SOME OBSERVATIONS UPON THE FORMATION

OF CARIOUS LESIONS IN HUMAN DENTAL ENAMEL.
Thesis submitted to the University of Sydney,
for admission to the degree of Doctor of Dental Science.

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The original work presented in this thesis has been carried out by me as an individual research project.

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I desire to express my sincere thanks for this assistance.

[Signature]
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"Let us first understand the facts, and then we may seek for the cause".

Aristotle
## PART I.

**Introduction and Review of Relevant Literature.**

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Chapter 1.
THE PROBLEM.

At a recent meeting of research workers who were interested primarily in the cause of dental caries, this question was posed: "What is the initial caries lesion as it can be seen under the microscope? What can an histologist tell us about what happens as he interprets what he sees magnified by the microscope as caries attacks the enamel?" The speaker replied: "I shall say frankly that I do not know". The questioner's reply was: "I think that this knowledge is so fundamental that we cannot let that question rest" (Basilisk, 1948). This is part of the discussion at a "workshop" held at the University of Michigan during 1947 to discuss the mechanism and to evaluate methods of control of dental caries. Such discussion as that set out above shows all too clearly that, whilst much work has been done upon the problem of dental caries, the etiology and histopathology of this disease remain obscure.

Before proceeding, it is essential that we define the term "dental caries". It has been defined in a number of ways and indeed it is no easy task to give a concise definition of this disease. It should be possible, provided we have adequate knowledge, to define dental caries either in terms of its clinical features or in terms of its pathogenesis and pathology. Macphie (1956) gave a so-called clinical definition, thus: "Dental caries is the progressive destruction of the tooth substance by a process which is neither erosion, abrasion or resorption". Most definitions, however, have been coloured by the personal beliefs of the authors. Thus we find completely divergent descriptions; for example Gottlieb (1947) stated: "Dental caries is a pathologic process found only in the teeth ... the 5% organic material of the enamel is so unfavourably arranged between walls of rigid calcification that it affords avenues for bacterial invasion leading to the dentine, ... the process by which this invasion occurs through the tooth surface is called dental caries". In contrast, Enright, Friessell and Trescher (1932) said: "Dental caries is a chemical dissolution of the calcium salts of the enamel and dentine of the teeth".
On examining the problem of finding an adequate definition, we realise how difficult this is. We cannot describe caries as a disease caused by a certain organism for, despite the voluminous output of work concerning L. acidophilus (Bunting, 1936) there is as yet no proof that it alone is the bacterial agent in dental caries. We are unable to describe the course of the disease fully for we are still undecided as to the histopathology of the disease. It would appear, then, that we must be satisfied at this juncture to accept a definition of a general nature and I suggest: Dental caries is a pathological process found in teeth, primarily of human beings. It begins on the external surface of the tooth and progressively involves the calcified tissues until the pulp is reached. The result is the dissolution of the enamel and dentine and the necrosis of the pulpal tissues. It might also be well at this point to recall the almost inspired definition given by Miller (1890): "Dental decay is a chemico-parasitical process consisting of two distinctly marked stages: decalcification, or softening of the tissue, and dissolution of the softened residue. In the case of the enamel, however, the second stage is practically wanting, the decalcification of the enamel practically signifying its total destruction".

It is an accepted fact that the cause of dental caries is one of the most baffling problems in medicine and dentistry and the disease is certainly one of the most widespread in the civilised world today. Cameron (1948), in a recent survey of the teeth of school children in New South Wales, showed that dental caries had occurred in the mouths of practically all the children by the time they reached the age of eight years. This is not a present day phenomenon by any means, for dental caries has existed throughout the ages as a disease accompanying the various epochs of civilisation. Pickering (1912) has discussed this point at length and showed that in groups of children located in different parts of the world some 95% suffered from carious teeth.

The work of past investigators has clarified many aspects of the problem but we have still no irrefutable evidence of the pathogenesis of the disease nor have we yet any acceptable and certain method of control. Until we have established the pathogenesis we shall be forced to rely on
empirical methods in our attempts to control the disease. The work presented in this thesis was carried out in an attempt to assist in the eludation of some of the many problems concerned with the pathogenesis of dental caries.

The incidence of caries is so great that it is simpler to study the abnormal rather than the normal. Thus certain accepted clinical facts have been established. One of these facts is that a carious lesion invariably starts on the external surface of the tooth. Because of this, it is not intended to refer in this work to dental tissues other than the enamel. Whilst it is undoubtedly important and interesting and instructive to be aware of the manner in which caries affects the deeper tissues, if we can solve the problem of the initiation of the lesions upon the enamel and prevent their development, the lesions in the deeper tissues will never occur.

As we know it, enamel has two major components - inorganic and organic - and caries is attributed primarily to an attack on either of these components. Any understanding of the process must rest on a sound knowledge of normal structure and composition. This dissertation is an attempt to appraise critically our present knowledge of this field and to make a contribution to that knowledge. The structure of the dissertation is therefore as follows: firstly, a review of the literature on the structure and composition of the enamel and the mechanism of dental caries; secondly, a report of experimental work carried out by me in relation to some of the problems and, thirdly, a discussion of the problem in general.
Chapter 2.

THE COMPOSITION OF NORMAL ENAMEL.

Enamel is a hard, inert tissue composed of inorganic and organic constituents. It invests those portions of the teeth which are normally exposed in the oral cavity.

The Inorganic Constituents.

Because of the structure of the enamel, it is comparatively easy to enumerate quite accurately the majority of its contents. This can be done because enamel is so highly calcified that the investigation of its composition amounts primarily to an analysis of inorganic-salts. Some variation occurred in the results of earlier investigations but more recent observations have shown a marked consistency. This can be seen from the results set out in the following table showing the concentration of calcium, phosphorus and magnesium in enamel:

<table>
<thead>
<tr>
<th>Source</th>
<th>Ca</th>
<th>P</th>
<th>Mg</th>
<th>Ca/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wainwright (1933)</td>
<td>39.0</td>
<td>17.5</td>
<td>-</td>
<td>2.26</td>
</tr>
<tr>
<td>Logan (1935)</td>
<td>35.8</td>
<td>17.03</td>
<td>0.25</td>
<td>2.09</td>
</tr>
<tr>
<td>Bowes and Murray (1935)</td>
<td>37.07</td>
<td>17.22</td>
<td>0.46</td>
<td>2.15</td>
</tr>
<tr>
<td>Armstrong and Brakhus (1937)</td>
<td>35.41</td>
<td>17.45</td>
<td>0.5</td>
<td>2.03</td>
</tr>
<tr>
<td>Bremer (1939)</td>
<td>36.64</td>
<td>16.82</td>
<td>-</td>
<td>2.19</td>
</tr>
<tr>
<td>French and others (1939)</td>
<td>37.1</td>
<td>17.6</td>
<td>-</td>
<td>2.12</td>
</tr>
<tr>
<td>(precolumbian Indian teeth)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ockerse (1943)</td>
<td>35.54</td>
<td>17.42</td>
<td>0.42</td>
<td>2.04</td>
</tr>
</tbody>
</table>

It will be seen from these figures that the results obtained are reasonably consistent. However all investigators reported that some variation occurs in the concentrations in individual specimens and thus it is difficult to do other than accept a statistical figure for what might be called normal enamel. It has also been suggested that the concentration of the salts varies, depending upon the area from which the enamel sample is obtained.

Other inorganic salts are present in smaller amounts than those already mentioned but on that account should not be ignored, because the
presence of trace elements in biological material is often of fundamental
importance. Sodium, potassium, lead, zine, iron, copper, chlorine,
fluorine, lithium and strontium have all been shown to be present quite
consistently in normal enamel. (Logan, 1955; Bowes and Murray, 1935b;
Pfrieme, 1934; Cruickshank, 1936; Engel, 1934; Harrison, 1937;
Armstrong and Brekhus, 1938).

