<td>BWE</td>
<td>S4 1g 23 CHE</td>
<td>S4 1g 21 CNF</td>
</tr>
<tr>
<td>Lower Right</td>
<td>S4 1g</td>
<td>AWD</td>
<td>S4 1g 12 AWD</td>
<td>S4 1g 12 AWE</td>
<td>S4 1g</td>
<td>BWE</td>
<td>S4 1g 23 CHE</td>
<td>S4 1g 21 CNF</td>
</tr>
</tbody>
</table>

Remarks:  
Fig. 16
**Information Recorded.**

1. The tooth.
2. The surface of the tooth on which the subsurface decalcification is evident.
3. The position of the subsurface decalcification on the respective surface.
4. The size of the decalcification area.
5. The shape of the decalcification area.
6. The condition of the surface of the lesion.
7. Pigmentation of the lesion.
8. The type and surface coverage of any relevant restoration or carious lesion.

To determine what information was needed to study the pattern change of subsurface decalcification, many 35 mm. coloured transparencies were studied prior to the clinical examinations. These transparencies had been taken of patients at the previous caries examination in the longitudinal study already quoted and were of excellent quality which allowed for detail to be retained when viewed in magnification on a screen. After the clinical examinations commenced, it was found that there was no need to introduce any alteration of the survey design.
Survey Design and Criteria for Coded Scheme.

The coding system was similar in part to that in use with the Department of Preventive Dentistry, University of Sydney, and which itself had been derived from the method introduced by Klein and Palmer in 1940 (78). On the recording chart the space allotted to each tooth was divided into two, the left-hand division or "box 1" being for the record of the existence and position of either caries, subsurface decalcification or restorations, and the right-hand division or "box 2" for the sole purpose of qualifying the subsurface decalcification scored in the left-hand division.

1. The tooth. If the tooth was observed to have an area of subsurface decalcification, this fact was recorded in box 1 and denoted by the symbol for caries, $S_4$. This would conform to the incipient stage of caries which is regarded by Backer Dirks (5) as "Caries I" where the outer surface of the lesion remains glossy in appearance after drying for three seconds. However, to conform to the standards of the Federation Dentaire Internationale Classification (6), "Caries I" is still listed as being "the white opaque area with loss of lustre" which involves the enamel layer only, i.e., Caries I, and Caries of 1st Degree. Should active caries which now involved the dentine (Caries
of 2nd Degree) be recorded also, the difference was
easily made by the fact that there were no accompanying
and qualifying symbols opposite the $S_4$ sign in box 2;
that this was a subsurface decalcification study and
these lesions were recorded first in the tooth space
allotted.

2. The surface of the tooth. The usual coded numbers
were used for designating the surfaces and these
were:

<table>
<thead>
<tr>
<th>Surface</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lingual</td>
<td>0</td>
</tr>
<tr>
<td>Buccal or Labial</td>
<td>1</td>
</tr>
<tr>
<td>Mesial</td>
<td>2</td>
</tr>
<tr>
<td>Distal</td>
<td>3</td>
</tr>
</tbody>
</table>

The occlusal surface was not included in the recording
of subsurface decalcification, firstly because the
lesion usually takes place within the fissures and
secondly, because if it was to occur as an original
attack elsewhere on the occlusal surface, it was
seen mainly on the inclines of the cusps of second
molar teeth in only a few cases, caused by apparent
stagnation areas produced from the overlying flap of
soft tissue present in the tardy eruption of these
teeth. The prevalence of these lesions was too small
a number to influence the survey and was of a maximum
0.2 per cent observable occurrence.
Naturally, the proximal smooth surfaces were seen best in the cases of appositional loss of contact and otherwise, were recorded as well as they could be observed and thus this was not complete recording as for buccal (labial)/lingual surfaces.

3. The position of the lesion. To describe the position of the lesion on the tooth surface it was originally designed to either sketch the lesion on a chart depicting tooth shapes and surfaces, or photograph the lesion on the basis of the Scandinavian Moulage System of comparative photography (86). Although both methods would be an accurate description, they were discarded for the reason that the first involved more-complicated charts and more time, while the second was a question of high cost. The method adopted was arbitrary but descriptive in that the author regarded each surface as an entity of three horizontal sections, gingival, middle and incisal, each section being divided again vertically into three sections giving a total of nine positions on any one surface. (The work "incisal" was used in preference to "occlusal" since the initial for the latter, "o", would be confused with the designation for the lingual surface.) This division is illustrated in Fig.17. The actual division of the surfaces was not done precisely but was similar to the differentiation
between buccal and lingual surfaces and proximal surfaces in clinical scoring of caries by surface.

**Diagram 1.** A lesion occurring around the buccal pit was considered to be in the middle buccal or m$^1$ position; if it extended up the buccal groove towards the occlusal surface then it was in the middle incisal buccal or m$^1$I$^1$ or simply m$^1$I position. When the lesion was regarded as extending towards the mesial or distal surfaces on this buccal surface, then the number of the direction of extent was merely added to that already scored, e.g., a lesion which extended around the gingival margin from the mesial to the distal embrasure was called as S$^4$I$^2$g$^1$ and written as such in box 1.

**Diagram 2.** The surface division of a smaller tooth such as the bicuspid is identical except that the g$^2$ and g$^3$ positions were much smaller in area and unless the lesion showed obvious extension in an incisal direction towards the distal and mesial embrasures, the position was mostly called as simply g$^1$.

**Diagram 3.** The surface divisions are the same as for those in diagram 1. Only the directional numbers are altered.

**Diagram 4.** This diagram illustrates the division of the distal surface of an upper central tooth and it can be seen that in the incisor teeth
Fig. 17. On opposite page. Illustrations of the surfaces of the teeth to show the division into surface positions. For history of the separate diagrams, refer to Section 3 on the position of the lesions.

Diagram 1. Top Left Side.
Buccal surface of lower first molar tooth.

Diagram 2. Top Right Side.
Lingual surface of lower first premolar tooth.

Diagram 3. Lower Left Side.
Mesial surface of lower second premolar tooth.

Diagram 4. Lower Right Side.
Distal surface of upper central tooth.
the incisal third is narrow and did not necessitate subdivision unless desired.

4. The size of the lesion. Thus the lesion could be depicted on a surface in a certain position and although this was sufficient information for epidemiological study it was deemed necessary to qualify the lesion further to observe possible pattern change. The size and shape of the lesion were called first and the size was denoted by the capital letter A, B, or C, which denoted merely small, moderate or large size, and this symbol together with the other capital letters called was written down in box 2.

5. The shape of the lesion. Four shapes were noted from the preliminary examination of the photographs and these were denoted by the letters W, X, Y and Z, depicting "crescent", "trapezoidal", "cloud" and "speckled" shapes respectively. The recording of the size and shape was a combined description and this provided twelve types of lesion which are summarised with the author's criteria below:

AW - the lesion was crescent-shaped or less often, a straight line shape and less than 1mm. in width. It could extend through any position, i.e., be of any length. The straight line lesions were small and appeared as crescents when they increased in length to follow the contour of the tooth surface.
BW - the lesion was approximately 1-2 mm wide and could extend in any position.

CW - the crescent shape was still retained but the lesion was wider than 2 mm, and extended in any position.

AX - this combined symbol represented a large crescent-shaped lesion which had lost its true crescent appearance by loss of the upper concavity. It was thought that this shape was nondescript and in some degree resembled a trapezium at the AX stage and the term "trapezoidal" shape was used for all sizes of X.

BX - this lesion was virtually the same as AX but was larger in size with involvement of two of the horizontal sections, e.g., the gingival and middle thirds combined or the middle and incisal thirds combined.

CX - the requirement for this shape was that it had been originally an obvious crescent shape and was now so large that it resembled a cloud-shaped lesion. The former sharper defined borders of the lesion had become uneven and spread in any direction. This change usually applied to the incisal border.

AY - a small cloud-shaped lesion which was simply equivalent to the "white or chalky" spot.
BY - the spot had increased in size, was still present in only one tooth position but showed more shape than the fleck seen and recorded as AY.

CY - a cloud-shaped lesion covering more than one surface position. The criterion for its being recorded as CY as opposed to a CX was based purely on the author's assumption of its origin and obvious less involvement at the gingival third.

AZ - when two "white spots" were observed on one tooth surface position they were recorded as AY and written down on the chart twofold. If there existed more than two "white spots" on one position, then the shape was considered as speckled and was recorded as AZ.

BZ - these two combined symbols were used to depict and C2 increasingly larger speckled lesions and were used somewhat arbitrarily, the emphasis being on size. It was more than probable that BY and CY lesions could be derived also from unification of the "spots" in any of the three speckled lesions, i.e., from either AZ, BZ or C2.

(examples of the above shapes are given in Part VI, the Appendix.)

6. The condition of the surface of the lesion. The condition was tested by drawing the probe lightly to and fro across the surface and was recorded with any
pigmentation present in box 2 directly following the
symbols for size and shape.
D - smooth surface; the outer surface layer was intact
and "glassy" both in appearance and feel.
E - etched surface; upon drying with compressed air
the surface had lost its shine and there was a
chalk-like feel to the probe so that the surface
could be scratched visibly. This was evidence of
microscopic cavitation and is termed as an
"initial lesion" under the F.D.I. classification
of dental caries (6).
F - surface broken; this symbol was recorded if there
was a catch of the probe which showed physical
collapse of the outer surface layer, without
detection of a softened cavity floor on light
pressure of the probe. The F symbol was modified
by the usual code numbers 0, 1, 2, 3, to show the
position of the break or breaks found on that area
of subsurface decalcification recorded with no
regard to the number of breaks present. Under the
F.D.I. classification of caries (6) this lesion
was now defined as "clinical caries". If there
were more than two or three breaks in the surface,
it usually meant that the caries had progressed to
dentinal involvement and thus it would be scored
solely as a clinical cavity rather than as a region
of subsurface decalcification.
7. **Pigmentation.** The main concern in recording the pigmentation of the lesion was its prevalence and not the degree of pigmentation. It was acknowledged that there would exist some extrinsic pigmentation in the decalcified regions and since the author's observations were being conducted on students taking part in a fourfold study involving the availability and non-availability of stannous ions, there would also be some additional extrinsic pigmentation (94) (97). The symbol P was recorded only when the pigmentation was observed and was modified by the numbers 1, 2, or 3 to show the degree of colour:

- $P^1$ - slight brown colour as opposed to the "white spot".
- $P^2$ - moderate brown colour, or greenish brown colour, or perhaps a darker brown shade present only at the margins of the lesion.
- $P^3$ - very dark brown to black colour present uniformly throughout the decalcified region.

8. **Relevant restorations and carious cavities.** If an area of subsurface decalcification was associated by contact with either a carious cavity or a restoration, the + sign was written in box 1 below the recorded area, and underneath this the required information depicting the cavity or restoration, this being recorded in the code as normally used by the department. On the other hand, if it was desirous to record a
cavity or restoration that was not associated with
the observed "white spot" area, then the - sign was
merely substituted for the + sign. To summarise
the + and - signs, the former denoted "association
with" and the latter denoted "no association with"
and these signs always applied to the area of
subsurface decalcification recorded prior to the sign.
Other examples of the variations of the + sign are
as follows:

+1 = association buccally.
+2 = association mesially
+3 = association distally.
Ø = association around, i.e., the area of
decalcification surrounds the cavity
or restoration.
+g²13 = association gingivally from the mesial
to the distal embrasure on the buccal
surface.

With regard to the recording of relevant
restorations, there existed one other variation with
the symbol S₅. This symbol denoted any restoration
in the departmental coding system but was used in this
programme exclusively to denote amalgam restorative
work.

It was modified to Sil₅ and Sau₅ to register
silicate-type and gold restorations respectively,
and this differentiation was made in order to provide
a more accurate identification of the lesions under
scrutiny at the following examination.
The usual appropriate coded surface numbers for restorations were called in conjunction with these symbols and were:

5 = occlusal.
6 = lingual.
7 = buccal.
8 = mesial.
9 = distal.

For further description of the coded scheme, an appendix of photographs is given as Part VI of this thesis. The photographs shown have been taken of lesions observed in the author's examinations and are accompanied by the coded recording and a brief explanation of this recording.
Method of assessment of data.

Part 1.

Following completion of the first subsurface lesion examination, possible computations of the four main categories of shape which could be found clinically were evolved and the distribution of all these resultant types noted. This distribution was done on the basis of being either maxillary or mandibular, and in both arches, with respect to the buccal and lingual surfaces.

It had been stated previously in the survey design that if proximal surfaces revealed subsurface lesions which could be recorded accordingly, this would be carried out. It was found that only 27 and 7 proximal surfaces were so involved at the 1st and 2nd examinations respectively and thus observations on the lesions of the proximal surfaces were disregarded and the study confined to the buccal/lingual surfaces.

When the information had been recorded on the charts at each of the two examinations, the charts were distributed into their respective fluoride-treatment groups and a mean figure, along with the standard error and the range of measurements, was calculated for each group at each stage of examination. A mean figure was calculated for the following prevalences at both examinations:

(i) the number of buccal/lingual surfaces per subject affected by subsurface decalcification lesions.
(ii) the number of actual decalcification lesions per subject on all the affected buccal/lingual surfaces, and

(iii) the number of teeth per subject affected by decalcification lesions on their buccal/lingual surfaces.

The mean figures were calculated for the above three prevalences after the fluoride treatment groups were combined into their "topical solution" groups irrespective of the dentifrice being used and alternatively, into their "dentifrice" groups irrespective of the topical solution being received; i.e., \((1B + 1C, 2B + 2C)\) and \((1B + 2B, 1C + 2C)\).

The mean changes between examinations were also calculated for the above prevalences \((i), (ii)\) and \((iii)\), and an analysis of these mean changes was made, using the within-class error as an estimate of the residual variance, to determine statistical significances.

Furthermore, to determine whether there was any sex difference, a mean figure of only the number of affected buccal and lingual surfaces was also calculated for the combined males and combined females, irrespective of their fluoride-treatment groups.

The various degrees of pigmentation of those lesions involved were totalled for all groups, separate and combined, and to enable any significant differences in the overall degree of pigmentation to be shown,
the $p^1$, $p^2$ and $p^3$ scores were again totalled to ascertain the sum of all the pigmented subsurface lesions present. This was then given as a percentage of subsurface lesions affected by pigmentation and the differences between proportions tested for statistical significance.

The same survey design and criteria were used for a dental examination (subsurface lesions) on another group of 70 students (12 to 15 years old) who were not participating in the Topical Fluoride High School study and were attending one High School outside the metropolitan area (Tamworth, N.S.W.). The same three prevalences for subsurface lesions, surfaces and teeth affected, as well as the percentage of pigmentation were calculated and comparisons made.

In addition to the author's examinations, the prevalence of areas of subsurface decalcification as observed by another departmental examiner (N.D.M.) during his routine examination for dental caries experience, is given. Detection of these areas was not aided by prior prophylaxis or drying with compressed air. The incidence of these observed lesions at three examinations over a period of twelve months is shown.
An assessment was made of the clinical change that had occurred with decalcification lesions on the buccal/lingual surfaces to learn how far these lesions had either progressed or to what degree their progress was arrested significantly in the various experimental groups. The completed charts for the same group of 209 subjects who were seen at the two examinations were compared for each individual and the information recorded is listed below.

It should be noted that estimations of all the scores of those characteristics which dealt with conditional changes, i.e., size, surface condition, caries arrestment or caries progression, (g) to (n) inclusive), were carried out only on lesions which were the same subsurface lesions at both examinations. Identification for these was aided by the surface-position score plus any associations marked down.

To determine the size changes (g) and (h), use was made not only of the size and shape denominations (A, B, C and W, X, Y, Z) but also of the various recorded extensions and associations (carious or restorative).

In all cases, a decrease of score was disregarded if it had occurred by restorative work or extraction of the tooth having been carried out since Exam. 1.
Information summarised for the same group of 209 subjects at the two examinations and shown in Tables 21, 22, 23 was:

(a) the increase number of surfaces affected 
(b/l), which was obtained by subtracting 
the sum of affected surfaces at Exam. 1 from 
those seen at Exam. 2.

or alternatively,

(b) the decrease number of surfaces affected 
(b/l), i.e., the number at Exam. 1 less 
the number of Exam. 2.

(c) the number of new separate lesions now 
seen at Exam. 2 but not seen at Exam. 1,

or alternatively,

(d) the number of separate lesions not seen at 
Exam. 2 but seen at Exam. 1.

(e) the increase number of teeth affected by 
subsurface lesions on their buccal/lingual 
surfaces,

or alternatively,

(f) the decrease number of teeth so affected.

(g) the number of lesions that have increased in size, 
and,

(h) the number that have apparently decreased in 
size and are still obvious.
(i) the number of surfaces of the subsurface lesions that have apparently hardened or "remineralised", i.e., their surface-condition designations have altered from E to D, F to E or F to D. If part of the lesion was recorded at Exam. 2 as having become a clinical carious cavity now involving dentine, then F to E and/or F to D designations for these subsurface lesions were not included as showing the surface had "remineralised" since the broken surface (F) had in part progressed to dentinal involvement.