Whilst it has been a comparatively simple matter to decide upon
the basic constituents of the inorganic portion of the enamel, it has by
no means been a simple task to determine the form in which the inorganic
enamel substance exists. Bassett (1917) originally showed the ability
of calcium and phosphorus to form a stable, basic phosphate
$3 \text{Ca}_3(\text{PO}_4)_2 \cdot \text{Ca} \text{(OH)}_2$ with a structure characteristic of the mineral
apatite. This apatite structure, because of its stability, was accepted
as the type most likely to exist in bone and by analogy it was assumed
that it is also the form in which the inorganic salts exist in enamel.
This evidence was supported by the fact that the calcium/phosphorus ratio
for $\text{Ca}_3(\text{PO}_4)_2$ is 1.94, whilst that for hydroxyapatite is 2.15, a figure
which it will be seen is very similar to those given in the previous table.
This supposition was supported by the work of Taylor and Sheard (1929)
who showed that the refractive index of enamel is 1.62 and that of apatite
minerals is 1.63.

The difficulties associated with the study of such a highly
calcified tissue are great, but the technique of X-ray diffraction proved
to be most valuable and gave apparently accurate results. X-ray
diffraction diagrams for enamel and mineral apatites were made and
compared by Cape and Kitchin (1930), Thewlis (1934) and Bale, Le Fevre
and Hodge (1936) who all supported the view that great similarity exists
between the structure of enamel and that of the mineral apatites. The
main variation that was found by some of these workers was that whereas
pure hydroxyapatite contains only calcium and phosphorus anions, in
enamel it was possible to find substitution of magnesium, chlorine,
fluorine and sodium without any major alteration in the unit cell of the
crystal.
Dallemagne and Melon (1945, 1946), after studying exhaustively the composition of bone salts, threw further light on the structure of enamel salts. They showed that, whereas bone and dentine consist primarily of calcium phosphate and calcium carbonate, the enamel is composed of about 60% carbonate apatite, 30% tricalcium phosphate and 5% calcium carbonate and that the remainder consists of other minerals and organic matter. The carbonate apatite is in the enamel prisms whilst the free tricalcium phosphate is in the interprismatic substance. This location is most important as tricalcium phosphate is not as dense as the apatites and also is more soluble in acids.

The work of Kitchin (1933) who employed polarised light to study the enamel, showed that the completely calcified enamel consists of submicroscopic crystallites of hexagonal shape. These are arranged within the enamel prisms with their long axes lying roughly parallel to the long axis of the prism. Thewlis (1940) showed that the crystallites making up the enamel possess two angles of orientation, one being 5° to the prism direction and the other 40°. It is thought that the arrangement of the crystallites in the interprismatic substance is not essentially different from the arrangement within the prisms although Thewlis did say that the majority of crystals in the interprismatic substance have their axis at 40° to the prism direction.

Wyart and Tournay (1949) examined the orientation of the apatite crystallites in enamel by using X-rays and concluded that the axes of the apatite prisms are contained within a cone of 30°. The axis of this cone lies in a longitudinal section and makes an angle of 10° with the enamel surface towards the tip of the tooth. The apatite prisms appear to be independent of one another. They suggested, however, that their results gave only a statistical value.

The Organic Constituents.

At the beginning of this century, the belief that enamel was wholly inorganic was held by some of the foremost workers of the time. Williams (1896) expressed the opinion that enamel was composed entirely of inorganic matter and described the findings of some of his contemporaries (Heitmann, 1887) as "vain imaginings". Support came
from Tomes (1897), and Hopewell-Smith (1926, 1927) also claimed enamel to be an inorganic, inert tissue.

However, as early as 1887, Thompson had stated that "the salts of lime are deposited in an organic matrix of horny matter", and in 1906, Bodecker very ably demonstrated the presence of an "organic matrix" by means of a "celloidin decalcification" method of preparing sections of enamel for microscopical examination. This brought about much discussion and eventually by 1925 the existence of organic matter in enamel was accepted (Bodecker, 1924-1926).

Because of the extremely flimsy nature of the organic matrix, estimations of its concentration in enamel have varied. Karlström (1927) reported 0.3%, Sprawson and Bury (1928a & b) 0.15%, Rosebury (1930) 0.3-0.5%, Bowes and Murray (1935b) 0.9%, Le Fevre and Manly (1938) 1.2% and Deakins and Volkow (1941) 1.46% (average), as the amount of organic matter present in enamel.

The nature of the organic material, likewise, has not been readily ascertained because of the difficulty of obtaining suitable samples for investigation. It was assumed that, because enamel is of ectodermal origin, the protein should bear some relationship to the keratins which are the least reactive of all proteins. Bodecker and Gies (1924) were amongst the first to offer any histochemical proof of the presence and nature of protein matter in the enamel. They showed that a positive biuret test is given whilst the Molièch test for carbohydrates and the ammoniacal silver test for aldehydes are negative, indicating that the material is of a protein nature (Gies, 1924-1926). This conclusion was corroborated by the fact that a typical red colouration was obtained with Millon's reagent. This protein substance stains deeply with tryphen blue, a dye which Gies had previously demonstrated could be absorbed into the enamel during its formation but not after it had become fully calcified. This work was confirmed later by Rosebury and Gies (1929) who indicated that the protein shows numerous characteristics similar to those of keratin. They believed that it is somewhat closely allied to the neurokeratins. They also stated that there appears to be neither soluble diffusible nor indiffusible protein in the enamel.
Chase (1929) used histological staining to investigate the nature of the matrix and agreed that it is a protein, probably a keratin. Rosebury (1930) re-stated that there is no soluble protein in enamel, and this view has been supported by Karshan and others (1934) and Kenner (1939). It was further shown that enamel protein resists pepsin and trypsin and gives a weak reaction for the presence of sulphur which made Rosebury consider that enamel protein resembles neurokeratin.

Pincus commenced an investigation of the nature of the organic material in 1935 and has published numerous papers since that time (1935, 1936, 1939). Whilst he found that the matrix possesses many properties in common with the keratins, its X-ray diffraction diagram differs from that of most keratins. He also stated that it does not give a reaction for the presence of cystine and that it has a lower sulphur content than most keratins, there being no evidence of a sulphur-sulphur bond, the sulphur being bound only to carbon. His work indicated that tyrosine and methionine may be present. Wellings (1940), however, later suggested that the tyrosine was an impurity. Kenner (1939), by use of the polarograph, confirmed that cystine is absent.

Weinmann and others (1942) showed that enamel which has just completed its maturation (as judged by conversion of the acid-insoluble matrix to acid-soluble, calcified enamel) has a protein content of about 4%. This value is much higher than that given for older teeth. Frisbie and others (1944) assumed that the changes which occur in developing enamel continue after eruption - the process of progressive dehydration or deaciation eventually giving a protein which is more resistant to hydrolysis than is the protein in younger teeth.

Losee and Hess (1949) carried out a series of investigations on enamel removed from non-curious, permanent molars. They demonstrated a variation in protein content in different types of teeth and claimed that cystine, methionine and phenylalanine are present in enamel protein. Work by Wislocki and Sognnaes (1949) using histochemical reactions, suggested the presence in the organic framework of the enamel of an acid mucopolysaccharide. They also suggested that there is no glycogen in the organic framework of the enamel.
Hutton and Muckolls (1950a) indicated that they were unable to demonstrate the presence of free amino acids in normal enamel but after hydrolysis they tentatively identified cystine and glutamic acid, glycine, aspartic acid, serine and tyrosine in the enamel matrix. Further, they indicated the presence of an unidentified carbohydrate which they thought may be a glycoprotein. Looze and others (1950) suggested that the mol ratio of histidine, lysine and arginine (which they calculated to be 1:3:9) is in fairly close agreement with the ratio of 1:4:12 which has been suggested for a keratin.
Chapter 3.

PHYSICAL AND PHYSIOLOGICAL PROPERTIES OF NORMAL ENAMEL.

Permeability and "Vitality".

A question that arose quite early during the investigations into the structure and composition of enamel was whether the enamel could be considered a truly "vital" tissue or whether it were completely inert. For many years there were two schools of thought as to whether enamel had any function as a living tissue or whether it acted purely as a passive and protective surface. Black, in 1916, stated that "the tissues of the teeth are not changed in any by physiological processes after they are once formed". However, even in 1887, Thompson had said that "it has been conclusively demonstrated that there are areas of living matter in the enamel, and that this living matter is in direct connection by an anastomosis more or less regular and continuous with the contents of the dentine tubules. If this be true, then indeed there is osmosis by which nutrition is conveyed to the enamel however minute and inappreciable it may be".

The permeability of enamel, in a variety of conditions, was easily shown. Pickerill (1912) placed teeth in a 10% solution of silver nitrate for 24 hours, exposed them to bright sunlight for a day and then washed them in salt water and prepared ground sections. By this means, he found that he was able to observe penetration of the silver nitrate to a depth varying between 0.13 mm and 0.27 mm. He stated that, firstly, the amount of penetration bore a direct relationship to the structure of the tooth, depending upon its being "malacic" or "sclerotic"; secondly, the degree of permeability of the enamel surface bore an inverse relationship to the length of time the tooth had been erupted. He stated further that the stain penetrates at first along the interprismatic substance and then seems to spread laterally into the prisms. He was not prepared to state specifically what materials are stained but said "the fact remains that a fluid carrying a solid in solution can pass into the outer layers of enamel and that the solid is there fixed either by chemical combination of by precipitation".
Bunting and Hickert (1918) and later Klein and Amberson (1929) and Klein (1929, 1930, 1932) demonstrated a dialytic flow of sodium and chloride ions through the enamel of extracted teeth. This was corroborated by an impressive mass of work by different investigators (Beust, 1912; Fish, 1926, 1927; Applebaum, 1929; Bodecker, 1937, 1941; Lefkowitz and Bodecker, 1938; Berke, 1940; and Berggren, 1943) using such dyes as alcoholic fuchsin, methyl blue, trypan blue and lithium carmine; they clearly demonstrated that it is possible to stain enamel by various means both in vivo and in vitro. They attempted to stain the enamel by penetrating from both the external and the dentino-enamel junction. They were able to do this and they also showed that the more imperfect the enamel, the deeper the stain penetrated.

Jeffery (1952) showed that the enamel is permeable to stain by using the method of staining by cataphoresis. The outer layers of enamel seem to be least permeable and there is an apparent variation as the post-eruptive tooth age becomes greater. A number of these workers stated that the penetration occurs through prism sheaths.

Entin (1929) described a method of electro-endosmotic transport of water across enamel. Bodecker and Lefkowitz (1937) stated that the bands of Schreger and rod sheaths are the main areas stained in enamel. They suggested the existence of an afferent and efferent flow of dental lymph in the dentinal tubules which are connected with the enamel. They agreed that the enamel is less permeable than the dentine and that its permeability varies with age. They also pointed out that in non-vital teeth the staining of enamel is far more uniform than in vital teeth and they assumed that a vital pulp exerts some influence on the dental tissues, one factor being the regulation of diffusion of dental lymph. Lefkowitz and Bodecker (1938) suggested that stain can penetrate normal enamel from the dentino-enamel junction outwards but not from the external surface inwards. They compared the enamel to skin in having a protective function in not allowing the escape of lymph.

Berggren (1947) carried out investigations, using radio-active isotopes, bacteria and bacterial toxins and dyes, to test the permeability of the enamel. Atkinson (1948), using a method of cataphoresis, indicated that young enamel is permeable from both without and within to
certain dyes when they are transported by means of an electric current. Under the same conditions, old enamel appears to have an outer layer which, in parts, is impermeable to these dyes but which is permeable to water. He also stated that the dye, whether transported by dialysis or cataphoresis, was always found in the prism sheath. He further suggested the difference in staining between the old and the young enamel consists only in the reduction in the "pore-size" of the prism sheath.

These results were interpreted by some to indicate that enamel possessed "vital" properties whilst others more cautiously suggested that it merely showed that enamel, under certain conditions, was permeable and that chemical interchange was a possibility. Williams (1923a) considered this problem one of the most important to be solved in deciding the course of further investigations. He said: "Now obviously there is no more important problem before our profession today than this. If it could be conclusively demonstrated that metabolic or even so-called inorganic, chemical changes take place in the enamel of erupted teeth, the door would be open for investigations which might result in the discovery that such chemical changes could be controlled in a manner to improve the quality or resisting power to decay of that tissue. That indeed would be a momentous day in the history of dentistry and, I may add, in the history of humanity ... the question, you see, really involves the whole problem of the prevention of decay."

A series of papers were produced by Andresen (1923) in which he claimed that it is possible, both by physiological and artificial means, to remineralise enamel. His reasoning was rather vague in many respects but it is obvious that he believed that enamel is a vital structure which not only is permeable but also can be subjected to ionic exchange. Weber (1930) questioned the possibility of remineralisation in relation to the flow of salts from the pulp or saliva. He stated that where decalcification has been started, not only is the enamel not remineralised but that it will go on losing calcium ions to the saliva.

The work of Bodecker (1906), Malleson (1924) and Mummery (1927), concerning the organic matrix, offered considerable support for the view that vital processes may be possible within the enamel structure.
Bodecker, particularly, was convinced that this matrix could provide a medium for metabolic changes. However, many other workers considered that the matrix was sufficiently calcified to prevent such activities. Williams (1923b) (who by now admitted the presence of an organic structure in the enamel) was one who was not prepared to assume that any metabolic activity could occur in the enamel. Gies (1928) and Fish (1933) both suggested that, even if organic matter is present in enamel, it does not follow that it is a vital structure. The fact that the matrix resembles a keratin would indicate that it is not capable of most changes that are usually termed metabolic.

Chase (1929) believed that as enamel forms in the developing tooth it undergoes a series of changes which he termed maturation and which are the analogues of the changes found in the various layers of the skin. These changes consist essentially of an increasing degree of keratinisation of the tissue with steady loss of solubility and reactivity. Evidence in support of this work came from several investigators who indicated that the changes of maturation can continue even after the tooth has erupted. Fish (1933) showed that the enamel of dogs' teeth is very permeable to methylene blue when a dog is young but it becomes less permeable, particularly on the outer surface, when the animal is older. He found some evidence of a similar phenomenon in human teeth.

Beaup (1932) suggested that most organic structures are calcified by the time the tooth has fully erupted. "This renders those theories of resistance to caries that are based on the action of a buffer afforded by a lymph through the enamel paradoxical and no longer tenable".

Lura (1949) favoured the idea of an enamel metabolism and stated that all the conditions for an exchange of the enzymic processes which form a regular metabolism are present in the hard tissues of the tooth.

Ostby (1941) has reviewed a large amount of literature in relation to this matter and suggested that the vitality of hard structures of the teeth is decided by definitions. Those who believe that vitality is dependent upon the presence of phenomena characteristic of cell life naturally would regard the tooth structures as non-vital. However, those
who consider the physical and chemical changes in the intercellular substance as representative of vital activities in the broader sense, will regard enamel as participating in the vital processes of the body.

Gstby's observations appear to be very pertinent for, indeed, most discussions concerning "vitality" have revolved around the interpretation of permeability. It is possible to accept one of the two interpretations in this regard. Widdowson (1946), quoting Gies' experiments, stated that where an injection of trypan blue is made into a dog shortly before it is killed, the gum and skin are stained blue; also that if injections are made soon after birth and discontinued two months before the animal is killed, all the blue colour will have disappeared from the skin and gums but it will have been retained by the enamel of all the permanent teeth. He concluded that, since the dye is permanently retained in the enamel, so also will substances such as lime salts, circulating in the blood during enamel formation, be retained permanently. This observation would be in line with the concept of a mere physical permeability where, although it may be possible to show the passage of different substances through the calcified tissue, there is nothing to indicate that it is a controlled body interchange as we know it in other parts of the body. Such an opinion is held by many, including Enright, Friesell and Trescher (1952), who stated: "The results of an experiment carried out by Gies for a period of nearly ten years should convince us that there is no vital circulation in enamel".