(j) the number of surfaces that had now etched from being hard surfaces, (D to E).

(k) the number of subsurface lesions whose surfaces now registered a break, (D to F, E to F).

(l) the number of subsurface lesions which had become complete clinical cavities involving dentine and were now scored solely as such. In other words, a subsurface lesion was recorded on a b/l surface at Exam. 1, with or without a break in its surface (F); at Exam. 2 a clinical cavity was obvious and no subsurface lesion.

(m) the number of subsurface lesions that had become involved with clinical cavities at Exam. 2, i.e., part of the lesion had become a clinical cavity
with dentinal involvement (either on the buccal or lingual surface of the tooth) and there was still evidence of the original subsurface decalcification.

(n) the number of subsurface lesions that showed involvement with clinical cavities at Exam. 1 and which were now at Exam. 2 involved with what appeared to be an arrested carious lesion.

The scores for (a) to (n) inclusive were transferred to I.B.M. punch cards for processing and the required means, standard deviations, standard errors as well as correlation coefficients were obtained. Calculations for the numbers of buccal/lingual surfaces affected, of separate lesions on the buccal/lingual surfaces and of teeth affected by lesions on their buccal/lingual surfaces, had already been made so that for further increments or decrements to be shown, the relevant number of subsurface lesions present at the original Exam. 1 were added also to the I.B.M. punch cards, as well as the number of subsurface lesions which had hard glossy surfaces at Exam. 1 (D), the number of subsurface lesions which had etched surfaces at Exam. 1 (E), the number of subsurface lesions which had a surface break(s) at Exam. 1 (F), and the number of subsurface lesions which were involved already with a carious cavity at Exam. 1.
The obtained means in each fluoride treatment group were compared to those obtained for the control Group 1B and the probability of any differences being due to chance was tested using the Values of $t$, a table of areas in two tails of the normal curve.
PART IV.
RESULTS.
A. Epidemiological Aspects.

Prevalence and distribution of subsurface lesions.

At Exam. 1 2,671 separate subsurface lesions were recorded on a group of 228 subjects whose mean age was 14 years 5 months. The prevalence of subsurface lesions was 100 per cent as the subjects had been selected for study for precisely this reason, however, it was found that the range was 2 to 28 lesions for the total group at the first examination. At Exam. 2 which was six months later for the total group, 19 subjects were absent from examination so that only 209 subjects were seen and for this total group, 2,654 lesions were recorded and the range was 1 to 29 lesions present. (Table 2).

When the scoring for those absent 19 subjects was excluded from the values obtained at Exam. 1, it was found that the mean age for the 209 subjects seen at both examinations was still 14 years 5 months, however, only 2,464 lesions had been observed for the same group at the first examination. (Table 1).

Because an insufficient number of lesions on proximal smooth surfaces was recorded for any comparisons to be made between the two examinations,
and because it was incomplete recording with these lesions, observations were restricted to the buccal (labial)/lingual smooth surfaces to study the distribution of lesions in the respective groups and the prevalences of surfaces and teeth affected by such lesions.

Tables 1 and 2, 4 and 5, 7 and 8 present the mean values, for the same group observed at both examinations, of all the subsurface decalcification lesions present on the buccal/lingual surfaces in each mouth, of the buccal/lingual surfaces in each mouth affected by such lesions and, of the teeth in each mouth that are affected by subsurface decalcification lesions on their buccal/lingual surfaces.

Table 1 shows that the mean number of lesions able to be observed on the buccal/lingual surfaces of the teeth of the total group was 11.9 ± 0.38, where the mean values for the respective fluoride treatment groups were 12.7 ± 0.71 for Group 1B, 11.1 ± 0.61 for Group 2B, 11.2 ± 0.74 for Group 1C and 12.3 ± 0.79 for Group 2C. Although these means reveal some trend in the inhibitory effectiveness of the three fluoride treatment groups when compared to the control Group 1B, the differences between values were not large enough to be statistically significant at the 5 per cent level.
The groups were combined to determine the figures for the entire group receiving Solution 1 as compared to those receiving Solution 2, irrespective of the toothpaste being used (B or C) and combined again to compare the group using Dentifrice B to that using Dentifrice C, irrespective of the topical solution being applied. The mean value for the combined Group 1B + 1C (Solution 1) was 12.1 ± 0.52 and for the combined Group 2B + 2C (Solution 2) it was 11.7 ± 0.57, revealing an insignificant difference of 0.4 lesions. The mean values for combined Group 1B + Group 2B (Dentifrice B) and for combined Group 1C + Group 2C (Dentifrice C) were 12.0 ± 0.51 and 11.8 ± 0.52 respectively, revealing no significant difference.

At Exam. 2 six months later (Table 2) the mean number of subsurface lesions for the total group was calculated at 12.7 ± 0.39 which meant an increase of 0.8 lesions per individual on the buccal/lingual surfaces over a period of six months for this age group, however this increment was not statistically significant.

The mean values recorded at Exam. 2 for the three fluoride treatment groups were less than the control Group 1B value and again portrayed a possible inhibitory effect in these groups, where the mean number of subsurface lesions on buccal/lingual
surfaces per subject was 13.4 ± 0.70 for Group 1B, 12.0 ± 0.75 for Group 2B, 12.2 ± 0.91 for Group 1C and 12.9 ± 0.80 for Group 2C: again the differences were not large enough to show a statistical significance. There was at Exam. 2 an insignificant difference of 0.6 lesions between the total group receiving Solution 1 (1B + 1C) and that receiving Solution 2 (2B + 2C), and an insignificant difference of 0.2 lesions between the total group using Dentifrice B (1B + 2B) and that group using Dentifrice C (1C + 2C).

The means were 13.0 ± 0.55 and 12.4 ± 0.54 for combined Group 1B + Group 1C and for combined Group 2B + Group 2C respectively; and 12.8 ± 0.51 and 12.6 ± 0.59 for combined Group 1B + Group 2B and for combined Group 1C + Group 2C respectively.

The values of the mean change per individual between examinations for the four fluoride treatment groups are given in Table 3. The variance analysis of the differences between the two examinations revealed that, although there was a significant change between examinations (P<.001), the interactions of either the dentifrice, the solution or the dentifrice x the solution were all insignificant.
Table 1. The number of subsurface decalcification lesions on all the affected buccal/lingual surfaces of the teeth in 209 high school subjects observed at Exam. 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Subjects</th>
<th>Total No. of Lesions</th>
<th>Range</th>
<th>Mean No. of Lesions ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>66</td>
<td>839</td>
<td>3 to 28</td>
<td>12.7 ± 0.71</td>
</tr>
<tr>
<td>2B</td>
<td>54</td>
<td>576</td>
<td>3 to 28</td>
<td>11.1 ± 0.81</td>
</tr>
<tr>
<td>1C</td>
<td>41</td>
<td>459</td>
<td>4 to 22</td>
<td>11.2 ± 0.74</td>
</tr>
<tr>
<td>2C</td>
<td>48</td>
<td>590</td>
<td>2 to 22</td>
<td>12.3 ± 0.79</td>
</tr>
<tr>
<td>1B + 1C</td>
<td>107</td>
<td>1,298</td>
<td>3 to 28</td>
<td>12.1 ± 0.52</td>
</tr>
<tr>
<td>2B + 2C</td>
<td>102</td>
<td>1,166</td>
<td>2 to 28</td>
<td>11.7 ± 0.57</td>
</tr>
<tr>
<td>1B + 2B</td>
<td>120</td>
<td>1,415</td>
<td>3 to 28</td>
<td>12.0 ± 0.51</td>
</tr>
<tr>
<td>1C + 2C</td>
<td>89</td>
<td>1,049</td>
<td>2 to 22</td>
<td>11.8 ± 0.52</td>
</tr>
<tr>
<td>Total Group</td>
<td>209</td>
<td>2,464</td>
<td>2 to 28</td>
<td>11.9 ± 0.38</td>
</tr>
</tbody>
</table>
Table 2. The number of subsurface decalcification lesions on all the affected buccal/lingual surfaces of the teeth in 209 high school subjects observed at Exam. 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Subjects</th>
<th>Total No. of Lesions</th>
<th>Range</th>
<th>Mean No. of Lesions</th>
<th>± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>66</td>
<td>887</td>
<td>3 to 27</td>
<td>13.4</td>
<td>0.70</td>
</tr>
<tr>
<td>2B</td>
<td>54</td>
<td>649</td>
<td>2 to 29</td>
<td>12.0</td>
<td>0.75</td>
</tr>
<tr>
<td>1C</td>
<td>41</td>
<td>499</td>
<td>2 to 27</td>
<td>12.2</td>
<td>0.91</td>
</tr>
<tr>
<td>2C</td>
<td>48</td>
<td>619</td>
<td>1 to 24</td>
<td>12.9</td>
<td>0.80</td>
</tr>
<tr>
<td>1B + 1C</td>
<td>107</td>
<td>1,386</td>
<td>2 to 27</td>
<td>13.0</td>
<td>0.55</td>
</tr>
<tr>
<td>2B + 2C</td>
<td>102</td>
<td>1,268</td>
<td>1 to 29</td>
<td>12.4</td>
<td>0.54</td>
</tr>
<tr>
<td>1B + 2B</td>
<td>120</td>
<td>1,536</td>
<td>2 to 29</td>
<td>12.8</td>
<td>0.51</td>
</tr>
<tr>
<td>1C + 2C</td>
<td>89</td>
<td>1,118</td>
<td>1 to 27</td>
<td>12.6</td>
<td>0.59</td>
</tr>
<tr>
<td>Total Group</td>
<td>209</td>
<td>2,654</td>
<td>1 to 29</td>
<td>12.7</td>
<td>0.39</td>
</tr>
</tbody>
</table>
Table 3. Analysis of the Differences between Tables 1 and 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>1B</th>
<th>2B</th>
<th>1C</th>
<th>2C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>66</td>
<td>54</td>
<td>41</td>
<td>48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>(\bar{x})</th>
<th>(\bar{x}^2)</th>
<th>(\Sigma x)</th>
<th>(\Sigma x^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.79</td>
<td>52</td>
<td>718</td>
<td>677.03</td>
</tr>
<tr>
<td></td>
<td>0.91</td>
<td>49</td>
<td>649</td>
<td>604.54</td>
</tr>
<tr>
<td></td>
<td>0.98</td>
<td>40</td>
<td>352</td>
<td>312.98</td>
</tr>
<tr>
<td></td>
<td>0.56</td>
<td>27</td>
<td>467</td>
<td>451.81</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>(\Sigma (x-\bar{x})^2)</th>
<th>(S^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>677.03</td>
<td>10.42</td>
</tr>
<tr>
<td></td>
<td>604.54</td>
<td>11.41</td>
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<tr>
<td></td>
<td>312.98</td>
<td>7.82</td>
</tr>
<tr>
<td></td>
<td>451.81</td>
<td>9.61</td>
</tr>
</tbody>
</table>

Variance for testing comparisons = 0.76
Standard deviation = 0.87

Comparisons:

Examinations
\[\bar{x}_{1B} + \bar{x}_{2B} + \bar{x}_{1C} + \bar{x}_{2C} = 3.23\]
Statistical Significance
S.S. P<.001

Dentifrice x Examinations
\[\bar{x}_{1B} + \bar{x}_{2B} - \bar{x}_{1C} - \bar{x}_{2C} = 0.16\]
N.S.

Solution x Examinations
\[\bar{x}_{2B} + \bar{x}_{2C} - \bar{x}_{1C} - \bar{x}_{1B} = 0.29\]
N.S.

Dentifrice x Solution x Examinations
\[\bar{x}_{2C} - \bar{x}_{2B} - \bar{x}_{1C} + \bar{x}_{1B} = 0.53\]
N.S.

Abbreviations:

\(\bar{x}\) = the mean change between examinations.
\(\Sigma x\) = the sum of the differences.
\(\Sigma x^2\) = the sum of the squares of the differences.
\(\Sigma (x-\bar{x})^2\) = the sum of the squares of the deviations of the differences from the mean change.
\(S^2\) = the square of the standard deviation of the mean change (variance).
S.S. = Statistically significant.
N.S. = not statistically significant.
Tables 4 and 5 show the prevalence of buccal (labial) and lingual surfaces affected by subsurface decalcification. At Exam. 1 (Table 4), the mean number of buccal/lingual surfaces affected in each mouth was $11.5 \pm 0.63$ in Group 1B, $10.0 \pm 0.79$ in Group 2B, $10.2 \pm 0.67$ in Group 1C, $11.2 \pm 0.73$ in Group 2C and $10.7 \pm 0.36$ for the total group of 209 subjects. All the groups, 2B, 1C and 2C, showed a trend towards a slight inhibitory effect on the number of surfaces affected by lesions when compared to Group 1B, however, the differences between these means and that of Group 1B were not great enough to show a statistical significance.

When the groups were combined, the mean value for the combined Group 1B + Group 1C (Solution 1) was $11.0 \pm 0.49$ and for the combined Group 2B + Group 2C (Solution 2) it was $10.6 \pm 0.54$, the difference being 0.4 and non-significant. The mean value for the combined Group 1B + Group 2B (Dentifrice B) was $10.8 \pm 0.52$ and for the combined Group 1C + Group 2C (Dentifrice C) it was $10.7 \pm 0.50$, the difference being only 0.1 affected surfaces and not statistically significant.

Table 5 shows the figures obtained at Exam. 2 for the prevalence of buccal/lingual surfaces affected and the mean values for the respective groups were:
Group 1B, 12.1 ± 0.66; Group 2B, 10.8 ± 0.67; Group 1C, 10.8 ± 0.79; Group 2C, 11.6 ± 0.72. Although the mean values for groups 2B, 1C and 2C were all below that of Group 1B, none of the differences from Group 1B was statistically significant.

Comparing the two topical solutions, the mean for combined Group 1B + Group 1C (Solution 1) was 11.6 ± 0.50 and for combined Group 2B + Group 2C (Solution 2) it was 11.2 ± 0.49, a non-significant difference of 0.4 surfaces. For combined Group 1B + Group 2B (Dentifrice B) the mean was 11.5 ± 0.47 and for combined Group 1C + Group 2C (Dentifrice C) it was 11.2 ± 0.53, the difference of 0.3 again being not statistically significant. For the total groups in Table 5, the mean value was 11.4 ± 0.35 buccal/lingual surfaces affected which showed an increase of 0.7 affected surfaces in six months above the mean value obtained at Exam. 1 but this increment was not statistically significant.