However, if we favour the idea of a physiological permeability, it virtually implies that we believe that the enamel protein is living organic matter capable of transporting and giving up ions even after the enamel has been fully developed. Some studies in more recent years have added weight to the suggestion that some form of interchange may be possible within the enamel. In 1941, Wassermann and others injected radio-active phosphorus into the blood stream of experimental animals and then demonstrated that it was taken up by the dental tissues including the enamel. The portal of entry was through the pulp or cemento-dentinal junction and there was no absorption from the saliva by the enamel in any of the experiments. Barnum and Armstrong (1942) indicated that some
traces of radio-active phosphorus are able to penetrate the enamel surface. This view was confirmed by Pedersen and Schmidt-Neilsen (1942). However, both workers indicated that the amount is very small compared with that taken up by the dentine via the pulp.

In 1946, Forskhrud injected haemolysed red corpuscles into exsanguinated dogs. He claimed that sections of the teeth showed staining in areas that corresponded to the Hunter-Schreger lines and suggested that this was evidence of a series of nutritional canals throughout the enamel. He then advanced the theory that there is yet another part of the circulatory system which he termed "the ultra-capillary system" which is made up of reticular fibres. He further suggested that the tufts, lamellae and prism sheaths which form the "enamel stroma" are part of this reticular, fibrous system.

Bartelstone and others (1947) used a technique of injecting radio-active iodine into cats, then sacrificing them and making radio-autographs to determine the distribution of the iodine. The main disadvantage in this technique is that it does not lend itself to examination at any great degree of magnification.

Wainwright and Lemoine (1950) demonstrated the rapid diffuse penetration of intact enamel and dentine by carbon<sup>14</sup>-labelled urea. These newer methods, using radio-active isotopes have been well reviewed by Hevesy (1946) who confirmed the existence of a slight but definite ionic interchange within formed enamel.

Whilst it is obvious that, because of the disappearance of the ameloblasts, it is impossible for any tissue repair in the ordinary sense to occur, we feel that we cannot subscribe to any theory that assumes that chemical interchange within the formed enamel is impossible. It is quite possible that any ionic interchange or penetration of lymph that may occur in the enamel may be, for all intents and purposes, purely of a chemical nature and even fortuitous, but in such a specialised type of tissue as enamel this may be of paramount importance.
Micro-hardness.

It is obvious that such a highly calcified tissue as enamel will be extremely hard and it is known that enamel can resist the action of most abrasives quite easily. There is some doubt, however, as to whether there is any variation in hardness at the different levels of enamel. Hodge and McKay (1933) showed a variation in the hardness at the different depths of enamel. Progressing from the dentine they obtained measurements (in Bierbaum hardness numbers) ranging from 350 to 910 through about nine-tenths of the thickness of the enamel. In the extreme outer zone of both occlusal and buccal enamel they found a constant value of 2,050. Thewlis (1934) confirmed this work by the use of X-ray examinations and suggested that there is a highly calcified layer of enamel about 0.1 mm thick which invests the subjacent enamel like a skin. Hollander and Saper (1935) denied the existence of this dense margin claiming that it was an artefact but Thewlis, in 1937, confirmed his work to the satisfaction of most people.

Hodge (1939) attempted to correlate micro-hardness of the enamel with the existence of carious lesions but he was unable to find any association at all. Applebaum (1940), using Grenz rays, admitted the existence of a zone of hypercalcified enamel. He suggested that so opaque is it that even when early lesions occur in the enamel they do not remove sufficient calcium to change the Grenz ray picture.

Hinds (1943) said that there is no difference in the hardness of enamel in erupted and non-erupted teeth. He attempted to explain the variation in the hardness of the outer and inner layers by contending that in the process of maturation the enamel has to reduce its organic content and that it seemed that the enamel most remote from the surface would find most difficulty in getting rid of water and organic matter. This does not seem a particularly satisfactory explanation. Gustafson and Kling (1948) carried out numerous investigations of the methods of testing micro-hardness and, whilst they did not deny the correctness of any of the results that have been previously published, they sound a note of warning concerning the interpretation of many of the comparative results obtained. Earlier Gustafson (1945) had suggested that the strong double refraction (which other workers previously had suggested is an indication of a high degree of mineralisation) which occurs at the dentino-enamel
junction is due to the fact that the prisms in this area are all travelling in the same direction. He said that in the more superficial portions of the enamel the observations are more difficult due to the fact that the prisms in the various layers of the preparation run in different directions.
Figure 1.

A transverse section of enamel in which the enamel prisms or rods can be seen extending throughout the length of the field. The striated effect, giving the appearance of "varicosities" is quite marked. It is not common, however, for such striations to be so obvious in normal enamel. (X 120. Transmitted light).

Figure 2.

An enamel surface stained lightly with silver nitrate. It will be seen that the general outline of the prisms is roughly hexagonal but numerous deviate forms exist even in this field. (X 540. Incident light).
Chapter 4.

THE STRUCTURE OF NORMAL ENAMEL.

Both the inorganic and organic materials found within the human dental enamel form a pattern which, in normal teeth, varies very slightly. Quite marked differences are seen in the enamel tissue of different animals (Tomes, 1923) but in teeth of human beings, no matter what their race or background, a similar enamel structure seems to exist throughout. The main constituents of such enamel are the enamel rods or prisms, prism sheaths, interprismatic substance, enamel cuticle, tufts and lamellae. Within the enamel occur such phenomena, caused by the arrangement of the constituents, as the striae of Retzius and the Hunger-Schréger lines. As we will be concerned from time to time with the changes in all of these constituents, I intend to discuss each structure separately and then attempt to present a composite picture of normal enamel.

Enamel Prisms. (Figures 1 - 5)

Enamel prisms were first described by Retzius in 1837. It was soon realised that the enamel was fimbriated and could quite readily be split into long, calcified rods. These rods have been described as being built up of a row of calcified discs slightly separated from one another. Widdowson (1946) termed these discs “varicosities”. He stated that those of one prism lie side by side with those of its neighbours and do not interdigitate. Thus an appearance of cross striation is produced. Bodecker in 1876 described the prisms as columns of calcified substance which as a rule possess a hexagonal shape. Nasmyth (1839) had thought that the structure of the prism was not so much a column but a series of cells arranged in regular rows and forming composite fibres placed nearly at right angles to the surface of the dentine. However the concept of the columnar form is now universally accepted.

The pattern of a cross section of the rods varies in different areas of enamel and in different teeth. Round, square, hexagonal and heptagonal forms have all been described in addition to a peculiar
Figure 3.
A longitudinal section of enamel showing marked cross striation of the enamel prisms. In this photomicrograph the striations of the various prisms are lying adjacent to those of the adjoining prisms. (X 120. Transmitted light).

Figure 4.
A higher magnification of part of the section shown in figure 3. (X 300).

Figure 5.
A tangential section of enamel showing the granular appearance of the interior of the prisms. This has been interpreted by some to indicate the existence of organic structures within the prisms. (X 1,000. Incident light).
"arcade" form (Retzius, 1837; Neumynt, 1839; Bodecker, 1878; Tomes, 1877). Faber (1928) suggested that the polygonal types are found in the outer enamel layers and Eisenberg (1953) stated that they also occur in the incisal portion of the enamel. At times the existence of arcade-shaped forms has been denied. Meyer (1952) suggested that it is an artefact, whilst Wolf (1942) stated that it is a form brought about by displacement of the prisms. Gustafson (1945) believed that the arcade forms are artefacts due to one side of the enamel sheath staining more readily than the other. Hopewell-Smith (1926) put forward the suggestion that a perfectly formed enamel prism is hexagonal in transverse section because it is normally surrounded by six other prisms, whilst Gustafson (1945) said "where the interprismatic substance is well developed the prisms are always round or nearly round. The hexagonal prisms only occur in such places where the interprismatic substance is poorly developed and the prisms consequently lie close to each other".

The majority of investigators, (e.g., Hopewell-Smith, 1927; Meyer, 1952) believed that each prism passes from the dentino-enamel junction to the enamel surface as a discrete formation. Mummery (1919) doubted this possibility and even Gottlieb, in 1947, suggested the presence of branching or supplementary rods, but it is the common belief that these do not occur. If, however, there are no supplementary rods, some explanation must be made to account for the variation in surface area at the dentino-enamel junction and the enamel surface. This has been explained by the fact that there is a variation in the width of the enamel rods at the various levels of enamel.

Measurements by Pickerill (1913) indicated that the relationship of the diameter of prisms at the dentino-enamel junction to the diameter at the outer surface of the enamel is 1 to 1.85 and that measurements of the two surfaces involved indicate a relationship of 1 to 1.76. Thus, he suggested that there would be no room for the existence of secondary or supplementary prisms. He stated that the prisms increase in diameter immediately over the cusps more than elsewhere.

Williams (1929a) stated: "I have found that the ratio of diameter between dento-enamel border and surface is, in a general average, as 5.5 to 10".
Despite these observations, Jaccard and Grosjean (1941) still maintained that there is little variation in the diameter of prisms and so they assumed that supplementary inserted prisms must fill out the interspace.

The average width of a prism is about five microns (Hopewell-Smith, 1928). Scott and Wyokoff (1946), using electron microscopy, confirmed the general appearance of the cross section of the rods and gave measurements of from 5μ - 10μ as the diameter of the prisms.

The enamel prisms possess transverse striations which are seen quite regularly throughout the length of the rod. They can, however, be more easily observed in a decalcified or etched section. They were originally described by Nasmyth in 1841 who, however, thought that they were merely artefacts. The appearance of this cross striation has been interpreted as being indicative of a rhythmical calcification. Firstly, there is the phase during which inorganic material is mainly deposited and, secondly, there is a rest phase during which organic material is deposited (Mallison, 1924). They lie some 3μ - 4μ apart and the regularity of the cross striation is bound to a rhythm of 24 hours (Schour and Massler, 1940).

Andreson (1923) has suggested that the cross striations bear some relationship to the interprismatic substance, whilst Bodeker (1909) believed them to be offshoots from the prism sheaths. Their appearance has been claimed to be a sign of incomplete calcification or decalcification by external factors (Noyes and others, 1938). Forslund (1941) has interpreted these striations as a massing of organic substances forming a diaphragm in the enamel prism. The striations can be seen quite clearly under polarised light (Cape and Kitchin, 1930). Gustafson (1945) said: "The cross-striation of the prism is more complicated than has earlier been assumed. Thus it cannot be ascribed to a simple variation in the calcification ... In the one instance where a variation of the cross-striation has been observed earlier, viz. in the striae of Retzius, I found it impossible to perceive any such change. I had, on the other hand, the impression that the cross-striation was quite independent of the striae of Retzius. This indicates that mineralisation within the prisms is independent of possible disturbance of the direction of the prisms".
Figure 6.
A tangential section of enamel stained with silver nitrate. The prism sheaths are seen as dark areas surrounding the unstained prisms. (X 1,000. Incident light).

Figure 7.
A tangential section of enamel stained with silver nitrate. Spaces can be seen between the stained prism sheaths which, presumably, contain interprismatic substance. (X 1,000. Incident light).
The interior of the prisms was originally considered to be homogeneous but some fibrillar structures have been described within them. Gustafson (1945) described the presence of spiral threads passing through the prisms at an angulation of approximately some 50 degrees. His observations were based on the examination of polished and etched sections of enamel examined by reflected light. Other writers, mainly of earlier periods, have reported similar structures (Kantorh and Benedict, 1932). They are believed to be of an organic nature. Quite recently, Frank (1950), reporting his findings after examining enamel structure by means of the electron microscope, spoke of a fine network of ultra microscopic fibrils within the prism, but connected to the prism sheath.

Suggestions have also been made that tubular enamel may exist. Widdowson (1946) referred to this but his ideas on the nature of the enamel seem somewhat confused. Originally, it would appear that tubular enamel referred to the presence of a poorly calcified type of prism containing a thin central tube but one gains the impression from Widdowson's writings that he interpreted it as meaning the presence of dentinal tubules penetrating within the enamel.

**Prism Sheaths. (Figure 6)**

The initial description and investigation of prism sheaths was carried out primarily by Bodecker (1906) who demonstrated a sheath rich in organic material surrounding each enamel prism.

Rosebury (1930) and Bodecker and Glus (1924) produced evidence that the sheaths are made up of a keratinous substance. Their permeability to dye-substances and the possibility of lymphatic flow through them have already been discussed.

There appears to be little known concerning the extent to which the sheaths are calcified and opinions vary greatly in this matter.

Chase (1929, 1931), using Bodecker's cellolidin decalcification technique, supported the contention that the material is in the nature of a keratin and also stated that the organic content is present as often, and in equal amounts, in both old enamel (up to 48 years) and in very young enamel (3 months to 1 year). This does not support the earlier
investigations of Hoppe-Seyler who considered that the enamel of infants sometimes contained as much as 15% organic matter compared with the normal 3.6% in the enamel of adults. Gustafson (1945) said that the two halves of the sheath are unequally developed, one half being more easily stained and resisting acid better than the other half. This has already been mentioned as a possible origin of the arcade forms.

From time to time doubt has been cast on the existence of prism sheaths and some have denied that there is any variation at all in the periphery of the prisms (Hopewell-Smith, 1927; Chase, 1927). Meyer (1935) has further suggested that the border line between the prisms and the interprismatic substance is not distinct. Cape and Kitchin (1930) stated that the rod proper is enclosed in a layer of inorganic material and is separated from other rods by the organic sheath. This inorganic peripheral layer is the same material as the rod proper but with slightly different orientation and structurally different. It is also less susceptible to decomposition by acids. This description was made after their observing the enamel by polarised light. Gustafson (1945) said that the prism sheath is "double refracting with orientation in the direction of the sheath, i.e. round the prism".

Interprismatic Substance. (Figure 7)

Baumgartner (1911) suggested, along with others, that enamel prisms lay in close apposition with each other without any interposed substance but this idea has long been discarded. Although the presence of a cementing substance is now accepted, some diversity of opinion exists concerning its nature. It was described by Malleson (1924) and Fish (1927) as a material which could not be stained, and Jaccard and Grosjean (1941) believed it to be homogeneously calcified. Other investigators, however, amongst whom was Bodecker (1923), believed that this substance is easily stained. However it should be noted in this regard that Bodecker speaks of "the interprismatic substance or enamel prism sheaths". It can thus be seen that the reason for so much diversity of thought upon this matter is probably due to the fact that a number of the earlier investigators failed to make any distinction between the prism sheath and the interprismatic substance. Applebaum (1931) also speaks of the
"interprismatic substance, or organic matrix" being stained. This confusion probably arose, firstly, because interprismatic substance is often quite meagre; secondly, because it is difficult to demonstrate its presence except in sections which are cut through the prisms at right angles.

The distribution of the interprismatic substance throughout the enamel seems to vary greatly. Anderson (1902) thought that it is better developed at the dentino-enamel junction. The variation in its distribution is mentioned by Gustafson (1945) who stated: "Where the interprismatic substance is well developed the prisms are always round or nearly round. The hexagonal prisms occur only in such cases where the interprismatic substance is poorly developed and the prisms consequent-ly lie close to each other". Bodeker (1941) has postulated that one can normally see interprismatic substance far better in the teeth of young subjects and that it more or less disappears in older enamel. It is difficult, however, to understand how one of the portions of the enamel could be lost without any change in the form of the enamel.

It has also been pointed out that the interprismatic substance has a greater refractive index than the substance of the enamel rods and that it is more soluble in acids. Because of these facts, it is possible to distinguish the individual structure of rods when they are viewed in unstretched, longitudinal or transverse sections. Likewise, when a thin section of the enamel is cut parallel with the direction of the enamel rods and then subjected to a solution of 2% hydrochloric acid, it will be seen that the interprismatic substance is attacked more readily; the acid dissolves it out from between the enamel rods and attacks their sides. Eventually the ends of the rods will be seen to be projecting as they are freed from the surrounding interprismatic substance (Noyes and others, 1938). They also noted that the acid produced in a carious lesion dissolves the interprismatic substance to a greater extent than has been possible by laboratory methods.

Chase (1924) indicated that the interprismatic substance plays no part in the increase of the surface area of the enamel at the free surface as compared with that at the dentino-enamel junction.
Figure 8.
A longitudinal section of enamel at the dentino-enamel junction showing the variation in direction of the enamel prisms in this area. (X 120. Transmitted light).