The mean number of surfaces affected at Exam. 1 for the total number of males was 11.6 ± 0.51 whereas it was only 9.7 ± 0.48 for the total number of females. This was a significant difference of 1.9 surfaces (P=.01). At Exam. 2 the mean values were 12.2 ± 0.50 for total males and 10.6 ± 0.48 for total females, a significant difference of 1.6 affected surfaces (P=.02).
The analysis of variance for the mean change between examinations showed (Table 6) that, although there was an overall significant change ($P<.01$) the interactions of either the dentifrice, the solution or the dentifrice $\times$ the solution were all insignificant.
Table 4. The number of buccal/lingual surfaces affected by subsurface lesions in 209 high school subjects observed at Exam. 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Subjects</th>
<th>Total No. of Surfaces</th>
<th>Range</th>
<th>Mean No. of Surfaces</th>
<th>± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>66</td>
<td>760</td>
<td>3 to 24</td>
<td>11.5</td>
<td>0.68</td>
</tr>
<tr>
<td>2B</td>
<td>54</td>
<td>523</td>
<td>2 to 26</td>
<td>10.0</td>
<td>0.79</td>
</tr>
<tr>
<td>1C</td>
<td>41</td>
<td>417</td>
<td>3 to 21</td>
<td>10.2</td>
<td>0.67</td>
</tr>
<tr>
<td>2C</td>
<td>48</td>
<td>539</td>
<td>1 to 21</td>
<td>11.2</td>
<td>0.73</td>
</tr>
<tr>
<td>1B + 1C</td>
<td>107</td>
<td>1,177</td>
<td>3 to 24</td>
<td>11.9</td>
<td>0.49</td>
</tr>
<tr>
<td>2B + 2C</td>
<td>102</td>
<td>1,062</td>
<td>1 to 26</td>
<td>10.6</td>
<td>0.54</td>
</tr>
<tr>
<td>1B + 2B</td>
<td>120</td>
<td>1,283</td>
<td>2 to 26</td>
<td>10.8</td>
<td>0.52</td>
</tr>
<tr>
<td>1C + 2C</td>
<td>89</td>
<td>956</td>
<td>1 to 21</td>
<td>10.7</td>
<td>0.50</td>
</tr>
<tr>
<td>Total Group</td>
<td>209</td>
<td>2,239</td>
<td>1 to 26</td>
<td>10.7</td>
<td>0.36</td>
</tr>
<tr>
<td>Total Males</td>
<td>109</td>
<td>1,270</td>
<td>3 to 26</td>
<td>11.6</td>
<td>0.51</td>
</tr>
<tr>
<td>Total Females</td>
<td>100</td>
<td>969</td>
<td>1 to 22</td>
<td>9.7</td>
<td>0.48</td>
</tr>
</tbody>
</table>
Table 5. The number of buccal/lingual surfaces affected by subsurface lesions in 209 high school subjects observed at Exam. 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Subjects</th>
<th>Total No. of Surfaces</th>
<th>Range</th>
<th>Mean No. of Surfaces</th>
<th>± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>66</td>
<td>300</td>
<td>3 to 26</td>
<td>12.1</td>
<td>0.66</td>
</tr>
<tr>
<td>2B</td>
<td>54</td>
<td>584</td>
<td>2 to 26</td>
<td>10.8</td>
<td>0.67</td>
</tr>
<tr>
<td>1C</td>
<td>41</td>
<td>443</td>
<td>2 to 23</td>
<td>10.3</td>
<td>0.79</td>
</tr>
<tr>
<td>2C</td>
<td>48</td>
<td>555</td>
<td>1 to 21</td>
<td>11.6</td>
<td>0.72</td>
</tr>
<tr>
<td>1B + 1C</td>
<td>107</td>
<td>1,243</td>
<td>2 to 26</td>
<td>11.6</td>
<td>0.50</td>
</tr>
<tr>
<td>2B + 2C</td>
<td>102</td>
<td>1,139</td>
<td>1 to 26</td>
<td>11.2</td>
<td>0.49</td>
</tr>
<tr>
<td>1B + 2B</td>
<td>120</td>
<td>1,384</td>
<td>2 to 26</td>
<td>11.5</td>
<td>0.47</td>
</tr>
<tr>
<td>1C + 2C</td>
<td>89</td>
<td>998</td>
<td>1 to 23</td>
<td>11.2</td>
<td>0.53</td>
</tr>
<tr>
<td>Total Group</td>
<td>209</td>
<td>2,382</td>
<td>1 to 26</td>
<td>11.4</td>
<td>0.35</td>
</tr>
<tr>
<td>Total Males</td>
<td>109</td>
<td>1,325</td>
<td>1 to 26</td>
<td>12.2</td>
<td>0.50</td>
</tr>
<tr>
<td>Total Females</td>
<td>100</td>
<td>1,057</td>
<td>2 to 22</td>
<td>10.6</td>
<td>0.48</td>
</tr>
</tbody>
</table>
Table 6. Analysis of the Differences between Tables 4 and 5.

<table>
<thead>
<tr>
<th>Group</th>
<th>1B</th>
<th>2B</th>
<th>1C</th>
<th>2C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>66</td>
<td>54</td>
<td>41</td>
<td>48</td>
</tr>
</tbody>
</table>

\[ \bar{X} \]

\begin{align*}
\bar{X} & = 0.68 & 0.78 & 0.61 & 0.33 \\
\Sigma X & = 45 & 42 & 25 & 16 \\
\Sigma X^2 & = 583 & 544 & 217 & 306
\end{align*}

\[ \Sigma (x-\bar{x})^2 \]

\begin{align*}
\Sigma (x-\bar{x})^2 & = 552.32 & 511.33 & 201.76 & 300.67 \\
S^2 & = 8.50 & 9.65 & 5.04 & 6.40
\end{align*}

Variance for testing comparisons = 0.56

Standard deviation = 0.76

**Comparisons:**

**Examinations**

\[ \bar{X}_{1B}+\bar{X}_{2B}+\bar{X}_{1C}+\bar{X}_{2C} \]

\[ = 2.40 \quad S.S. \ P<.01 \]

**Dentifrice x Examinations**

\[ \bar{X}_{1B}+\bar{X}_{2B}-\bar{X}_{1C}-\bar{X}_{2C} \]

\[ = 0.52 \quad N.S. \]

**Solution x Examinations**

\[ \bar{X}_{2B}+\bar{X}_{2C}-\bar{X}_{1C}-\bar{X}_{1B} \]

\[ = 0.18 \quad N.S. \]

**Dentifrice x Solution x Examinations**

\[ \bar{X}_{2C}-\bar{X}_{2B}+\bar{X}_{1C}+\bar{X}_{1B} \]

\[ = 0.37 \quad N.S. \]

**Abbreviations:**

\[ \bar{X} = \text{the mean change between examinations.} \]

\[ \Sigma X = \text{the sum of the differences.} \]

\[ \Sigma X^2 = \text{the sum of the squares of the differences.} \]

\[ \Sigma (x-\bar{x})^2 = \text{the sum of the squares of the deviations of the differences from the mean change.} \]

\[ S = \text{the square of the standard deviation of the mean change (variance).} \]

**S.S.** = statistically significant.

**N.S.** = not statistically significant.
Tables 7 and 8 present the prevalence of the teeth affected by subsurface lesions on their buccal/lingual surfaces. At Exam. 1 (Table 7), the mean number of teeth affected in the total group was $8.8 \pm 0.30$ where the mean value for the respective groups were: Group 1B, $9.2 \pm 0.57$; Group 2B, $8.2 \pm 0.65$; Group 1C, $8.4 \pm 0.57$ and Group 2C, $9.2 \pm 0.61$. These values showed no statistically significant differences.

In comparing the combined groups, the values were for combined Group 1B + Group 1C (Solution 1) and combined Group 2B + Group 2C (Solution 2), $8.9 \pm 0.41$ and $8.7 \pm 0.45$ respectively. The mean value for combined Group 1B + Group 2B (Dentifrice B) was $8.8 \pm 0.43$ and for combined Group 1C + Group 2C (Dentifrice C) it was $8.8 \pm 0.42$. Again there were no statistically significant differences.

At Exam. 2 (Table 8), the mean value for the total group was $9.2 \pm 0.33$ teeth affected which was an insignificant increment 0.4 teeth. The mean values for the individual groups were: Group 1B, $9.8 \pm 0.53$; Group 2B, $8.5 \pm 0.62$, Group 1C, $8.6 \pm 0.66$ and Group 2C, $9.5 \pm 0.63$. Although the figures for Groups 2B, 1C and 2C were all less than that for Group 1B, and showed a slight trend towards an inhibition effect, the values of the differences were not of statistical significance.
The value for the combined Group 1B + Group 1C (Solution 1) was $9.2 \pm 0.41$ and for combined Group 2B + Group 2C (Solution 2) it was $9.0 \pm 0.44$ showing an insignificant difference of 0.2 teeth affected. For combined Group 1B + Group 2B (Dentifrice B) the value was $9.2 \pm 0.40$ and for combined Group 1C + Group 2C (Dentifrice C) it was $9.1 \pm 0.45$, an insignificant difference of only 0.1 affected teeth.

It is of interest to note here that at the time of Exam. 1 the number of permanent teeth present in the dentition of each subject in the respective groups was: Group 1B, $26.9 \pm 0.21$; Group 2B, $27.0 \pm 0.22$; Group 1C, $26.1 \pm 0.24$ and Group 2C, $26.4 \pm 0.24$ teeth. This meant that for the total group of 209 subjects, approximately 33 per cent of the permanent teeth per individual were affected by visible signs of subsurface decalcification on the buccal/lingual surfaces of the teeth.

The analysis of variance for the mean change between examinations showed (Table 9) that, although there was an overall significant change ($P < .05$) the interactions of either the dentifrice, the solution or the dentifrice $\times$ the solution were all insignificant.
Table 7. The number of teeth affected by subsurface lesions on their buccal/lingual surfaces in 209 high school subjects observed at Exam. 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Subjects</th>
<th>Total No. of Teeth</th>
<th>Range</th>
<th>Mean No. of Teeth</th>
<th>± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>66</td>
<td>609</td>
<td>2 to 22</td>
<td>9.2</td>
<td>0.57</td>
</tr>
<tr>
<td>2B</td>
<td>54</td>
<td>446</td>
<td>2 to 23</td>
<td>8.2</td>
<td>0.65</td>
</tr>
<tr>
<td>1C</td>
<td>41</td>
<td>346</td>
<td>2 to 17</td>
<td>8.4</td>
<td>0.57</td>
</tr>
<tr>
<td>2C</td>
<td>48</td>
<td>442</td>
<td>1 to 18</td>
<td>9.2</td>
<td>0.61</td>
</tr>
<tr>
<td>1B + 1C</td>
<td>107</td>
<td>955</td>
<td>2 to 22</td>
<td>8.9</td>
<td>0.41</td>
</tr>
<tr>
<td>2B + 2C</td>
<td>102</td>
<td>888</td>
<td>1 to 23</td>
<td>8.7</td>
<td>0.45</td>
</tr>
<tr>
<td>1B + 2B</td>
<td>120</td>
<td>1,055</td>
<td>2 to 23</td>
<td>8.8</td>
<td>0.43</td>
</tr>
<tr>
<td>1C + 2C</td>
<td>89</td>
<td>788</td>
<td>1 to 18</td>
<td>8.8</td>
<td>0.42</td>
</tr>
<tr>
<td>Total Group</td>
<td>209</td>
<td>1,843</td>
<td>1 to 23</td>
<td>8.8</td>
<td>0.30</td>
</tr>
</tbody>
</table>
Table 8. The number of teeth affected by subsurface lesions on their buccal/lingual surfaces in 209 high school subjects observed at Exam. 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Subjects</th>
<th>Total No. of Teeth</th>
<th>Range</th>
<th>Mean No. of Teeth</th>
<th>± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>66</td>
<td>645</td>
<td>3 to 21</td>
<td>9.8</td>
<td>0.53</td>
</tr>
<tr>
<td>2B</td>
<td>54</td>
<td>484</td>
<td>2 to 23</td>
<td>8.5</td>
<td>0.62</td>
</tr>
<tr>
<td>1C</td>
<td>41</td>
<td>355</td>
<td>2 to 21</td>
<td>8.6</td>
<td>0.66</td>
</tr>
<tr>
<td>2C</td>
<td>48</td>
<td>458</td>
<td>1 to 18</td>
<td>9.5</td>
<td>0.63</td>
</tr>
<tr>
<td>1B + 1C</td>
<td>107</td>
<td>1,000</td>
<td>2 to 21</td>
<td>9.2</td>
<td>0.41</td>
</tr>
<tr>
<td>2B + 2C</td>
<td>102</td>
<td>942</td>
<td>1 to 23</td>
<td>9.0</td>
<td>0.44</td>
</tr>
<tr>
<td>1B + 2B</td>
<td>120</td>
<td>1,129</td>
<td>2 to 23</td>
<td>9.2</td>
<td>0.40</td>
</tr>
<tr>
<td>1C + 2C</td>
<td>89</td>
<td>813</td>
<td>1 to 21</td>
<td>9.1</td>
<td>0.45</td>
</tr>
<tr>
<td>Total Group</td>
<td>209</td>
<td>1,942</td>
<td>1 to 23</td>
<td>9.2</td>
<td>0.33</td>
</tr>
</tbody>
</table>
Table 9. Analysis of the differences between Tables 7 and 8.

<table>
<thead>
<tr>
<th>Group</th>
<th>1B</th>
<th>2B</th>
<th>1C</th>
<th>2C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>66</td>
<td>54</td>
<td>41</td>
<td>48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>1B</th>
<th>2B</th>
<th>1C</th>
<th>2C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{x}$</td>
<td>0.56</td>
<td>0.33</td>
<td>0.22</td>
<td>0.33</td>
</tr>
<tr>
<td>$\Sigma x$</td>
<td>37</td>
<td>18</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>$\Sigma x^2$</td>
<td>391</td>
<td>325</td>
<td>135</td>
<td>184</td>
</tr>
<tr>
<td>$\Sigma (x-\bar{x})^2$</td>
<td>370.26</td>
<td>319.00</td>
<td>133.02</td>
<td>178.67</td>
</tr>
<tr>
<td>$S^2$</td>
<td>5.70</td>
<td>6.02</td>
<td>3.32</td>
<td>3.90</td>
</tr>
</tbody>
</table>

Variance for testing comparisons = 0.36

Standard deviation = 0.60

Comparisons: Statistical Significance

Examinations
\[ \bar{x}_{1B}+\bar{x}_{2B}+\bar{x}_{1C}+\bar{x}_{2C} \]
= 1.45
S.S. P < .05

Dentifrice x Examinations
\[ \bar{x}_{1B}+\bar{x}_{2B}-\bar{x}_{1C}-\bar{x}_{2C} \]
= 0.34
N.S.

Solution x Examinations
\[ \bar{x}_{2B}+\bar{x}_{2C}-\bar{x}_{1C}-\bar{x}_{1B} \]
= 0.11
N.S.

Dentifrice x Solution x Examinations
\[ \bar{x}_{2C}+\bar{x}_{2B}+\bar{x}_{1C}+\bar{x}_{1B} \]
= 0.34
N.S.

Abbreviations:

$\bar{x}$ = the mean change between examinations.
$\Sigma x$ = the sum of the differences.
$\Sigma x^2$ = the sum of the squares of the differences.
$\Sigma (x-\bar{x})^2$ = the sum of the squares of the deviations of the differences from the mean change.
$S^2$ = the square of the standard deviation of the mean change (variance).
S.S. = statistically significant.
N.S. = not statistically significant.
Another group of 70 students attending High School at Tamworth, N.S.W., and who were not therefore included in the Topical Fluoride Survey, was examined in the interval between the two Topical Fluoride High School examinations; this group was selected similarly to the High School Group with respect to the presence of subsurface decalcification and not gross caries. This was not a large group and the results in the following tables (Tables 10, 11, 12) present the same prevalences as in the previous tables (Tables 1 and 2, 4 and 5, 7 and 8). The total group of 70 subjects was distributed into four age groups.

The mean number of subsurface lesions per subject in this total Tamworth Group was \(10.3 \pm 0.76\) (Table 10); the mean number of buccal/lingual surfaces affected by such lesions was \(9.2 \pm 0.70\) (Table 11); the mean number of teeth involved per subject was \(7.6 \pm 0.56\) (Table 12). These mean values were all less than the mean values shown for the same three prevalences (lesions, surfaces and teeth) in both examinations of the Topical Fluoride High School groups. The differences between the means of the total Tamworth Group and the total Topical Fluoride High School Groups at the two examinations and their statistical significance were:

<table>
<thead>
<tr>
<th></th>
<th>Topical Fluoride Exam.1</th>
<th>Topical Fluoride Exam.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesions</td>
<td>1.6, not significant</td>
<td>2.4, significant ((P=.01))</td>
</tr>
<tr>
<td>Surfaces</td>
<td>1.6, not significant</td>
<td>2.2, significant ((P=.01))</td>
</tr>
<tr>
<td>Teeth</td>
<td>1.2, not significant</td>
<td>1.7, significant ((P=.01))</td>
</tr>
</tbody>
</table>
It was noted that although there were only 19 subjects of the Tamworth Group who were in the 15 to 16 year old age group, the mean values for this group for lesions, affected surfaces and affected teeth were 12.4 ± 1.1, 11.2 ± 1.1, and 9.0 ± 0.9 respectively (Tables 11, 11, 12). These latter mean values were however very close to those obtained for the total Topical Fluoride groups at the two examinations and with no statistically significant differences at all.

On the whole it appeared that the means obtained of the three prevalences for the Total Tamworth Group were similar to those for the total Topical Fluoride High School Group at Exam. 2. The prevalence of subsurface decalcification lesions was in general less for the total Tamworth Group than it was for the city High School Group of children of the same chronologic age. This finding could have resulted from three factors: (i) the Tamworth Group was smaller and possessed the same (or a larger) range of values; (ii) there was a beneficial effect of 1 ppm. of fluoride ion which had been introduced into the community's water supply in 1962; (iii) the specific condition was not examined under identical conditions to those for the Topical Fluoride High School Groups since a thorough prophylaxis was not able to be done before scoring.
Because the various values for this Tamworth Group were similar to those of the High School Group at Exam. 1, reference has been made to the Tamworth Group in the later discussion of the distribution of the different shapes of lesions and the pigmentation of the lesions.

Furthermore, it could be observed from Tables 10, 11, 12 that subsurface decalcification susceptibility appeared to increase with the age of the individual.
Table 10. The number of subsurface decalcification lesions on the affected buccal/lingual surfaces of the teeth in 70 subjects attending high school outside the metropolitan area - Tamworth.