Figure 9.
Enamel tufts arising at the dentino-enamel junction. (X 504. Transmitted light).
Gustafson (1946) found no double refraction in the interprismatic substance. He declared that the double refraction which other workers have ascribed to the interprismatic substance is actually a property of the prism sheath which he states shows strong positive double refraction.

Scott and Wyckoff (1946), using replicas of enamel surfaces and examining them by electron microscopy, have indicated that rod ends are separated from each other by from 1μ - 3μ of interprismatic substance. The thickness varies quite considerably in a single area and on different sides of a single rod end.

**Enamel Tufts and Lamellae.**

It has long been the custom to group tufts and lamellae together, primarily because of the fact that they both have a relatively high organic content. Enamel tufts occur at the dentino-enamel junction and therefore presumably cannot play a part in the earliest stages of carious lesions. On the other hand, lamellae may pass through the entire width of the enamel.

**Enamel tufts.** (Figures 8 - 11). Enamel tufts arise at the dentino-enamel junction and may penetrate the enamel for up to one-third of its width. Originally they were thought to resemble tufts of grass in form but Orban (1928) and Baut (1932) have shown that they are ribbon-like structures arising from the dentine. The plane of the ribbon lies in the long axis of the crown. They are composed of hypocalciified enamel rods and interprismatic substance.

The original idea that tufts might be artefacts produced by cracking of the sections during preparation was quickly dismissed when the use of decalcification techniques for the study of the organic structure of enamel became common.

Forshufvud (1941) has described them as consisting of a comparatively thick fibril running into the enamel for a short distance and not branching out into more than one or two ramifications. They are seen only in transverse sections and their numbers vary according to the type of tooth and the position within the enamel.
Figure 10.
A transverse section of enamel, stained with methyl green, showing tufts and a lamella passing from the dentino-enamel junction to the external enamel surface. (X 75. Incident light).

Figure 11.
A transverse section of enamel showing numerous tufts arising from the dentino-enamel junction. (X 175. Polarised light).
Those who have considered enamel to have some circulatory system have often identified the tufts as one of the main tissues through which such a system might function (Beust, 1932a), particularly as quite early their apparent connection with the dentine was demonstrated after teeth had been decalcified in toto (Bodecker, 1937b).

Gustafson (1945) maintained that the tufts are areas of a low degree of mineralisation which extend over a much greater area than that which is seen by direct staining. He said that these "areas" divide the enamel into alternately narrow, fairly soft layers (the tufts) and broad, harder layers (the areas between the tufts). They consist of less mineralised but, for the rest, normal enamel substance and are consequently no new structure in the architecture of the enamel. He suggested that such an arrangement would be advantageous, both from the mechanical and nutritional point of view.

**Enamel lamellae.** (Figure 12). As in the earlier interpretations of the structure of the enamel tufts, so did many of the earlier workers consider lamellae merely to be cracks or artefacts introduced into the enamel during the preparation of a specimen. However, it can be quite readily shown by decalcifying a ground section and watching the progress under a microscope that they are organic structures extending from the dentine to the enamel surface (Orban, 1944).

It has been suggested that lamellae are malformations and consequently cannot be counted among the normal structures of the enamel (Brammer and Breuer, 1926) but Beust (1930) affirmed that their regular appearance in apparently normal enamel warrants their inclusion among normal anatomical structures.

Some workers (Pickerill, 1918; Chase, 1927b) have linked tufts and lamellae together stating that the lamellae are merely tufts which continued to the outer surface of the enamel. However Orban (1927) denied this relationship.

Fish (1935) considered that lamellae are formed by the inability of some ameloblasts to lay down calcium salts and so instead of prisms, traces of organic substances are found.
Figure 12.

A transverse section of enamel showing a lamella passing from the dentino-enamel junction to the external enamel surface. Striae of Retzius can be seen meeting the external enamel surface at an angle. (X 175. Polarised light).
Frisbie and others (1944) believed that lamellae are groups of prisms in which both the maturation and the calcification of the matrix are incomplete.

The most accepted concept of lamellae formation (Orban, 1944) is that planes of tension develop in the maturing enamel. In some cases the planes do not calcify and in others actual cracks occur which are then filled in from the soft tissue surrounding the crown. Thus, two types of lamellae are formed: those composed of poorly calcified prisms and interprismatic substance and those composed of different tissue elements which have invaded the cracks from without during the pre-eruptive stage. The first of these is always limited to the enamel, whereas the second type may even reach into the dentine. Depending upon the form of the lamellae, it is possible to obtain a hornified, outer, secondary cuticle in the cleft (Göttliob, 1921) or to have a deposition of cementum (Orban, 1944).

Gustafson (1945) still maintained that the lamellae are almost identical with tufts. He suggested that the dentinal portions are misinterpretations of cracks which allow dye to penetrate into the dentine. He stated: "the lamellae must be considered as tufts which have retained a tendency to less mineralisation during the whole development of the enamel".

**Enamel Spindles.** (Figures 13 & 14).

In ground sections examined by transmitted light, a series of fairly thick projections can be seen extending from the dentino-enamel junction for a short distance into the enamel. These occur primarily around the cusps of teeth and, because of their shape, they have been termed spindles.

There is little variation in expression of opinion concerning the formation of these spindles and it is generally accepted that they originate from processes of ameloblasts and extend into the enamel before the enamel is calcified. They usually lie at right angles to the surface of the dentine and therefore are not always parallel with the enamel prisms (Orban, 1944). This formation is of little importance as it does not arise from ameloblastic tissue.
Figure 13.
A longitudinal section of enamel showing enamel spindles arising from the dentino-enamel junction. (X 120. Transmitted light).

Figure 14.
A longitudinal section of enamel showing numerous enamel spindles. In the enamel, Hunter-Schreger lines can be seen extending towards the outer surface of the enamel. (X 42. Transmitted light).
The Enamel Cuticle.

A delicate membrane normally covers the crowns of newly erupted teeth. Because it was originally described by Nasmyth in 1839, it is commonly called Nasmyth's membrane. It is only approximately 1/200 in thickness (Glickman and Bibby, 1943) and it has been suggested that it is continuous with the lamelles (Fish, 1933). Earlier workers considered that this membrane consisted only of one layer which was formed by the ameloblasts after their production of the enamel rods had been completed. This view has now been modified and it is believed that there are two layers known as the primary and secondary enamel cuticles. It is the first of these which is identical with the layer described by the earlier workers. The primary cuticle is believed to be formed from the ameloblasts, whilst the secondary cuticle arises from the reduced enamel epithelium which covers the crown. The primary enamel cuticle usually calcifies but it may be more resistant to acids and alkalis than the true enamel structure (Orban, 1944). The secondary enamel cuticle, however, is of a keratinous nature, a property which has been amply demonstrated by its marked birefringence in polarised light (Cape and Kitchin, 1930; Kitchin, 1933).

It is generally assumed that the cuticle is lost from most portions of the crown soon after the eruption of the tooth due to mastication and other mechanical influences. It is possible, however, that in protected areas, such as the approximal surfaces for fissures, the cuticles may remain intact throughout life. In this regard it might be noted that Pinous (1949) described a groove protein in connection with his theory of the aetiology of dental caries. He has also identified the secondary cuticle as a scleroprotein.

Gustafson (1946) had great difficulty in isolating the two cuticles - indeed so much so that he stated: "although I did not succeed in observing any secondary membrane, I have the impression that the authors which have described it earlier are in the right".

Gottlieb (1947) drew attention to work previously reported by Bodecker which indicated that a connection exists between the cuticle and the enamel lamellas and the prism sheaths.
Figure 15.

Striae of Retzius in a longitudinal section of an incisor tooth. The striae can be seen curving upwards as they follow the contour of the tooth. (X 150. Transmitted light).
Widdowson (1946) made an attempt to analyse the structure, origin and function of Nasmyth's membrane. His findings upon the structure and origin are in keeping with those already reported and his remark upon the function is that it is unknown. He referred to various works which indicate that the role of the membrane may be to act as: (i) a protection against acids; (ii) a dialysing membrane, or (iii) a medium in the formation of the epithelial attachment.