<table>
<thead>
<tr>
<th>Group Age</th>
<th>No. of Subjects</th>
<th>Total No. of Lesions</th>
<th>Range</th>
<th>Mean No. of Lesions</th>
<th>± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>19</td>
<td>173</td>
<td>1 to 28</td>
<td>9.1</td>
<td>1.43</td>
</tr>
<tr>
<td>13</td>
<td>18</td>
<td>156</td>
<td>1 to 15</td>
<td>8.7</td>
<td>0.90</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td>153</td>
<td>2 to 33</td>
<td>10.9</td>
<td>2.76</td>
</tr>
<tr>
<td>15</td>
<td>19</td>
<td>236</td>
<td>2 to 19</td>
<td>12.4</td>
<td>1.11</td>
</tr>
</tbody>
</table>

Total Group 70 718 1 to 33 10.3 0.76

Table 11. The number of buccal/lingual surfaces affected by subsurface lesions in 70 subjects attending high school outside the metropolitan area - Tamworth.

<table>
<thead>
<tr>
<th>Group Age</th>
<th>No. of Subjects</th>
<th>Total No. of Surfaces</th>
<th>Range</th>
<th>Mean No. of Surfaces</th>
<th>± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>19</td>
<td>151</td>
<td>1 to 25</td>
<td>8.0</td>
<td>1.29</td>
</tr>
<tr>
<td>13</td>
<td>18</td>
<td>141</td>
<td>1 to 13</td>
<td>7.8</td>
<td>0.82</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td>137</td>
<td>2 to 29</td>
<td>9.8</td>
<td>2.44</td>
</tr>
<tr>
<td>15</td>
<td>19</td>
<td>212</td>
<td>2 to 19</td>
<td>11.2</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Total Group 70 641 1 to 29 9.2 0.70
Table 12. The number of teeth affected by subsurface lesions on their buccal/lingual surfaces in 70 subjects attending high school outside the metropolitan area - Tamworth.

<table>
<thead>
<tr>
<th>Group Age</th>
<th>No. of Subjects</th>
<th>Total No. of Teeth</th>
<th>Range</th>
<th>Mean No. of Teeth</th>
<th>± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>19</td>
<td>131</td>
<td>1 to 22</td>
<td>6.9</td>
<td>1.12</td>
</tr>
<tr>
<td>13</td>
<td>18</td>
<td>119</td>
<td>1 to 13</td>
<td>6.6</td>
<td>0.75</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td>111</td>
<td>1 to 22</td>
<td>7.9</td>
<td>1.82</td>
</tr>
<tr>
<td>15</td>
<td>19</td>
<td>171</td>
<td>2 to 15</td>
<td>9.0</td>
<td>0.92</td>
</tr>
</tbody>
</table>

| Total Group | 70              | 532                | 1 to 22 | 7.6              | 0.56  |
So far the tooth prevalence studies shown in Tables 1 to 12 inclusive have all dealt with a 100 per cent affected group. It was not possible from the author's figures to gain any knowledge of the prevalence of the observable subsurface lesions on buccal/lingual surfaces. For three stages of the Topical Fluoride High School Study, one examiner (N.D.M.) had been recording the presence of subsurface lesions in conjunction with his dental examinations. His findings are shown in Table 13. It must be remembered that while these figures are certainly indicative of a high prevalence of subsurface lesions on the buccal/lingual surfaces of teeth, the observations were not aided by prior prophylaxis nor by drying by compressed air and were observed during a shorter examining period (approximately 1 to 2 minutes for caries and subsurface decalcification scoring). The first time that the affected surfaces were recorded was at Stage III of the High School study and these were recorded, if present on buccal/lingual surfaces, on examination of 1,000, 13 to 14 year old male and female subjects combined: at Stage III, the subjects had received only one topical fluoride treatment six months beforehand. At Stage IV (two topical fluoride treatments) there remained only 966 subjects from the original group of 1,000 and at Stage V (three topical fluoride treatments), 940 subjects
remained whose ages were now in the 14 to 15 year old age group.

The prevalence percentage of subsurface lesions present was 50.7 ± 1.58 when first seen at Stage III and was as high as 76.5 ± 1.38 when 940 subjects of the original group of 1,000 were examined one year later at Stage V. The mean numbers of buccal/lingual surfaces affected for the totally-affected groups over the three stages were 2.6, 4.0, and 6.3 surfaces respectively; these mean values were much below the author's findings for his totally-affected groups, the lower values being attributed to the fact that the recording was done prior to prophylaxis.
Table 13. The prevalence of subsurface decalcification lesions observed (N.D.M.) on buccal/lingual surfaces. (8 High Schools).

<table>
<thead>
<tr>
<th>Topical F. High School Study</th>
<th>No. of Subjects</th>
<th>No. with Lesions</th>
<th>No. of b/l surfaces affected.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage III</td>
<td>1,000</td>
<td>507</td>
<td>1,342</td>
</tr>
<tr>
<td>6 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>966</td>
<td>644</td>
<td>2,585</td>
</tr>
<tr>
<td>6 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage V</td>
<td>940</td>
<td>719</td>
<td>4,516</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean Surfaces Affected Total Group.</th>
<th>Mean Surfaces Affected Group with Lesions</th>
<th>Prevalence total group Per cent</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage III</td>
<td>1.34</td>
<td>2.65</td>
<td>50.7</td>
<td>1.58</td>
</tr>
<tr>
<td>6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>2.77</td>
<td>4.01</td>
<td>66.7</td>
<td>1.52</td>
</tr>
<tr>
<td>6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage V</td>
<td>4.80</td>
<td>6.28</td>
<td>76.5</td>
<td>1.38</td>
</tr>
</tbody>
</table>

Discussion.

The total number of buccal/lingual surfaces affected by subsurface lesions was evaluated separately for males and females to ascertain any sex difference in the distribution. This prevalence was selected in preference to that of the number of lesions, or of the number of affected teeth because it could, if necessary, be related to the DMFS already scored in the major caries survey and because of closer relationship to the DMFS index score in general.

At both Exam. 1 and Exam. 2 (Tables 4 and 5) the males and females were distributed almost evenly with 109 males and 100 females. There was at Exam 1 (Table 4) a difference of 1.9 affected buccal/lingual surfaces, the males portraying 11.6 ± 0.51 surfaces and females 9.7 ± 0.48 surfaces, and this difference was statistically significant (P=.01) so that in the total group of 209 subjects whose mean age was 14 years 5 months, it appeared that approximately 20 per cent more subsurface decalcification could be observed in males than females.

Six months later at Exam. 2 (Table 5) of the author's 100 per cent affected group, there was still a significantly less number (P=.02) of affected surfaces in the females, the males showing 12.2 ± 0.50 surfaces and the females 10.6 ± 0.48 surfaces, the difference being 1.6 surfaces.
The fact that the teeth of females erupt at an earlier age than those of males means that they are exposed to the risk of caries attack at an earlier average age; and therefore, if this sex difference is taken into consideration the caries susceptibility of the males and females of the author's of any other group could be said to be relatively comparable for the purpose of this type of study and hence no further comparisons were made throughout the study for determining sex differences.

It is pertinent to refer again to Table 13 where it has been shown that in the period of six months from Exam. 1 (Stage IV) to Exam. 2 (Stage V), the proportion of individuals with affected buccal/lingual surfaces had risen by a significant amount of approximately 10 per cent in a total group of 940 young teenage subjects all of whom were not necessarily showing any signs of subsurface decalcification at the beginning of the major Topical Fluoride Study.

The proportion had increased significantly (16 per cent) in the six months prior to the author's first examination (Stage III to Stage IV) and at the end of the duration of the author's study (Stage IV to Stage V), although the prevalence had still increased, the rate had decreased. However, regardless of the rate, there was no doubt that at this chronologic
age the evidence of subsurface decalcification was of an active process for the total group of 940 subjects. As far as the examiner's (N.D.M.) totally-affected group was concerned, it was shown (Table 13) that at Stage IV, the mean number of buccal/lingual surfaces affected by decalcification had risen by 65 per cent to 4.0 affected surfaces per subject and again by 63 per cent at Stage V to 6.3 affected surfaces. However, the author was unable to show such a large increase in the number of affected buccal/lingual surfaces, and showed an increase of only 6 per cent in affected surfaces for the period of Exam. 1 (Stage IV) to Exam. 2 (Stage V) as seen in Tables 4 and 5 where the mean number of affected surfaces increased in six months from 10.7 surfaces to 11.4 surfaces and showed a non-significant increase.

The difference between the results of these two examinations was essentially due to the conditions under which the examination was carried out, the author always observing the teeth in a cleaned dry field and being a total examination of a specific condition: also, the distribution of subjects was not the same where the author's group was constant while in the other examiner's group (N.D.M.) the number of subjects who were included in the totally-affected group increased with time and the observed prevalence
rate. Nevertheless, if the number of affected buccal/lingual surfaces of any one individual is disregarded, the fact still remains that in a group of subjects of over 900 young males and females selected from the general community and whose average age was 14 years 5 months, approximately 67 per cent of subjects were affected by visible signs of subsurface decalcification on the buccal/lingual surfaces of the teeth and by the time the same group had attained the average age of 14 years 11 months, approximately 77 per cent were so affected.

The number of subjects who showed visible signs of such subsurface decalcification was certainly increasing significantly with the time which was indicative of increasing caries activity in general. In the author's smaller 100 per cent affected group this activity of subsurface decalcification in relation to the smooth buccal/lingual surfaces could not be shown to alter significantly in the same time so that further study on the same group or a similar study of the prevalence of affected buccal/lingual surfaces would be unlikely to yield any conclusive results from the preventive aspect with regard to the use of various experimental agents; the rate at which new buccal/lingual surfaces were being affected by decalcification was not sufficiently high enough to show this.
Of the three distribution prevalences scored and calculated in Tables 1 and 2, 4 and 5, 7 and 8 it was found that all three showed higher mean values at the 2nd examination, however, these values were not found to be significantly different to those obtained at the 1st examination. This meant that the values given at either Exam. 1 or Exam. 2 would be indicative in this group of subjects of either the number of subsurface lesions on buccal/lingual surfaces, the number of buccal/lingual surfaces affected, or the number of teeth affected by subsurface lesions on buccal/lingual surfaces; whichever the case may be. On the other hand, the mean values for all prevalences of the three fluoride-treatment groups were below those of the control groups (Tables 1 and 2, 4 and 5 and 7 and 8) and this finding was consistent at both examinations. Although this portrayed only a possible trend towards an inhibitory effect, the differences between the mean values of experimental groups and the control groups at either examination were not sufficient to reveal which fluoride-treatment group was exerting the greater inhibitory effect on the subsurface decalcification.

At Exam. 1, if there had been no differential effect on the progress of lesions in the other three fluoride-treatment groups (1C, 2B, 2C as compared with the
control group 1B), then the mean values of the
number of lesions present per subject in each group
would be expected to be the same and the total number
of lesions in each treatment group would be influenced
by the number of subjects examined. However, such
findings are feasible only on the assumption that
the four groups of subjects possessed an equal number
of subsurface decalcification lesions on the buccal/
lingual surfaces of their teeth and an equal caries
activity rate prior to the application of any
treatment at all.

This was not the case; thus, by analysing the
significance of the mean change between the examinations
(Tables 3, 6, 9) it was found that there was a
significant change between Exam. 1 and Exam. 2 for
all three prevalences. Therefore it was significant
that there had been an increased change between
examinations for the total group but it was evident
that the effects of the interaction of either the
dentifrice, the solution or the dentifrice in
combination with the solution was too small in each
case to be of significance in affecting this change.

In Tables 3 and 6, it would seem that Group 2C,
which showed the smallest mean change, might give a
significant result with increasing elapsed time or
increased sample size; however, it is not valid to
test this a posteriori.
Conclusion.

It was found that, of over 900 young males and females who attended various high schools in the Sydney metropolitan area and had attained the approximate age of 15 years, approximately 77 per cent of this group showed visible signs of subsurface lesions on the buccal(labial)/lingual surfaces of their teeth.

In a smaller sample of 209 subjects which was selected from the above for more detailed study, it was observed that approximately 33 per cent of the permanent teeth per individual possessed macroscopic subsurface lesions on the buccal(labial)/lingual surfaces.

By comparing the values relating to the distribution of the subsurface lesions in the group of 209 subjects with those observed in another smaller group of 70 subjects who were outside the metropolitan area, it would appear that all values found would be generally indicative of the number of subsurface lesions, affected surfaces and affected teeth in similar age groups.

It was considered that the study of the incidence of the separate subsurface lesions on the buccal/lingual surfaces of the teeth would be the best indicator of significant changes to the rate of formation of new
subsurface decalcification in the teeth of various
groups. This was concluded from study of the
prevalence of separate subsurface lesions on the
buccal(labial)/lingual surfaces of the teeth, the
prevalence of buccal/lingual surfaces affected by
subsurface decalcification and by the study of the
prevalence of teeth possessing subsurface lesions
on their buccal/lingual surfaces in a group of 209
subjects, all of whom were affected by regions of
subsurface decalcification on their teeth. It was
also based on the fact that it was a more detailed
prevalence and it showed the largest significant
mean change over a period of six months (P<.001).

The mean change values for the individual
experimental groups, 2B, 1C and 2C as compared to
the control group, 1B, were not significant in
showing the merit of the use of either 10 per cent
stannous fluoride topical solution or of 0.4 per cent
stannous fluoride dentifrice for the control of the
incidence of subsurface lesions on the smooth buccal/
lingual surfaces of the teeth. Such findings could
have resulted from (i), an insufficient number of
subjects to show statistically significant differences
and/or (ii), too short an interval between examinations
and/or (iii), no effect on subsurface decalcification
lesions by the experimental agents used.
Distribution of the various shapes of lesions.

The four main classifications of the shape of the lesion have been explained earlier and were designated by the letters W, X, Y and Z. It was natural to expect different combinations of different shapes of lesions on the different surfaces depending upon the available surface area. Each surface affected by subsurface decalcification had one, two or three lesions and 16 different combinations of shapes of lesions were found in both the subjects from the Tamworth Group and from the Topical Fluoride High School Group at Exam. 1.

Table 14 is a summation of the various shapes recorded on the Topical Fluoride High School Group of 228 subjects at Exam. 1 and also shows the distribution of these shapes as well as the prevalence percentage of the buccal/lingual surfaces that are affected by the shape in question. In this group, 2,430 buccal/lingual surfaces were affected and of these surfaces, 1,061 were of maxillary teeth and 1,349 of mandibular teeth, i.e., 45 per cent of affected buccal/lingual surfaces occurred in the maxillary arch while 55 per cent were in the mandibular arch. In totalling the number of buccal surfaces as compared to the lingual surfaces involved, it was found that of the 2,430 surfaces assessed, 1,738 or
72 per cent were buccal surfaces and 692 or 28 per cent were lingual surfaces. Out of the wide range of combinations of shapes of lesions that could exist it was observed that 91.2 per cent of buccal/lingual surfaces were classified as being affected by only one shape of lesion, either W, X, Y or Z. On considering the buccal surfaces alone, it was found that slightly more of these surface shapes were recorded on the mandibular teeth (51 per cent), there being 49 per cent on the maxillary teeth. Of the lingual surfaces, only 34 per cent were recorded on the maxillary teeth whereas there were approximately twice as many (66 per cent) recorded on the mandibular teeth. Alternatively when the two dental arches were considered separately it was calculated that of the maxillary teeth, 78 per cent were buccally affected and 22 per cent lingually affected. Of the mandibular teeth, 66 per cent were buccally affected and 34 per cent lingually affected.

Table 15 reveals the equivalent values (as in Table 14) which were assessed on the data for the Tamworth Group of 70 subjects and this involved 641 affected buccal/lingual surfaces. Of these, 292 or 45 per cent were in the maxillary arch and 349 or 55 per cent in the mandibular arch; of the 641 affected surfaces, 470 or 73 per cent were on buccal
surfaces while 171 or 27 per cent were on lingual surfaces. Again in this group it was found that the single lesions, W, X, Y, or Z accounted for 39.1 per cent of all the lesions present. When the distribution of the buccal surface shapes was considered separately, it was noted that 48 per cent were on the maxillary teeth and 52 per cent on the mandibular teeth. Of the lingual surfaces, 39 per cent were on the maxillary teeth and 61 per cent recorded on the mandibular teeth; and likewise, of the maxillary teeth, calculations showed that 77 per cent were buccally affected and 23 per cent lingually affected while of the mandibular teeth, 70 per cent were buccally affected and 30 per cent lingually affected.

Disregarding the actual surface that was affected, the percentages of the total number of lesions examined for each fluoride-treatment group were tabulated (Tables 16 and 17) to show their surface position on either the buccal (labial) or lingual surfaces of the teeth according to whether they were on the gingival, middle or incisal thirds of the anatomical crown of the tooth. On the whole approximately 60 per cent of the lesions were observed on the gingival third and approximately 30 per cent on the middle third of the teeth surfaces, and the remaining
10 per cent accounted for the incisal as well as
the intermediate positions. This finding was
consistent at both examinations with the exception
of Group 2C at Exam. 1 where roughly 70 per cent
were on the gingival third and 20 per cent on the
middle third; this finding could have resulted
from a greater number of teeth in this group still
in a state of eruption or difficulty in determining
gingival as compared to middle third positions of
the lesions.
Discussion.

There was definite similarity between the larger High School Group and the smaller Tamworth Group for the prevalence and distribution of the various shapes of lesions. The finding of such similar prevalence figures (and in some cases, the same figures) for these two different groups suggested that in any young teenage group of children affected by subsurface decalcification, the distribution of those lesions and/or shapes would conform to the pattern drawn here and unless otherwise stated, the results from the two groups are discussed as generalities.

From the assessment of the two groups, the following approximate ratios for the distribution of the numbers of lesions are given for each case under consideration:

Both dental arches - Mandible to Maxilla ratio = 1.1 to 1

Both dental arches - Buccal surfaces to lingual surfaces ratio = 2.6 to 1

Mandible alone - Buccal surfaces to lingual surfaces ratio = 2.1 to 1

Maxilla alone - Buccal surfaces to lingual surfaces alone = 3.4 to 1

Buccal surfaces alone - Mandible to Maxilla ratio = 1 to 1

Lingual surfaces alone - Mandible to Maxilla ratio = 1.8 to 1
The teeth in the mandibular arch were slightly more affected by subsurface decalcification on their buccal/lingual surfaces, there being 10 per cent more affected surfaces recorded in the mandibular arch than in the maxillary arch.

There were also significantly many more buccal surfaces affected by subsurface decalcification than lingual surfaces and there appeared to be twice as many mandibular teeth with affected buccal surfaces than maxillary teeth.

Of those affected surfaces in the maxillary arch, there were approximately three times as many recorded on buccal surfaces as there were on lingual surfaces.

The distribution of affected buccal surfaces as compared to lingual surfaces showed that there was roughly the same amount of affected buccal surfaces on the maxillary teeth as there was on the mandibular teeth but twice as many affected lingual surfaces were seen on the mandibular teeth as on the maxillary teeth.

Thus by applying the above approximate ratios of distribution to a known number of existent lesions, an approximate distribution could be determined. In the practical cases of the High School Group at Exam. 1 and the Tamworth Group, the approximate mean
number of surfaces affected were 11 and 9 respectively, so that the following approximate distributions could be expected using estimates based on Tables 14 and 15:

<table>
<thead>
<tr>
<th>High School Group</th>
<th>Tamworth Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 surfaces.</td>
<td>9 surfaces.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Max.</th>
<th></th>
<th>Max.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bucc.</td>
<td>4</td>
<td>Ling.</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Man.</td>
<td>4</td>
<td>2</td>
<td>Man.</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

It would seem from such distributions that, if subsurface lesions are to be prevented from occurring at all, and the application of good oral hygiene is recognised as a preventive factor, then this should be carried out with more regard to the buccal (and labial) surfaces than otherwise expected. A further explanation of such a distribution observed in the two groups examined could lie in the existence of more restorative work having been done on the lingual surfaces while at the same time, the buccal surface lesions were now in self-cleansing and accessible-to-brushing areas by virtue of eruption. However the washing action of saliva and the tongue could be as equally as effective for self-cleansing on the lingual surfaces, so that, other than a possible morphological factor, no explanation can be given here as to why the distribution should exist.
On the other hand, coefficients of correlation were calculated for the High School Group at Exam. 1 to determine whether the prevalence of buccal/lingual subsurface lesions was proportional to the amount of debris observed. These coefficients for the respective fluoride treatment groups were: $1B = +0.26$, $2B = +0.005$, $1C = +0.17$ and $2C = +0.04$. This showed very low positive correlation between the debris and the subsurface decalcification scores for Group 1B alone. The coefficients of correlation between the incidence of new buccal/lingual lesions and the amount of debris at Exam. 1 were; $1B = -0.03$, $2B = +0.17$, $1C = -0.17$ and $2C = +0.04$, which revealed no relationship. If it is assumed that the subsurface lesions occurred in an environment of similar or larger amounts of debris, then it did not appear that formation of the lesions could be shown to be influenced to a marked degree by debris.

Nevertheless, by virtue of the distribution of these lesions, it could be said that if topical application of fluoride solutions is used in the treatment of subsurface decalcification lesions, then diligence and technique are relatively more important with respect to the buccal (labial) surfaces than is necessary for the lingual surfaces.
With regard to the possible variability of combination of shapes of subsurface decalcification lesions that could occur, it was of marked interest to note that this was not so, since the majority (approx. 90 per cent) of smooth buccal/lingual surfaces that were affected possessed only one lesion and this was one of the four main shapes described previously, W, X, Y, or Z.

Perusal of the prevalence of buccal/lingual surfaces affected by the respective shapes in both Tables 14 and 15 revealed very similar values. It was natural to expect that the more unusual the pattern, the less numbers of buccal/lingual surfaces so-affected; however, this applied to only 10 per cent of the surfaces. When two shapes were present on the one surfaces, these could have been connected to each other or separate, as this information has not been included here.

It was observed that the crescent lesions were the most common, comprising nearly 70 per cent of all lesions recorded. The trapezoidal lesion (X) was regarded previously as an overall incisal extension on a tooth surface of the crescent lesion (W) so that the combined prevalence figure for these two types was in the region of 73 per cent.
The single cloud lesion (Y) existed on approximately 12 per cent of buccal/lingual surfaces and the speckled lesion (Z) on approximately 6 per cent of the surfaces. It was more likely that speckled lesions progressed with time to become spot or cloud-shaped ones and this would give a combined figure of 17 per cent for these two latter types. Thus there were two main basic types of lesions, the crescent (W) and cloud lesions (Y) in a proportional ratio of at least 4 crescent lesions to 1 cloud lesion (r = 4.2:1).

To reiterate on the other 10 per cent of combination shaped lesions, only five combinations i.e., WY, WZ, XY, WXY, and WYY were disregarded in obtaining any final percentage figures and this was because of difficulty in assigning them to their respective basic-shaped groups. However, after the prevalence percentages for the remaining combination shaped lesions which could be assigned to one of the two basic groups had been considered, it was calculated that the prevalence of the crescent lesions present throughout the two groups of subjects was increased to approximately 78 per cent while the prevalence of the cloud lesions increased to only 13 per cent. This meant that the inclusion of the combination shaped lesions did not alter significantly
the ratio of the number of crescent lesions as compared to the cloud lesions \((r = 4.3:1)\) so that the recording of the four single-shaped lesions \((W, X, Y, Z)\) was a sufficient indicator of the proportional distribution of the two main basic shapes and perhaps sufficient for the study of any clinical changes to these lesions; the one proviso being the assumed association of crescent shapes with trapezoidal shapes, and cloud shapes with speckled shapes respectively.

At this juncture it is to be recalled that the four experimental groups in the total High School Group had, by the time of the author's Exam. 1, already received the use or non-use of a fluoridated toothpaste for one year with or without the addition of two six-monthly topical applications of 10 per cent \(\text{SnF}_2\); therefore it was decided to compare any possible effect that the two dentifrices and the two topical solutions might have had upon the existence of the various shapes of lesions. The numbers of the various shapes observed are given for the pooled groups in Table 16. The prevalence percentages all lay fairly close to if not virtually the same as one another which meant that the possible differing effects by the four experimental groups upon the variability of shapes of lesions were negligible.
Moreover, the percentages obtained were all comparable to the total High School Group as well as the total Tamworth Group. Thus any assessing (in a later section of the results) of the clinical changes in subsurface lesions in the experimental groups could be carried out with the prior knowledge that, although the condition of the lesions might be altered in time by topical agents, it did not seem likely that in the oral cavity the existent shapes of the lesions had been influenced by these agents.

The formation of crescent lesions has been associated with attack along the gingival margin (99) (153) and the very shape is indicative of this occurrence. Alternatively, the cloud lesions could be said not to be influenced by such anatomy and would probably arise from caries attack at enamel "faults" or repeated attacks at the one location, both factors influencing the variegated cloud shape. From the previous distribution ratio of 4 crescent to 1 cloud lesion, it would appear that if a mouth is affected by multiple subsurface lesions on the buccal/lingual smooth surfaces of the teeth, it is more likely that they have been associated with attack along the gingival margin and therefore reasonable to assume that the crescent lesions are better indicators of present or past subsurface decalcification and any future treatment plan.
Conclusion.

By recording the various types or shapes of subsurface lesions on the buccal/lingual surfaces of the teeth in two groups of teenage subjects, it was observed that although the two groups were relatively dissimilar for examination purposes, the distributions and the numbers of types of lesions were remarkably alike. This suggested that such a distribution found here could apply to any young teenage group of subjects whose teeth showed visible signs of subsurface decalcification on their buccal/lingual surfaces. Approximate ratios were calculated to show the distribution of the lesions on the buccal and lingual surfaces and whether these were located in the mandibular or maxillary arches. Although the mandible had only 10 per cent more subsurface lesions than the maxilla, the dominating fact was that the buccal surfaces of the oral cavity were affected two to three times more so by such lesions than the lingual surfaces. Assuming that this latter proportion was not masked by restorative work having depleted the number of lesions that would have been observed otherwise on the lingual surfaces, and if treatment of present or prevention of future subsurface decalcification is contemplated by the applications of fluoride, then with respect to the buccal/lingual
surfaces it would be two to three times as important to treat buccal surfaces than lingual surfaces.

The variability of shapes was not as evident as originally anticipated, the most common shape being the crescent lesion. If it is assumed that this shape was influenced by attack along the gingival margin, it was found that there was a 78 per cent occurrence of attack initiating from this clinical area. It was also noted that at least 60 per cent of all lesions examined (apart from shape) were observed to be in the area of the gingival third of the anatomical crowns of the teeth, irrespective of whether they were on the buccal or lingual surfaces (Tables 17, 18).

When topical fluoride agents had been used over one year to help control the subsurface lesions, it was found that the prevalences of the different shapes of lesions in the control group as well as in the other three groups who had received varying degrees of topical fluoride, were consistently similar to one another. In addition, the prevalence values corresponded closely to another relatively dissimilar group of subjects so that the only conclusion to be deduced was that any influence by topical fluoride (10 per cent SnF$_2$ solution or a 0.4 per cent stannous fluoride toothpaste) upon the actual shapes of the lesions was not evident after one year of their application.
Table 14. The distribution of the various shapes of subsurface lesions with respect to the buccal and lingual surfaces as well as the maxillary and mandibular arches.

<table>
<thead>
<tr>
<th>Shape of Lesion</th>
<th>No. on buccal surfaces</th>
<th>No. on lingual surfaces</th>
<th>Totals</th>
<th>Prevalence percentage of b/l surfaces affected by shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>454</td>
<td>630</td>
<td>175</td>
<td>343</td>
</tr>
<tr>
<td>X</td>
<td>76</td>
<td>26</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>Y</td>
<td>174</td>
<td>93</td>
<td>31</td>
<td>40</td>
</tr>
<tr>
<td>Z</td>
<td>96</td>
<td>35</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>WX</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>WX</td>
<td>17</td>
<td>31</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>WZ</td>
<td>5</td>
<td>8</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>XY</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>UW</td>
<td>12</td>
<td>51</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>UX</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WUX</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WXY</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>XY</td>
<td>7</td>
<td>7</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>YYX</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>ZY</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>UYX</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Totals</th>
<th>845</th>
<th>898</th>
<th>231</th>
<th>456</th>
<th>2,430</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freq. (%)</td>
<td>49</td>
<td>51</td>
<td>34</td>
<td>66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total Buccal = 1,743 72%  Total Maxilla = 1,976 45%
Total Lingual = 687 28%  Total Mandible = 1,354 55%
Table 15. The distribution of the various shapes of subsurface lesions with respect to the buccal and lingual surfaces as well as the maxillary and mandibular arches.

Tamworth Group of 12 to 15 year olds; No. in group = 70.

<table>
<thead>
<tr>
<th>Shape of Lesion</th>
<th>No. on buccal surfaces</th>
<th>No. on lingual surfaces</th>
<th>Totals</th>
<th>Prevalence percentage of b/l surfaces affected by shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>120</td>
<td>160</td>
<td>58</td>
<td>90</td>
</tr>
<tr>
<td>X</td>
<td>24</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Y</td>
<td>39</td>
<td>20</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Z</td>
<td>23</td>
<td>5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>WX</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>WY</td>
<td>3</td>
<td>10</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>WZ</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>XY</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>WY</td>
<td>5</td>
<td>23</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>XY</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WX</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WY</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>XY</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WY</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ZY</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WY</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Totals</td>
<td>226</td>
<td>244</td>
<td>66</td>
<td>105</td>
</tr>
<tr>
<td>Prev. (%)</td>
<td>48</td>
<td>52</td>
<td>39</td>
<td>61</td>
</tr>
</tbody>
</table>

Total Buccal = 470 73%       Total Maxilla = 292 45%
Total Lingual = 171 27%      Total Mandible = 349 55%
Table 16. The distribution of the various sizes of sub-surface lesions in terms of totals for the two Dentifrice Groups, B & C, and the two Topical Solution Groups, 1 & 2.

<table>
<thead>
<tr>
<th>Type of Lesion</th>
<th>Dent. B</th>
<th>Dent. C</th>
<th>Prevalence per cent</th>
<th>Top. Sol. 1</th>
<th>Top. Sol. 2</th>
<th>Prevalence per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>250</td>
<td>652</td>
<td>66.3</td>
<td>65.3</td>
<td>811</td>
<td>791</td>
</tr>
<tr>
<td>X</td>
<td>278</td>
<td>541</td>
<td>5.3</td>
<td>5.4</td>
<td>62</td>
<td>63</td>
</tr>
<tr>
<td>Y</td>
<td>124</td>
<td>144</td>
<td>13.5</td>
<td>14.4</td>
<td>198</td>
<td>140</td>
</tr>
<tr>
<td>Z</td>
<td>83</td>
<td>65</td>
<td>5.8</td>
<td>6.5</td>
<td>74</td>
<td>74</td>
</tr>
<tr>
<td>W2</td>
<td>5</td>
<td>1</td>
<td>0.3</td>
<td>0.1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>W2</td>
<td>33</td>
<td>25</td>
<td>2.3</td>
<td>2.5</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>V2</td>
<td>10</td>
<td>7</td>
<td>0.7</td>
<td>0.7</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>V2</td>
<td>4</td>
<td>2</td>
<td>0.3</td>
<td>0.2</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>V3</td>
<td>58</td>
<td>35</td>
<td>4.1</td>
<td>3.5</td>
<td>51</td>
<td>42</td>
</tr>
<tr>
<td>V3</td>
<td>3</td>
<td>1</td>
<td>0.2</td>
<td>0.1</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>V3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>W3</td>
<td>3</td>
<td>2</td>
<td>0.2</td>
<td>0.2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>W2</td>
<td>9</td>
<td>10</td>
<td>0.6</td>
<td>1.0</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>W2</td>
<td>1</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>V2</td>
<td>1</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>V2</td>
<td>2</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Totals</td>
<td>1,432</td>
<td>998</td>
<td>99.9</td>
<td>99.9</td>
<td>1,289</td>
<td>1,171</td>
</tr>
</tbody>
</table>
Table 17. Surface positions involved by all shapes of lesions on the buccal/lingual surfaces observed at Exem. 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Lesions</th>
<th>Percentage of lesions on the various surface positions.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>s.  gm.  m.  ml.  l.  gmL.</td>
</tr>
<tr>
<td>1B</td>
<td>839</td>
<td>57.3  8.0  29.4  2.0  0.9  2.5</td>
</tr>
<tr>
<td>2B</td>
<td>576</td>
<td>54.9  6.5  31.9  2.3  1.4  3.0</td>
</tr>
<tr>
<td>1C</td>
<td>459</td>
<td>56.0  5.6  31.4  2.7  1.4  2.9</td>
</tr>
<tr>
<td>2C</td>
<td>590</td>
<td>68.3  5.9  21.9  1.8  1.0  0.8</td>
</tr>
<tr>
<td>Total Group</td>
<td>2,464</td>
<td>59.6  6.6  28.2  2.2  1.1  2.2</td>
</tr>
</tbody>
</table>

Table 18. Surface positions involved by all shapes of lesions on the buccal/lingual surfaces observed at Exem. 2

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Lesions</th>
<th>Percentage of lesions on the various surface positions.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>s.  gm.  m.  ml.  l.  gmL.</td>
</tr>
<tr>
<td>1B</td>
<td>887</td>
<td>53.6  7.7  29.2  1.1  1.4  2.0</td>
</tr>
<tr>
<td>2B</td>
<td>649</td>
<td>54.3  8.2  31.6  1.6  1.6  2.5</td>
</tr>
<tr>
<td>1C</td>
<td>499</td>
<td>57.0  7.3  32.1  1.6  0.8  1.2</td>
</tr>
<tr>
<td>2C</td>
<td>639</td>
<td>55.7  6.3  33.9  1.5  0.8  1.8</td>
</tr>
<tr>
<td>Total Group</td>
<td>2,654</td>
<td>56.6  7.4  31.5  1.4  1.2  1.9</td>
</tr>
</tbody>
</table>
The pigmentation of subsurface lesions.