The Striae of Retzius. (Figure 18).

In 1837 Retzius described the appearance of brownish, parallel bands in ground sections of the enamel. They can be seen best by transmitted light. In longitudinal sections they can be seen passing from the cervical margin in oblique fashion from the dentino-enamel junction to the surface of the enamel, deviating occlusally as they approach the enamel surface. In a transverse section of the enamel the striations appear as concentric circles. From the first they were considered to be poorly calcified areas within the enamel and also to be an indication within the fully developed enamel of "pauses" that may have occurred during the development of the enamel. Schour and Hoffman (1939) suggested that the cross striations of the enamel prisms appear more clearly in the striae of Retzius and that the intervals between the striae are in multiples of sixteen, from 32μ - 128μ.

Any idea that the striae were formed by a layer of pigment was disproved by the work of Applebaum and his colleagues (1933) and Hollander and others (1935) who, using Grenz rays, showed that the striae illustrate the successive apposition of layers of enamel matrix during the formation of the crown. They assumed also from their results that there is a difference in density of calcification between the striae and the remainder of the enamel.

Barker (1931) also believed that the striae indicate the border line between different layers of calcification.

Orban (1944) described the striae thus: "They may be compared to the growth rings in the cross section of a tree. The term 'incremental lines' designates these structures appropriately, for they do, in fact, show the advance of growth of the enamel matrix. The
Figure 16.
A longitudinal section of enamel photographed by polarised light with crossed prisms. The Hunter-Schreger lines can be well seen in the enamel structure. (X 65.)

Figure 17.
A longitudinal section of enamel, photographed by polarised light with parallel prisms, showing Hunter-Schreger lines in normal enamel. (X 175.)
incremental lines are an expression of the rhythmically recurrent variation in the formation of the enamel matrix". He pointed out that metabolic disturbances can cause a pathological thickening of the striae rendering them more prominent.

Gustafson (1946) claimed that he could not demonstrate any variation in hardness between the striae and the surrounding enamel. He also stated that investigations using polarised light indicated that the rhythm of cross striation of the prisms does not seem to be disturbed in the striae. He suggested that the striae are caused by a displacement of the prisms during the development of the enamel which is manifested by "bends" in the prisms facing towards the cemento-enamel junction. This view, however, is not in accord with that of the majority of workers.

Sognness (1949b) used decalcified paraffin sections to examine the areas in which striae of Retzius were observed. He reported that "the organic framework has a pattern of its own in the regions of the incremental lines, which ... satisfactorily explains the morphological appearance of the incremental lines of the enamel". He showed that the prism sheaths are actually thickened at the sites of the striae.

Remembering Bodecker's confusion between prism sheaths and interprism substance, this latter observation is probably in agreement with the earlier observations of Bodecker (1906) who indicated that the inter-rod substance is thickened at the site of the striae at the expense of the prisms.

The Hunter-Schreger Lines. (Figures 16 & 17).

These optical phenomena were observed and described over one hundred years ago. They appear in longitudinal sections of the enamel as broad bands passing from the vicinity of the dentino-enamel junction in a slightly curved line towards the enamel surface. The convex surface is always towards the gingival margin of the tooth. They can best be seen when viewed by reflected light and appear as alternating dark and light strips of varying width. Orban (1944) described the cause of these bands in the following words: "In a sagittal section the rods are generally cut obliquely. If the bundles of rods are traced from the surface of such a section into the depth it will be observed
that they run obliquely in one disc to the right, in the next disc to
the left. If such a section is illuminated from the right side the
rays pass without being reflected through the rods which run in the same
direction; such discs appear dark. The discs in which the rods run
in the opposite direction appear light because the rays are reflected
by the lateral surfaces of the rods. This explanation is borne out by
the fact that a 180° rotation of the slide reverses the phenomena; the
strips which were dark in the first position appear light; those which
were light appear dark". This concept was supported by the work of
Gustafson (1945). The results of investigations in which soft X-rays
(Hollander and others, 1938) and vital staining (Bodecker and Lefkowitz,
1937; Lefkowitz and Bodecker, 1938) were used, indicated the
possibility of some variation in the calcification of the enamel which
is coincident with the distribution of the Hunter-Schreger lines. This
was also supported by the observation of Orban (1944) who stated that by
careful decalcification these alternate zones could be shown to possess
slightly increased permeability and a higher content of organic material.
However, Gustafson (1945) could not show any difference in the
mineralisation of the different zones.

Stas (1946) suggested that these bands or lines indicate some
stages in calcification. Emerquin (1949) stated that they correspond
to a hexagonal double curvature of the prisms in the inner two-thirds of
the enamel in the lateral plates and involve a complete ring of enamel.
In different rings, the deviation is to the left or right alternately.
Sognaes (1949a), using a low temperature demineralisation technique, has
shown that when viewed in a longitudinal plane the densely packed prism
sheaths which are arranged in longitudinal, corrugated segments give
rise to the pattern which makes the Schreger bands.

The Author's Concept of Normal Enamel.

With so many confusing claims and with so few that can be
unreservedly accepted as valid, it is obvious that the first task is to
attempt to ascertain the structure of normal human dental enamel. There
are certain structures about whose existence, nature and function the
majority of workers are in agreement. There are others, however, about which there is obvious disagreement and which are so important that some attempt must be made to clarify the position.

The majority of workers have confined their observations to one particular aspect, for example, either an attempt to identify the chemical properties of the enamel or else a description of its physical properties or structure. Others have attacked the problem by one method only, for example, the use of dyes. I intend to re-state the main properties of enamel and to discuss any controversial aspects. The embryology of enamel formation will be considered only when it is felt that it has some particular bearing upon the nature of structure.

**General characteristics of enamel.** Enamel is known to be a hard, rather brittle, calcified tissue. Its colour varies from yellowish-white to greyish-white depending primarily upon the degree of translucency of the enamel. It forms a protective covering for the dentine over the crowns of all teeth. Chemically, it has been consistently shown to contain (in dry weight) about 93% inorganic ash and about 1.7% of organic matter. This, however, gives a rather false idea of the possible importance of the organic matter as, by volume, the organic reticular substance (or stroma) is believed to occupy a space more nearly comparable with that occupied by the inorganic substance. The classical comparison of a stone and a sponge well illustrates this point (Bodecker, 1906): a stone and a sponge may have the same volume but their weights vary greatly, the weight of a sponge being less than 1% of that of a stone.

**Enamel prisms.** The main components of dental enamel are undoubtedly the enamel prisms. It is accepted almost universally that they commence at the dentino-enamel junction and pass in a wave-like course to the external enamel surface. In cross section they vary in shape from almost circular forms to fairly distinctive hexagons. They are independent of each other. They are usually considered to be almost totally inorganic, their submicroscopic structure consisting of hexagonal crystals of carbonate apatite lying at an angle to the long axis of the enamel rod.

Organic elements have been described in relation to their internal structure, and the appearance of "striations" is particularly well known. Some workers have described thread-like organic material
passing through the core of the rods.

Frisbie, Nuckolls and Saunders (1944) more recently have propounded the idea that each enamel-forming cell is responsible for the formation of a single organic rod and its adjacent inter-rod substance which subsequently undergoes a progressive mineralisation. They claim to have shown that this protein matrix remains within the enamel at least up to the time of the eruption of the tooth. If this assumption be correct, it could have a marked bearing upon the interpretation of the microscopic appearance of early carious lesions - indeed these investigators have put forward many new ideas which are quite at variance with the older and more accepted views. In this regard it is interesting to note the opinion of the Editor of the Journal of Dental Research (Robinson, 1947) who said: "...We believe that there is still some doubt as to the validity of their conclusions ... Such pictures have been seen in many authors' illustrations and have been interpreted entirely differently".

The demonstration of an organic component within enamel calls for the greatest care in the preparation of specimens and, indeed, the preparation of a good specimen of organic material is a rarity. Usually acid decalcification must firstly be employed and the fine matrix very often is almost completely lost. Preparations have been made, however, with reasonable accuracy ever since Bodecker introduced his double decalcifying method but Frisbie and Nuckolls are among the first to suggest that the entire rod substance has a hyaline matrix. The presence of this matrix must still be questioned but there seems little doubt that there is a concentration of organic material within the rods at the sites of the striations and also that organic fibres may exist within the body of the rods.