Scoring of the pigmentation of a subsurface lesion was carried out for the Topical Fluoride High School Groups at both examinations and followed a prophylaxis to remove debris and extrinsic surface stain. The intensity of colour from the pigment had been graded threefold as $P^1$, $P^2$, and $P^3$, i.e., mild, moderate and severe respectively, and the number of lesions scored for the four separate topical fluoride treatment groups at the two examinations is given in Tables 19 and 20. The number of graded, pigmented lesions was totalled to provide the overall prevalence percentage of lesions which were indeed pigmented to any degree. If there were significant prevalence differences of pigmentation between the various separate or combined topical fluoride treatment groups, then the prevalence of the extent of discolouration in that group, could be calculated from the graded pigmentation scores. The pigmentation scoring was also recorded for the total Tamworth Group and then compared to the Topical Fluoride Groups at both examinations.

At Exam. 1 (Table 19), 427 out of the total 2,464 lesions were pigmented to some extent, i.e., the prevalence of lesions pigmented was $17.3 \pm 0.76$ per cent.
The prevalence seen in the Tamworth Group was 10.6 ± 1.32 per cent where 76 out of a total 718 lesions were pigmented. There was a clear significant difference in the amount of pigmentation of subsurface lesions between the Tamworth Group and the High School Group (P<.003). It could be seen in the individual treatment groups that the percentage of lesions which were pigmented was 9.3 ± 1.00 per cent for Group 1B and 11.1 ± 1.47 per cent for Group 1C, while Groups 2B and 2C showed similar prevalence values of 25.0 ± 1.80 per cent and 26.1 ± 1.81 per cent respectively. Therefore it was evident that the combined Group 1B + 1C compared to the combined Group 2B + 2C would possess a significantly less number of pigmented lesions. Topical Solution 1 (1B + 1C) gave a prevalence of 9.9 ± 0.83 per cent of pigmented lesions and Topical Solution 2 (2B + 2C) had a prevalence of 25.6 ± 1.23 per cent of pigmented lesions, so that the difference between the two topical solutions was clearly statistically significant (P<.003). In comparing the Dentifrice B (1B + 2B) to Dentifrice C (1C + 2C) there was still a statistical significant, but much smaller, difference in prevalence of pigmented lesions (P = .02), and the prevalence percentages were 15.7 ± 0.97 per cent and 19.5 ± 1.22 per cent respectively.
In Table 20 the Topical Fluoride High School Group was evaluated for pigmentation six months later at Exam. 2 and also compared to the one Tamworth Group study. On the whole, the value for the prevalence of pigmentation observed was slightly less at this examination with regard to the two Topical Solution 1 Groups, 1B and 1C (5.6 ± 0.34 per cent and 6.2 ± 1.08 per cent respectively), and somewhat higher in the two Topical Solution 2 Groups, 2B and 2C (30.5 ± 1.81 per cent and 27.1 ± 1.79 per cent respectively). These changes meant that the prevalence of pigmentation observed in the total group of 209 subjects at Exam. 2 was 16.8 ± 0.73 per cent and the same as that of Exam. 1 (17.3 ± 0.76 per cent) since the slight difference shown was not significant. It was therefore still a significantly different prevalence of pigmentation to that of the Tamworth Group (P<.003). The prevalence of pigmented lesions for the total Topical Solution 1 Group (1B + 1C) was 5.8 ± 0.63 per cent and for the total Topical Solution 2 Group (2B + 2C) it was 28.9 ± 1.27 per cent which was a clearly significant difference (P<.003). On the other hand, Dentifrice B (1B + 2B) and Dentifrice C (1C + 2C) had similar values for the prevalence of pigmented lesions at Exam. 2, these values being 16.2 ± 0.94 per cent and 17.8 ± 1.14 per cent respectively.
Discussion.

In the small Tamworth Group approximately one out of every ten lesions observed was recorded as being pigmented and there was an 83 per cent (63 in 76) occurrence of its being graded as mild discolouration. This group was not receiving any high concentrations of topical fluoride (stannous) to the teeth; there was however always the possibility of the subject having received application of SnF₂ solution from his or her family dentist or of using SnF₂-containing toothpaste, but other than this, pigmentation was not considered to be acquired from stannous salts. The amount of pigmentation was found to be significantly less than that observed in the two High School Group examinations and in which, many subjects definitely were receiving the added effects of stannous fluoride. However the higher pigmentation figures in these High School Groups to provide the significant difference was influenced by the application of 10 per cent SnF₂ solution rather than the use of fluoride-containing dentifrice. In the Exam. 1 (Table 19) the Tamworth Group value was similar to the two groups using either a fluoride-containing dentifrice or a non-fluoridated dentifrice. At Exam. 2 (Table 20) the pigmentation associated with either of the two dentifrices was less than the Tamworth Group and the reason for this
is unknown. If the dentifrices were compared for their effect in causing pigmentation at either examination, it could only be said that the fluoridated dentifrice appeared to cause more. There were not sufficient subjects nor values to provide a statistical excess of pigmentation. In any event it would seem that all those values which were less or similar to that evaluated for the Tamworth Group would be indicative of the pigmentation not being the result of excessive stannous ions. Perusal of the grading of discolouration of the separate dentifrice groups in the High School Groups (Tables 19 and 20) shows also that the pigmentation was in general mild and this was roughly a 65 to 90 per cent occurrence (52 in 78, 37 in 51, 35 in 50, 28 in 31).

Muhler (94)(97) has frequently suggested that the presence of stannous ions will produce a type of stannous phosphate in the regions of the enamel which are decalcified and that this substance is light brown in colour and if stannous ions react with oxygen and/or sulphur in the organic plaque then the aesthetically unacceptable dark brownish black colour is produced. Although the true identity of the light brown colour is still debated (17) the more decalcified the subsurface lesion, the more pigmented stannous salt formed and hence more light brown colour; and the more porous this decalcified region has become,
the more likelihood of darkly-stained organic material being retained. This would not be removed easily by prophylaxing. From such knowledge and the fact that 65 to 90 per cent of lesions in the dentifrice groups were classified as being mildly pigmented, one could not state conclusively that stannous ions in preference to other substances had brought about the discoloration in the dentifrice groups. On the other hand stannous ions and colour are eventually lost from decalcified areas if they are continually cleaned with dentifrice, moreso if the dentifrice contains no free stannous ions (94)(97).

The total pigmentation prevalence, although slightly less, had not changed much over the six months between the two High School examinations. However the pigmentation observed in the two dentifrice groups (1B and 1C) which had received no topical solution of 10 per cent SnF₂, had been significantly reduced and other than possible examiner error, no reason is suggested.

It was shown from the observations that in all cases of use of the 10 per cent SnF₂ topical solution there was significant pigmentation and even after six months this had increased, (At the Exam. 2 the subjects had received 3 topical applications of 10 per cent SnF₂). If, because of insignificant
differences at both examinations, it is assumed that the pigmentation observed in both dentifrice groups (1B and 1C) which had received only topical application of 0.85 per cent saline, was brought about by the acquisition of other material then at least the difference in pigmentation between those groups receiving saline topical solution and the groups receiving 10 per cent SnF$_2$ topical solution could be said to be a result of the stannous action. In the first examination (Table 19) this action would have caused approximately 16 per cent of the pigmentation and at the second examination (Table 20) it caused approximately 23 per cent of the pigmentation. Since the prevalence of discolouration found in the total group receiving 10 per cent SnF$_2$ topical solution was more constant and in the region of 26 to 29 per cent, and the prevalence of the non-stannous fluoride topical solution group was (because of possible examiner error) so much less at the second examination, then the figure of 16 per cent of lesions being pigmented by the action of 10 per cent SnF$_2$ would be more likely to be closer to the true percentage.

In addition it was noted that the pigmentation was found to be darker in the 10 per cent SnF$_2$ topical solution groups and the range of occurrence over the two examinations where the discolouration
was considered to be only mild had increased from approximately 48 to 63 per cent (14.2 in 298, 229 in 366).
Conclusion.

It appeared from the findings that after three six-monthly applications of 10 per cent SnF₂ topical solution (30 secs. duration) there was only approximately 16 per cent more pigmentation than would have occurred normally and in this 16 per cent increase, approximately 17 to 28 per cent would be graded as darker than mild discolouration. If these facts are considered with the number of lesions per subject (approximately 12 lesions) in the total group receiving 10 per cent SnF₂ topical solution (Tables 1 and 2), then only two lesions are likely to be pigmented than otherwise would have been and there would be a 63 per cent occurrence of these two lesions showing only a mild discolouration. These results show that a large groups of subjects could be treated with 10 per cent SnF₂ topical solution without the occurrence of too much discolouration. This would be advantageous for a general dental public health programme in the 12 to 15 year old age group. Alternatively, there is no doubt that more severe and unaesthetic discolouration will take place in certain individuals included in such a programme and these cases can only be assessed on the amount and character of the subsurface decalcification portrayed in that individual. Such individuals should be treated no doubt by restorative
work as well, and the merits of using a topical fluoride solution without doing so should be questioned: their non-inclusion in the topical fluoride programme would no doubt have decreased further the prevalence of pigmented subsurface lesions.
Table 19. Pigmentation of Subsurface Lesions observed at Exam. 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Subjects (Table 1)</th>
<th>Total Lesions</th>
<th>Pigmentation</th>
<th>Total</th>
<th>Percentage of lesions pigmented</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>66</td>
<td>839</td>
<td>52 25 1</td>
<td>78</td>
<td>9.3</td>
<td>1.00</td>
</tr>
<tr>
<td>2B</td>
<td>54</td>
<td>576</td>
<td>71 59 14</td>
<td>144</td>
<td>25.0</td>
<td>1.80</td>
</tr>
<tr>
<td>1C</td>
<td>41</td>
<td>459</td>
<td>37 13 1</td>
<td>51</td>
<td>11.1</td>
<td>1.47</td>
</tr>
<tr>
<td>2C</td>
<td>48</td>
<td>590</td>
<td>71 69 14</td>
<td>154</td>
<td>26.1</td>
<td>1.81</td>
</tr>
</tbody>
</table>

|         |                  | 1B + 1C 107  | 1,298 89 38 2 129 | 9.9    | 0.83                           |
|         |                  | 2B + 2C 102  | 1,166 142 128 28 298 | 25.6   | 1.23                           |

|         |                  | 1B + 2B 120  | 1,415 123 84 15 222 | 15.7   | 0.97                           |
|         |                  | 1C + 2C 89   | 1,049 108 82 15 205 | 19.5   | 1.22                           |

| All Groups | 209 2,461 231 166 30 427 | 17.3 | 0.76 |

Tamworth Total Group | 70 713 63 12 1 76 | 10.6 | 1.32 |
Table 20. Pigmentation of Subsurface Lesions observed at Exam. 2

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Subjects</th>
<th>Total Lesions (Table 2)</th>
<th>Pigmentation p1</th>
<th>p2</th>
<th>p3</th>
<th>Total P</th>
<th>Percentage of lesions pigmented</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>66</td>
<td>887</td>
<td>35</td>
<td>14</td>
<td>1</td>
<td>50</td>
<td>5.6</td>
<td>0.84</td>
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<tr>
<td>2B</td>
<td>54</td>
<td>649</td>
<td>126</td>
<td>67</td>
<td>5</td>
<td>198</td>
<td>30.5</td>
<td>1.81</td>
</tr>
<tr>
<td>1C</td>
<td>41</td>
<td>499</td>
<td>28</td>
<td>3</td>
<td>0</td>
<td>31</td>
<td>6.2</td>
<td>1.08</td>
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<tr>
<td>2C</td>
<td>48</td>
<td>619</td>
<td>103</td>
<td>58</td>
<td>7</td>
<td>168</td>
<td>27.1</td>
<td>1.79</td>
</tr>
<tr>
<td>1B + 1C</td>
<td>107</td>
<td>1,386</td>
<td>63</td>
<td>17</td>
<td>1</td>
<td>81</td>
<td>5.8</td>
<td>0.63</td>
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<tr>
<td>2B + 2C</td>
<td>102</td>
<td>1,268</td>
<td>229</td>
<td>125</td>
<td>12</td>
<td>366</td>
<td>28.9</td>
<td>1.27</td>
</tr>
<tr>
<td>1B + 2B</td>
<td>120</td>
<td>1,536</td>
<td>161</td>
<td>81</td>
<td>6</td>
<td>248</td>
<td>16.2</td>
<td>0.94</td>
</tr>
<tr>
<td>1C + 2C</td>
<td>89</td>
<td>1,118</td>
<td>131</td>
<td>61</td>
<td>7</td>
<td>199</td>
<td>17.8</td>
<td>1.14</td>
</tr>
<tr>
<td><strong>All Groups</strong></td>
<td><strong>209</strong></td>
<td><strong>2,654</strong></td>
<td><strong>292</strong></td>
<td><strong>142</strong></td>
<td><strong>13</strong></td>
<td><strong>447</strong></td>
<td><strong>16.8</strong></td>
<td><strong>0.73</strong></td>
</tr>
</tbody>
</table>

Tamworth Total Group

<p>| | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
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</tbody>
</table>
PART IV.

RESULTS.

B. Qualitative surface changes of incipient caries lesions.

To enable the possible beneficial effects of the three methods of fluoride application used to control caries to be shown, the same group of 209 subjects as seen at Exam. 1 was re-examined at Exam. 2 six months later and the qualitative changes of the incipient caries lesions were noted. Qualitative changes were noted only for those lesions which were the same lesions seen at both Exam. 1 and Exam. 2.

Hence Table 21 provides the mean values and standard errors obtained for the information as discussed in Part 2 of the Method of Assessment of Data. The values are set out according to the individual fluoride treatment groups and thus compared with the control group, Group 1B. Statistical significance of any difference between the control group value and the other three treatment groups are quoted for the respective group involved together with the probability value, P.

With regard to the prevalence values shown on Table 21, it could be seen that only the values for two treatment groups were significantly different to those of the control groups. The first instance was with the mean number of separate lesions observed
at Exam. 1, but not observed at Exam. 2, where
Group 1C revealed a significant difference \((P = .01)\)
of 0.8 lesions less than that of the control group,
Group 1B: the values were \(2.9 \pm 0.28\) and \(2.1 \pm 0.24\)
for Groups 1B and 1C respectively. Secondly, it
was also seen that Group 2B had only \(2.2 \pm 0.27\)
lesions with etched surfaces at Exam. 1 whereas
Group 1B had \(3.0 \pm 0.27\) lesions, a significant
difference of 0.8 etched lesions \((P = .04)\).

In assessing the number of lesions which had
altered in size over the six months period, it was
revealed that in all four treatment groups the same
number of lesions had increased in size and the
same number had decreased in size. When the size
effect values were combined \((g) + (h)\), the
number of lesions for the respective groups which
showed a change in size was: 1B = 5.7 lesions, 2B =
5.3 lesions, 1C = 5.7 lesions and 2C = 6.0 lesions,
which meant that approximately 48 per cent of the
lesions observed at Exam. 1 and Exam. 2 for the total
group had either increased or decreased in size.

That information which revealed the mean values
for clinically-visible alterations to the condition
of the surfaces of the subsurface lesions, over the
six months period, showed that there were several
values which differed significantly from the respective
control Group 1B values.
There were in Group 2C significantly less of those lesions which had now etched from being hard surfaces, the values being for Group 1B, $1.1 \pm 0.17$ lesions and for Group 2C, $0.6 \pm 0.14$ lesions, a significant difference of 0.5 lesions ($P = .02$).

The number of subsurface lesions which had new surface breaks were $0.7 \pm 0.18$ for Group 1B and only $0.3 \pm 0.08$ for Group 2B. This was a significant difference of 0.4 lesions ($P = .05$).

The number of lesions which had become in part a carious cavity were all less in the three fluoride treatment groups than in the control Group 1B, whose value was $0.9 \pm 0.13$ lesions. Groups 2B and 1C both showed a significant difference of 0.4 lesions ($P = .04$), where the values were $0.5 \pm 0.13$ and $0.5 \pm 0.14$ lesions respectively and Group 2C showed a significant difference of 0.5 lesions ($P < .003$).

In the case where a subsurface lesion at Exam.1 had been scored now at Exam. 2 as only a carious cavity, it was seen that both Group 2B and Group 1C revealed values of $0.2 \pm 0.06$ and $0.1 \pm 0.06$ lesions respectively. These values differed significantly from the control Group 1B value by 0.1 ($P = .05$) and 0.3 ($P = .003$) lesions in Groups 2B and 1C respectively.
Finally it was observed that the number of lesions which were now involved with what appeared to be arrested carious cavities at Exam. 2 was clearly significantly more in Group 2C, where the control Group 1B showed only $0.04 \pm 0.026$ lesions which were arrested whereas Group 2C showed $0.3 \pm 0.09$ lesions ($P=0.005$).
Discussion.

It was seen that the distribution of the condition aspects of the surfaces of all the lesions studied at Exam. 1, whether they were hard, etched, broken or involved with a carious cavity, was essentially the same in each group. The one exception was Group 2B which had at Exam. 1 significantly less lesions possessing etched surfaces than any other group. However, at Exam. 2 the only group to show significantly less lesions which had become newly etched was Group 2C. Thus, from Group 2C, it appeared that the combined use of a 10 per cent stannous fluoride topical solution with the home-use of a fluoridated dentifrice effected a 45 per cent reduction in new subsurface lesions having etched surfaces than otherwise would have occurred if no inhibiting agents had been used.

Correlation coefficients between the number of lesions which had become etched and the actual number of subsurface lesions at Exam. 1 all showed that there was generally moderate positive correlation between these two factors in all four groups. The coefficients were +0.46 for Group 1B, +0.30 for Group 2B, +0.41 for Group 1C and +0.57 for Group 2C. Although these coefficients did not reveal high correlations, there was certainly a positive relationship between the number of subsurface
decalcification lesions per individual and the number of lesions whose surfaces would progress from a hard condition to an etched condition. This would imply that any agent used effectively to prevent the progress of subsurface lesions to further demineralisation would in general inhibit the further demineralisation of subsurface lesions as a whole.

With regard to the number of lesions that now possessed a break(s) in their surfaces, it was obvious that Group 2B which had less etched subsurface lesions initially, also would have less at Exam. 2 and when it was considered also at Exam. 2 that a certain number of etched lesions would progress to broken surfaces, such a finding could not contribute to any inhibitory effects having been caused by the sole use of the 10 per cent stannous fluoride topical solution i.e., Group 2B.

If an area of subsurface decalcification had been present on a buccal/lingual surface at Exam. 1 and had demineralised to the extent where only a clinical cavity was recorded at Exam. 2 and no subsurface decalcification, then it was found that this had occurred to a significantly lesser extent in Groups 2B and 1C as compared to Group 1B. The importance of Group 2B has already been disregarded so that the greatest inhibitory effect would appear
to come from the sole use of a fluoridated dentifrice, i.e., Group 1C. However, if comparisons were made on the number of subsurface lesions which had progressed to involvement with carious cavities and yet these subsurface lesions were still obvious, then it could be seen that the values of all three fluoride treatment groups were significantly less than the control group value. Once again, if Group 2B is ignored, then an inhibitory effect could be shown again by the use of the fluoridated dentifrice and this was evident whether a 10 per cent stannous fluoride topical solution had been used or not, i.e., Groups 1C and 2C.

The final condition to be studied was to show the extent to which the subsurface decalcification lesions were now involved at Exam. 2 with signs of arrested caries. These results were as would be expected and there was a significantly greater amount of arrested caries evident in that group in which the maximum type of fluoride treatment had been used, i.e., topical application of 10 per cent stannous fluoride and the added home-use of a fluoride dentifrice, Group 2C.

If the figures pertaining to the surface effect on the lesions are combined ( (j)+(k)+(l)+(m) ), it was found that in Group 1B, 3.1 lesions had progressed
towards developing into clinical cavities whereas 1.84 lesions ( (i)+(n) ) had apparently hardened and not progressed to cavity formation. This meant that in Group 1B, a nett total of 1.3 lesions showed continued carious breakdown. In Group 1C there was a nett total of 0.3 lesions which progressed towards cavity formation and in Group 2B and Group 2C, there was a nett total of only 0.1 and 0.2 lesions respectively which progressed to clinical cavities. Since the surface effects were observed on different lesions at different stages of their development into clinical cavities, these nett figures are not of statistical significance and only help to show a trend in an overall inhibitory effect of all three fluoride treatment groups on caries formation as compared to that group which was not receiving any fluoride ion.

It could be observed that there were no significant alterations in the size of the lesions over six months which could have been brought about by any fluoride action. This fact certainly agreed with the previous findings that the action of fluoride did not appear to affect the shape of the subsurface lesions. There were also no significant differences shown in any of the four fluoride treatment groups with regard to the number of subsurface lesions which had apparently hardened
or "remineralised". On the other hand, significant differences were shown in the remaining surface condition factors which were studied, but it could not be demonstrated which fluoride treatment group played the most predominating part in bringing about caries-inhibitory effects.

To reiterate on the previous statement on prevalence figures, it had been noted from Table 21 that there was a significant difference in Group 1C, as compared to Group 1B, in the number of lesions which had been recorded at Exam. 1 but not recorded at Exam. 2. This in fact represented either a remineralisation effect on the lesions to a point where they could be observed no longer, or it represented a reversal examiner error, or it was a combination of both factors. If the former assumption of remineralisation is accepted entirely, then the difference shown in Group 1C would represent the least number of remineralised lesions in this group as compared to the control group and this would now seem unlikely; however, if the former assumption is not accepted entirely, and this is probably closer to the truth, then the lower number of reversals in Group 1C would represent a smaller examiner error in this group in the observation of lesions at both examinations. It should be remembered
that, in assessing the clinical alterations to the subsurface lesions, only those lesions which could be observed at both examinations were used for these estimations.

Tables 22 and 23 have been abbreviated from the form of Table 21 and only give those values which relate to a possible clinically-visible effect on the surface of the subsurface lesions in the various combined groups and are referred to as (g) to (n): they have been compiled to show the combined values when comparing the efficacy of the dentifrice containing 0.4 per cent SnF₂ as compared to the control non-fluoridated dentifrice and the efficacy of a 10 per cent SnF₂ topical solution as compared to a control solution of 0.85 per cent saline.

In comparing the combined groups for testing the two solutions it could be seen from Table 22 that Solution 2 appeared to exert a significantly better inhibitory effect on the number of lesions which had etched by Exam. 2, on the number of lesions which had a surface break(s) by Exam. 2 and on the number of lesions which were now associated with carious cavities at Exam. 2.

However, when the dentifrices were compared (Table 23) there was no effect shown by either Dentifrice B or C on the number of newly-etched
lesions or on the number of lesions which had acquired a surface break(s). These effects would have been masked by the uneven distribution of individuals receiving the two different topical solutions within the two dentifrice groups. However it had already been shown (Table 21) that Group 2C or the use of a 10 per cent stannous fluoride topical solution plus the use of a fluoridated dentifrice was beneficial in reducing the number of lesions which had become etched. Nevertheless it appeared that statistically significant differences were shown in both Tables 22 and 23 with regard to the number of lesions which were newly associated with carious cavities. This meant that in this case, a beneficial inhibitory effect was exerted not only by Topical Solution 2 as compared to Topical Solution 1 but also by Dentifrice C as compared to Dentifrice B. This could be interpreted as Group 2C but it has already been shown with regard to factor (m) that study of the individual groups also revealed (Table 21) a beneficial inhibitory effect from Group 1C and Group 2C, i.e., the sole use of a fluoridated dentifrice with or without the use of the 10 per cent SnF₂ topical solution. Thus it was obvious that in comparing combined groups there could be a masking effect by
an uneven distribution of either the attributes or the variables whichever the case may be. However, by comparing the individual groups, it was seen that the study of the surface condition of the lesions which represented the progress to carious cavities or arrested caries provided a greater number of statistically significant group values and these values were interpreted as representing a positive action of caries inhibition.

It was not possible to state conclusively which fluoride treatment group was exerting a greater effect on inhibiting the progress of subsurface decalcification lesions.
Conclusion.

It was found that the values relating to the prevalence and incidence of subsurface lesions which were calculated in this section of the results and shown in Table 21, confirmed the findings of the previous epidemiological section. This meant that, generally, in relation to the prevalence of subsurface lesions, buccal/lingual surfaces affected by subsurface lesions and teeth affected by subsurface lesions on their buccal/lingual surfaces, it was not possible to show statistically significant differences between the four fluoride treatment groups examined at either examination: nor was it possible at the second examination to show significant differences in the incidence values for these subsurface lesions, affected buccal/lingual surfaces or affected teeth, when comparing the individual fluoride treatment group or total group values obtained at the first examination to those obtained at the second examination six months later. On the other hand, observations of the clinical quality of the lesions over the same period did provide sufficient values for differential effects to be shown with regard to the respective fluoride treatment groups when these were compared to the control group.
Study of the size (taking shape into account) of the lesions revealed that there were no significant alterations in the size of the same lesion, observed at the two examinations, which may have been caused by fluoride during the six months study period; but study of the actual condition of the surface of the lesions provided position information as to whether a lesion appeared to be progressing towards a carious cavity or not. When the three fluoride treatment groups were compared to the control group, in this respect, it was observed that the use of a fluoride dentifrice containing 0.4 per cent stannous fluoride, with or without a thirty seconds application of 10 per cent stannous fluoride solution to the teeth at six-monthly intervals, was a factor in inhibiting the progress of subsurface decalcification lesions into clinical cavities.
Abbreviations for Table 21.

\[ \bar{X} = \text{the mean value.} \quad \text{S.E.} = \text{the standard error.} \]

N.S. = no statistical significant difference.

P. = the probability of a difference as great or greater than the observed difference occurring due to chance alone and in expressed as so many times in 100 or in 1,000.

(a) = increase no. of surfaces affected.

(b) = decrease no. of surfaces affected.

(c) = new lesions seen at Exam. 2

(d) = lesions seen at Exam. 1 but not at Exam. 2.

(e) = increase no. of teeth affected.

(f) = decrease no. of teeth affected.

(g) = lesions which have increased in size.

(h) = lesions which have decreased in size.

(i) = lesions whose surfaces have hardened.

(j) = lesions whose surfaces have etched.

(k) = lesions whose surfaces now show a break(s).

(l) = lesions which are scored solely as carious cavity at Exam. 2.

(m) = lesions which are newly associated with a carious cavity.

(n) = lesions which are newly associated with arrested caries.

(o) = number of lesions with hard surfaces at Exam. 1.

(p) = number of lesions with etched surfaces at Exam. 1.

(q) = number of lesions with surface breaks at Exam. 1.

(r) = number of lesions associated with carious cavities at Exam. 1.
Table 21. Various Mean Values as processed for comparison of the individual groups within the total group of 209 subjects.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>1B</th>
<th>2B</th>
<th>1C</th>
<th>2C</th>
<th>Stat. Sig. Differences</th>
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</thead>
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<tr>
<td></td>
<td>X</td>
<td>S.E.</td>
<td>X</td>
<td>S.E.</td>
<td>X</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>1.5</td>
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<td>.25</td>
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</tr>
<tr>
<td>(b)</td>
<td>0.8</td>
<td>.19</td>
<td>0.8</td>
<td>.25</td>
<td>0.5</td>
</tr>
<tr>
<td>(c)</td>
<td>3.7</td>
<td>.28</td>
<td>3.4</td>
<td>.28</td>
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</tr>
<tr>
<td>(d)</td>
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<td>.28</td>
<td>2.6</td>
<td>.29</td>
<td>2.1</td>
</tr>
<tr>
<td>(e)</td>
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<td>.20</td>
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<td>(h)</td>
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<td>1.5</td>
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<td>.73</td>
<td>31.0</td>
<td>.79</td>
<td>32.2</td>
</tr>
<tr>
<td>D.M.S.</td>
<td>33.5</td>
<td>1.79</td>
<td>35.1</td>
<td>1.98</td>
<td>38.0</td>
</tr>
<tr>
<td>(o)</td>
<td>7.4</td>
<td>.60</td>
<td>7.4</td>
<td>.66</td>
<td>6.7</td>
</tr>
<tr>
<td>(p)</td>
<td>3.0</td>
<td>.27</td>
<td>2.2</td>
<td>.27</td>
<td>2.5</td>
</tr>
<tr>
<td>(q)</td>
<td>2.2</td>
<td>.38</td>
<td>1.4</td>
<td>.22</td>
<td>2.0</td>
</tr>
<tr>
<td>(r)</td>
<td>1.1</td>
<td>.20</td>
<td>0.9</td>
<td>.17</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Abbreviations for Tables 22 and 23:

\[ X \] = The mean value.

\[ S.E. \] = The standard error.

\[ H.S. \] = Not a statistically significant difference.

\[ S.S. \] = A clearly statistically significant difference.

\[ P. \] = The probability of a difference as great or greater than the observed difference occurring due to chance alone and is expressed as so many times in 100 or in 1,000.
Table 22. Various values as processed for comparison of combined groups using Topical Solutions 1 and 2.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Top._Sol.1</th>
<th></th>
<th>Top._Sol.2</th>
<th></th>
<th>S.E.</th>
<th>Stat. Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(g) inc. in size</td>
<td>3.5</td>
<td>0.24</td>
<td>3.1</td>
<td></td>
<td>0.23</td>
<td>N.S.</td>
</tr>
<tr>
<td>(h) dec. in size</td>
<td>2.1</td>
<td>0.14</td>
<td>2.5</td>
<td></td>
<td>0.20</td>
<td>N.S.</td>
</tr>
<tr>
<td>(i) surfaces hardened</td>
<td>1.8</td>
<td>0.18</td>
<td>1.7</td>
<td></td>
<td>0.15</td>
<td>N.S.</td>
</tr>
<tr>
<td>(j) surfaces etched</td>
<td>1.0</td>
<td>0.12</td>
<td>0.6</td>
<td></td>
<td>0.10</td>
<td>S.S. P = .005</td>
</tr>
<tr>
<td>(k) surfaces broken</td>
<td>0.6</td>
<td>0.12</td>
<td>0.4</td>
<td></td>
<td>0.06</td>
<td>S.S. P = .05</td>
</tr>
<tr>
<td>(l) new caries cavity</td>
<td>0.3</td>
<td>0.06</td>
<td>0.3</td>
<td></td>
<td>0.06</td>
<td>N.S.</td>
</tr>
<tr>
<td>(m) new with cavity</td>
<td>0.7</td>
<td>0.10</td>
<td>0.4</td>
<td></td>
<td>0.08</td>
<td>S.S. P = .04</td>
</tr>
<tr>
<td>(n) new arrested</td>
<td>0.04</td>
<td>0.020</td>
<td>0.2</td>
<td></td>
<td>0.05</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Table 23. Various values as processed for comparison of combined groups using Dentifrices B and C.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(g) inc. in size</td>
<td>3.2</td>
<td>0.22</td>
<td>3.5</td>
<td>0.26</td>
<td>N.S.</td>
</tr>
<tr>
<td>(h) dec. in size</td>
<td>2.3</td>
<td>0.17</td>
<td>2.4</td>
<td>0.18</td>
<td>N.S.</td>
</tr>
<tr>
<td>(i) surfaces hardened</td>
<td>1.7</td>
<td>0.15</td>
<td>1.7</td>
<td>0.15</td>
<td>N.S.</td>
</tr>
<tr>
<td>(j) surfaces etched</td>
<td>0.9</td>
<td>0.12</td>
<td>0.7</td>
<td>0.11</td>
<td>N.S.</td>
</tr>
<tr>
<td>(k) surfaces broken</td>
<td>0.5</td>
<td>0.10</td>
<td>0.5</td>
<td>0.06</td>
<td>N.S.</td>
</tr>
<tr>
<td>(l) new caries cavity</td>
<td>0.3</td>
<td>0.05</td>
<td>0.2</td>
<td>0.06</td>
<td>N.S.</td>
</tr>
<tr>
<td>(m) new with cavity</td>
<td>0.7</td>
<td>0.09</td>
<td>0.4</td>
<td>0.03</td>
<td>S.S. P &lt; .003</td>
</tr>
<tr>
<td>(n) new arrested</td>
<td>0.1</td>
<td>0.03</td>
<td>0.2</td>
<td>0.05</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
PART V.

SUMMARY AND CONCLUSIONS.

The literature on the histological and chemical changes in the subsurface decalcification process of the incipient caries lesion in dental enamel has been reviewed. In addition, an attempt has been made to establish a basis for the possibility of the clinical reaction of the fluoride ion being a factor in inhibiting the lesion's formation or progresses.

It has been noted that not all observers, even in recent times, have related white spot lesions to the actual caries process and in many of the earlier experiments and clinical records there has been no criteria for the incipient lesion. It would appear that the incipient lesions earlier workers were describing, were in fact fairly-advanced clinical lesions in contrast to what is classified now as incipient caries. The mechanism of caries can still be regarded as the end result of acid fermentation of refined carbohydrates by bacteria and either the carbohydrate or the bacteria may be termed an agent factor; it is a biochemical mechanism in the sense that the chemical reactions in the carious enamel occur under environmental conditions which are determined by biological factors and the pattern of the subsurface decalcification attack can be
explained as a physico-chemical diffusion process. It would seem that the commonly and adequately available agent for enamel attack is the hydrogen ion and the incipient caries lesion is defined as that process when the hydrogen ion diffuses into the enamel subsurface and there is a nett loss in the diffusion of the resultant reaction products away from the tooth surface. There are various levels at which the subsurface decalcification can be determined or recognised.

Histologically the incipient lesion has often been divided into zones for more-localised study and from these zones the levels, at which the lesion can be first recognised microscopically and macroscopically, can be determined. There appears to be no clear-cut evidence concerning specific points of entry for an attacking agent and it is stressed that the appearance of such points of entry or breaches of the enamel show that the striae are merely involved with the rest of the enamel (i.e., during dissolution of those apatite crystals on the borders of the striae and the prisms, and at the prism interfaces) and become exaggerated when viewed in the morphological picture. It has been shown also that the features of the smooth surface lesion and the pit and fissure lesions are similar, and since lesions developing in these two areas are the
result of the same physico-chemical diffusion process, there does not seem to be any reason for differentiating pit and fissure and smooth surface incipient caries as separate entities.

In contrast to the concept of the organic matrix being responsible for spaces in the normal enamel structure, it has been shown that a pore system exists in mature enamel where the "spaces" are determined by the distribution of the crystallites rather than by the organic matrix. Thus during caries attack, there is a non-specific dissolution of the apatite crystals so that some prisms have their centres unaffected with demineralisation at their peripheries while others reveal greater mineralisation of the peripheries than in the centre, and in the resulting progressive broadening of the intercrystalline spaces there is a deposition of amorphous organic material. The acquisition of organic material, the presence of the cuticle and the process of remineralisation are all shown to afford a certain amount of resistance of the enamel against incipient attack however the identity of the first substance to be altered in the dissolution process and the level at which the resistance of the enamel surface breaks down are still debated.
It is appropriate to point out that a description of the initial caries lesion can be made by the use of polarised and transmitted light and by microradiography before it is clinically and radiographically recognisable. Fortunately histological changes in the organic matrix are not found until the surface layer of the enamel is about to break down, which means that areas of subsurface decalcification present an obvious indication of dental caries prior to cavitation and it should be recognised as such.

It follows that it is of practical importance to the dentist to realise that carious lesions of enamel which are visible to the naked eye or detectable by x-ray are too far advanced to be likely to be remineralised unless there are environmental changes, e.g., loss of a neighbouring tooth or preventive measures taken, so that the susceptibility of attack is decreased. Hardwick (153) talks of the methyl red tests for indicating acidity in the oral cavity, where it has been shown that there is a temporary area of high acidity near the gingival margin which apparently disappears when the tooth has fully erupted or comes into occlusion. The lesion that has formed in these cases is closely related to the gingival margin so that subsidence of its progress is influenced by the eruption factor. Furthermore it is difficult to say whether interproximal caries is brought about
initially by the contact points or, whether the lesion was initiated at the eruption level of high acidity, was too early to be recorded, "remineralised" on further eruption, and then was re-attacked when blame could be placed upon the contact point.

In the final section of the review consideration is given to the ability of the apatite crystal for iso- and heteroionic exchange and the involvement of the hydration shell of the crystal in the exchange of ions. Presumably any ion present in the solution can and will penetrate into the hydration shell but only specific ions tend to concentrate there, and only a few ions such as strontium, radium, lead and fluoride are capable of intracrystalline exchange reaction. It would appear from the histology of the incipient caries lesion and from the evidence concerning the ionic exchange reactions that the only enamel reactions during the incipient attack are those of dissolution and recrystallisation. Studies on the organic and inorganic content of the normal and carious enamel have demonstrated that the organic content is of little significance in the solubility effect during the incipient caries lesion formation in vivo, but that the very presence of organic material at the time of incipient attack is attributed to a surface protection effect.
On the other hand, it has been pointed out that the equilibrium of the solubility mechanism of the inorganic apatite portion of the tooth enamel is complex since the apatite crystal is of a variable composition brought about by an indefinite series of nearly-perfect isomorphic ionic substitutions.

The incorporation of fluoride into the enamel is discussed and it is also logical to expect a better degree of crystallisation in the superficial enamel from its direct exposure to available calcium, phosphate and other ions in the tissue fluids: in terms of incipient caries, the most significant portion of the enamel would be those crystals at the surface. The actual persistence of crystals in advanced caries and the higher concentrations of fluoride in carious enamel could be explained on the basis of a crystallite composition change resulting in reduced solubility. The rate of enamel dissolution is determined by the diffusion of the hydrogen ion and undissociated organic acids at the tooth surface, and also to the diffusion of reaction products away from the surface and it has been shown that the initial solubility rate can be decreased by the presence of reaction products (calcium and phosphate ions), of most if not all cations, and especially by the presence of the anion, fluoride. With the exception of fluoride, no other element has been
shown to have as great a topical effect in reducing caries.

It is obvious that the dissolution process is indirectly proportional to the pH of the liquid phase but because of the variability of the enamel and saliva, it is not possible to know to what extent, however, the pH is the one variable, which by itself, can indicate whether dissolution might occur. Solubility studies in saliva over a considerable pH range have revealed that both hydroxyapatite and fluorapatite are controlled by the same activity product of CaHPO$_4$. Furthermore, as long ago as 1946, it has been demonstrated that slightly-decalcified enamel can remineralise from the inorganic constituents of saliva and more recently, the unique ability of the presence of fluoride to induce apatite formation in suspensions of calcium and phosphate ions has been reported. From the standpoint of the possible chemical reactions of the enamel it is fully acceptable that the availability of the fluoride ion during the dissolution process of incipient caries will play a major role in caries prevention and leaves no doubt as to the efficacy of the fluoride ion as a factor in providing the enamel with a degree of resistance to incipient caries attack.
Hence a clinical study was undertaken by the author to test the principle of the fluoride ion being a factor in incipient caries prevention by diagnosing the incipient lesion at the macroscopic level in 228 students in 17 Sydney Metropolitan High Schools, and which entailed the observation of 2,671 subsurface lesions using a coded system to record a quantitative and a qualitative description of the lesions over a six months period. The 228 students were selected within each school and the total group consisted of four smaller fluoride treatment groups in which there were two major variables, the one receiving no stannous fluoride topical application and the other receiving 10 per cent stannous fluoride topical application. To each of these variables was added the use or non-use of a 0.4 per cent stannous fluoride dentifrice. The findings were assessed in two sections with regard to the epidemiological aspects and to the qualitative surface changes of the lesions.

A summary of the findings of the clinical study is:

1. The susceptibility of subsurface decalcification appeared to increase with the age of the individual up to the age of 15 years. In the general community, 3 out of 4 individuals who were in the 14 to 15 year old age group showed visible signs of subsurface
lesions on the buccal (labial)/lingual surfaces of their teeth. Of those who were in the author's smaller study group, approximately 1 out of 3 permanent teeth possessed macroscopic subsurface lesions on the buccal (labial)/lingual surfaces.

(2) There were only 10 per cent more affected teeth in the mandibular arch than in the maxillary arch, however there were 2 to 3 times as many lesions on the buccal (labial) surfaces as there were on the lingual surfaces.

(3) The most common type of lesion observed was crescent-shaped and if it was assumed that this shape had been influenced by attack along the gingival margins of the tooth, then approximately 3 out of 4 lesions were initiated in this clinical area. It was also noted that approximately 2 out of 3 lesions studied were situated in the area of the gingival third of the anatomical crown, irrespective of their shape or whether they were on the buccal (labial) or lingual surfaces of the teeth. It was found that the use of 0.4 per cent stannous fluoride dentifrice and/or the use of two applications of 10 per cent stannous fluoride topical solution over a period of one year did not appear to have affected the actual shapes of the lesions.

(4) Attributing the pigmentation of subsurface lesions to the action of stannous ions, it was found
that a large group of subjects could be treated
over eighteen months with stannous fluoride topical
solution without the occurrence of too much
discolouration. There was only 16 per cent more
of pigmented lesions observed than would have been
observed without the use of stannous fluoride and
in this 16 per cent increase of pigmentation, only
17 to 28 per cent of the pigmentation would be
considered as unaesthetic, i.e., approximately 4 to
5 per cent of the total number of lesions studied
for a group would be expected to show unaesthetic
pigmentation from the action of stannous ions.

(5) By observing the incidence of the number of
subsurface lesions, the number of affected buccal
(labial)/lingual surfaces, and the number of affected
teeth over a six months period, it was not possible
to show the merit of using either a stannous fluoride
topical solution or a fluoridated dentifrice as a
control in reducing the rate of new lesion formation.

(6) It was revealed that in all four fluoride
treatment groups under study the same number of
lesions had increased in size and the same number
had decreased in size, and that for the total group
examined over the six months, approximately 1 out of
2 lesions had either increased or decreased in size.
However there were no significant alterations in
the size of the same lesions, observed at the two
examinations, which could have been caused by any fluoride action during the study period.

(7) By observing the surface qualities of the sure lesions for six months as to whether they were of a hard and glossy nature, were etched, had surface breaks, were associated with a clinical cavity, or were now observed as clinical cavities alone, it was concluded that the routine use of a fluoridated dentifrice containing 0.4 per cent stannous fluoride, with or without a thirty seconds application of a 10 per cent stannous fluoride topical solution at six monthly intervals, was a factor in inhibiting the progress of incipient caries lesions into clinical cavities.
PART V.

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<th>Title</th>
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<td>113</td>
<td>POSNER, A.S.</td>
<td>Mineralised Tissues.</td>
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<td>118</td>
<td>SCHMIDT-NIELSON, B.</td>
<td>The Solubility of Tooth Substance in Relation to the Composition of Saliva.</td>
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<td>119</td>
<td>SCHULE, H.</td>
<td>Chemische Zusammensetzung und Physikalische Eigenschaften des Schmelzoberhautchens.</td>
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</table>
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### Coded Recording

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>Upper Right Lateral</strong></td>
<td>Fig. I Two small separate crescent lesions and one white spot lesion all in the gingival third position and having hard glossy surfaces. The small white spot lesion is in the distal part of the gingival third.</td>
</tr>
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<table>
<thead>
<tr>
<th>$g^1$</th>
<th>AWD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g^{13}$</td>
<td>AWD</td>
</tr>
<tr>
<td>$g^3$</td>
<td>AYD</td>
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| **Upper Left Lateral** | Fig. II A crescent lesion in the middle-distal gingival third position with an etched surface and associated distally with a clinical cavity on the distal surface of the tooth. |

<table>
<thead>
<tr>
<th>$g^{13}$</th>
<th>AWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>+3</td>
<td></td>
</tr>
<tr>
<td>$g^3$</td>
<td></td>
</tr>
</tbody>
</table>

| **Upper Left Canine** | Fig. II A larger crescent lesion with an etched surface and situated in the mesial-to-distal gingival third. |

<table>
<thead>
<tr>
<th>$g^{213}$</th>
<th>BWE</th>
</tr>
</thead>
</table>
### Upper Left Central

<table>
<thead>
<tr>
<th>$S_{4.1g}^{13}$</th>
<th>BWD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_{4.1}$</td>
<td></td>
</tr>
<tr>
<td>$S_{5.1}$</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. III**

A crescent lesion in the middle-distal gingival third position with a clinical cavity in the middle of the lesion and it is also associated distally with a silicate restoration on the distal surface of the tooth.

### Lower Right Molar I

<table>
<thead>
<tr>
<th>$S_{4.1}m^{213}$</th>
<th>CWDP'</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_{5.7}$</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. IV**

A crescent lesion in the mesial-to-distal middle third position with an amalgam restoration in the middle of the lesion. The surface of the lesion is hard and glossy and there is a slight brown colour which is not clearly evident on this photograph.
Fig. V
Two separate crescent lesions both in the mesial gingival third position, both associated with a buccal amalgam restoration, and both having slight brown pigmentation; however, one had a hard surface whereas the other had an etched surface.

Fig. V
A crescent lesion in the mesial-to-distal gingival third with an etched surface, slightly pigmented and associated in the middle with a buccal clinical cavity.

Fig. VI
A crescent lesion in the gingival third with a hard surface and very dark brown pigmentation. Another separate smaller crescent lesion in the distal gingival third with a hard surface and associated buccally with a crescent arrested caries lesion which is again associated with a small hard white spot lesion.
**Lower Left Canine**

<table>
<thead>
<tr>
<th>$S_4^{213}g$</th>
<th>CWDP$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_4^{213}g$</td>
<td>CWDP$^2$</td>
</tr>
</tbody>
</table>

**Fig.VII**

A large crescent lesion occupying most of the gingival third from mesial to distal and which has a hard surface and dark pigmentation which is not present throughout the entire lesion. It does not appear to be associated with the clinical cavity on the mesial surface of the tooth.

**Lower Left Molar I**

<table>
<thead>
<tr>
<th>$S_4^{203}O_3^a$</th>
<th>BWD$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_4^{203}O_3^a$</td>
<td>BWD$^3$</td>
</tr>
</tbody>
</table>

**Fig.VIII**

A crescent lesion in the mesial-to-distal gingival third position on the lingual surface and which extends around to the mesial surface of the tooth. The lesion is hard and very dark brown throughout. The lesion on the mesial surface of the tooth has not been recorded.
Lower Left Molar I

<table>
<thead>
<tr>
<th>$S_4 \cdot 1 \cdot m^{2.1}$</th>
<th>BWD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$+1$</td>
<td></td>
</tr>
<tr>
<td>$S_5 \cdot 7$</td>
<td></td>
</tr>
<tr>
<td>$S_4 \cdot 1 \cdot m^{3}$</td>
<td>BZD</td>
</tr>
<tr>
<td>$+1$</td>
<td></td>
</tr>
<tr>
<td>$S_5 \cdot 7$</td>
<td></td>
</tr>
<tr>
<td>$+3$</td>
<td></td>
</tr>
<tr>
<td>$L_6$</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. IX**

A hard crescent lesion in the middle third associated with a buccal amalgam restoration, and in the distal middle third there is a medium speckled lesion with a hard surface and which is associated buccally with the same amalgam restoration and distally with a white developmental hypocalcification (6W).

Upper Right Molar II

<table>
<thead>
<tr>
<th>$S_7 \cdot 1 \cdot g^{2.13}$</th>
<th>AXD $P^1$</th>
</tr>
</thead>
</table>

**Fig. X**

A trapezoidal lesion with slight pigmentation and a hard glossy surface.
### Upper Right Canine

<table>
<thead>
<tr>
<th>$S_4,I_{gm}^{2,13}$</th>
<th>BXD P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>$+3$</td>
<td></td>
</tr>
<tr>
<td>$S_4,I$</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. XI**

A relatively larger trapezoidal lesion than shown in Fig. X. It has a hard surface with moderate dark brown pigmentation and is associated distally with a labial clinical cavity.

### Upper Right Lateral

<table>
<thead>
<tr>
<th>$S_4,I_{g}^{1}$</th>
<th>AXD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_4,I_{m}^{1}$</td>
<td>BZD</td>
</tr>
</tbody>
</table>

**Fig. XII**

A hard trapezoidal lesion in the gingival third and a separate hard speckled lesion in the middle third position.
**Lower Right Molar III**

<table>
<thead>
<tr>
<th>$S_4 1g^{213}$</th>
<th>$m_{13}^3$</th>
<th>$\text{CXE}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>+1</td>
<td>(6)</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. XIII**

This appears as a trapezoidal lesion which now involves all surface positions except $m^2$ and $i^{12}$ and resembles a cloud lesion. Its surface is etched and it is associated buccally with a developmental hypoplastic defect (6).

---

**Lower Right Lateral**

<table>
<thead>
<tr>
<th>$S_4 1g^3$</th>
<th>$\text{BYD}$</th>
</tr>
</thead>
</table>

**Fig. XIV**

A medium-sized cloud lesion on the distal gingival third position. Its surface is hard and glossy.
### Fig. XV
Both teeth show large cloud lesions with hard surfaces in the middle and gingival thirds of the lingual surfaces of the teeth. In the middle of both lesions there is a small developmental hypoplastic defect (6).

### Fig. XVI
A hard slightly pigmented cloud lesion in the gingival third and associated with a hard more-speckled lesion in the middle third of the labial surface of the tooth, and a separate small speckled lesion on the distal middle third position.
Upper Right Lateral

| $S_4 g^{12}$ | BYE $P^2$ |
| + | $S_4 g^{13}$ | BZD $P^2$ |

Fig. XVII

An etched cloud lesion on the mesial-middle gingival third with moderate dark brown colour at the margins of the lesion and it is associated with a medium-sized hard speckled lesion on the middle-distal gingival third which also has pigmentation. The lesion appears to be the one lesion where the speckled area has not yet coalesced to form an uniform cloud lesion.

Upper Right Canine

| $S_4 g^{12}$ | BZD |
| + | $2$ |
| $S_4$ |

Fig. XVIII

A hard speckled lesion on the middle-mesial gingival third and which has a small clinical cavity in the mesial part of the subsurface lesion.
Fig. XIX
A larger speckled lesion than shown in Fig. XVII and which occupies the mesial-to-distal gingival third. It is etched and pigmented and does not appear to be associated with the silicate restoration on the mesial surface of the tooth.