Interprismatic substance. The confusion between the interprismatic substance and prism sheaths still exists and at this juncture the most we can say is that both prism sheaths and interprismatic substance do exist, the former being mainly organic and the latter mainly inorganic. The inorganic material is believed to be a quite homogeneous substance and to be made up primarily of Ca tricalcium phosphate in contra-distinction to
the carbonate apatite structure in the enamel prisms. It should be noted that, whilst Dallemagne (1945) made this observation, he relied considerably on the work of Cape and Kitchin. This distribution of inorganic salts may have a great bearing on our problem as there is a big difference between the solubilities of the two substances, the \( \alpha \)-tricalcium phosphate being far more soluble than the carbonate apatite. It has been suggested (perhaps somewhat arbitrarily) that the interprismatic substance is less calcified than the prisms themselves but more calcified than the prism sheaths. Kanthak and Benedict (1932) showed that the rods decalcified more easily than the interprismatic substance but Dallemagne and Melon (1946) suggested that this was due to the presence of organic matter within the interprismatic substance and they were able to obtain greater penetration through the interprismatic substance than through the prisms themselves once they had removed the organic matter. It might again be queried whether these investigators were in fact referring to the interprismatic substance or the prism sheaths.

The true function and constitution of interprismatic substance, therefore, is still in doubt and until its nature has been ascertained its importance cannot be assessed.

**Prism sheaths.** The predominantly organic nature of prism sheaths is now an accepted fact and the majority of workers are prepared to agree that some permeation can occur by this medium. The degree of calcification of the sheaths and the type of inorganic salts present are still in doubt.

**Tufts and lamellae.** The organic nature of these structures can readily be demonstrated by subjecting a polished surface of enamel to Millon's reagent and then examining it under the microscope by reflected light. The acid reagent etches the surface somewhat and the tufts and any lamellae can be seen standing out above the decalcified surface having accepted the pink stain of the Millon's reagent to a marked degree. The penetration of the lamellae to the very surface of the enamel undoubtedly exists in some cases.
Figure 18.
A gold-shadowed collodion replica of etched enamel in the area of the dentino-enamel junction. The variation in the direction of the enamel rods corresponding to the Hunter-Schreger lines is plainly seen. (X 120).

Figure 19.
A gold-shadowed collodion replica of etched enamel. The variation in the direction of the enamel rods corresponding with Hunter-Schreger lines is plainly seen. (X 120).
Hunter-Schreger lines. The accompanying photomicrographs (Figures 18 & 19) are of a celluloid impression of an etched surface of enamel. They confirm the belief that Hunter-Schreger lines are formed by variation in direction of the rods.

Striae of Retzius. The majority of evidence indicates that Orban's comparison between striae of Retzius and incremental lines is correct. It is felt, however, that it should be stressed that these bands occur in concentric rings throughout the width of the enamel when it is viewed in a transverse section. The majority of sections of the enamel are made in a longitudinal plane so that not only are pictures of the striae of Retzius in transverse sections rarely seen, but also the relation of carious lesions to these structures in this plane is not usually studied. Williams (1923a) once remarked: "Transverse sections of the teeth are not often shown but such sections yield much new and valuable information."

Enamel cuticle. The existence of a primary and secondary enamel cuticle is now accepted. However the purpose of the cuticle in relation to erupted teeth and the period for which the cuticle remains in position after eruption, are points which are still under discussion.
Chapter 5.

ABNORMALITIES OCCURRING WITHIN THE STRUCTURE OF THE ENAMEL.

There are certain abnormalities which can occur within the structure of the enamel and which can arise from a variety of causes. As a study of these conditions may assist in an interpretation of the structure and the relative importance of the components of the enamel, I shall review them briefly.

The conditions which are of interest to us are those which originate before the calcification of the enamel is complete. Whilst these conditions sometimes cause an abnormality in the gross form of the enamel, in many cases the effect is on a microscopic level. It is possible for the abnormality to develop in either of the two developmental stages of the enamel, that is, in either the stage of matrix formation or that of maturation. If the interference occurs during the matrix formation, a condition known as enamel hypoplasia is found; that is, the enamel may show a defective configuration or be partially absent but its actual calcium content is normal. If the process of maturation is interfered with, hypocalcification of the enamel results: the enamel form will be quite normal but it may persist in the matrix stage and is comparatively soft and insoluble in acids (Diamond and Weinmann, 1940).

The confusion of these two terms "hypoplastic" and "hypocalcified" has existed for some time. As early as 1929, McKay criticised the use by Mollanby of the term " imperfectly formed". Hypoplasia is due to a defective formation of the enamel organic matrix whereas hypocalcification is due to the failure of crystallisation of the colloidal calcium in the organic matrix, so that the calcium content is abnormally low.

Enamel hypoplasia is by far the more common of the two conditions. There are two views of its formation (Kronfeld, 1949). The first or "collapse" theory suggests that any delay or disturbance in the deposition and calcification of the matrix causes it to lose its shape. This folding can be seen in the irregular grooves and depressions on the surface of the erupted tooth. The appearance of lines of injury ("Unterbrechungslinien") in adult hypoplastic teeth tends to confirm this theory. These lines run from the bottom of the hypoplastic defects to
the dentino-enamel junction and resemble exaggerated striae of Retzius, but whereas enamel rods pass undisturbed through the striae, they are interrupted at the lines of injury.

The second theory, which is now believed to be the more correct, is that the ameloblasts are disturbed during the period of amelogenesis, that is, during the functional stage. As a result the "enamel secretion" is expelled in degrees, varying with the degree of intensity of the irritation. Intensive irritation may destroy the ameloblasts, whilst on other occasions it may merely disturb them so that they recover and again form normal enamel matrix. The mechanism of the production of hypoplasia is the same, regardless of the nature of the disturbance (Diamond and Weinmann, 1946).

Pickett (1915) discussed departures from "normal" enamel structure at length. He concluded that it is the minute and not the gross defects which are most likely to bring about a greater degree of susceptibility to dental caries. However it is extremely difficult to find any detailed account of the histopathology of hypoplastic enamel. Although the basis of Hallamby's work (1929, 1930, 1934) was the formation of hypoplastic enamel through dietary deficiencies, she made little or no attempt to discuss the faults within the enamel structure. Kronfeld (1949) dismissed the subject in some few lines, merely pointing out the irregularity of the enamel (which is thinner and laid on the dentine in an uneven fashion) and the variation in the direction of the striae of Retzius. Colyer and Sprawson (1946) said: "it (enamel) may even be absent over pits and grooves, and there it shows a rounded contour where it rather suddenly fades to absence; the brown striae of Retzius follow such abnormal contour instead of the usual sweeping curve; and so are irregular and may even coalesce in places ... Striation of the enamel prism is said to be more marked than normal, but though this may sometimes be the case it is by no means always so, and indeed, frequently the reverse occurs. Similarly the brown striae are said to be more marked, and though this sometimes is so it is by no means constant ... Experimental permeation of such enamel by stains and silver salts from the pulp aspect demonstrates that it is often not so permeable via the interprismatic substance as enamel from apparently normal teeth of the same age; this indicates that though the enamel is small in amount and uneven in
distribution it is more highly calcified than normal."

Stones (1948) remarked upon the occurrence of enamel disturbances in relation to deficiencies of vitamins A, C and D and certain inorganic salts, notably magnesium. He also drew attention to the abnormalities which may occur in the enamel following upon exanthemata. In these, the enamel is seen to be deficient in the areas that were being calcified during the disturbance and defects follow the brown striae of Retzius. The extent of the affected area of the enamel varies with the duration of the fever. In the more acute conditions a line can sometimes be seen passing through the enamel at the zone of injury and lying parallel to the striae of Retzius. This has already been referred to in the suggested interpretations of the formation of hypoplasia.

Hodson (1949, 1950) described a further abnormality which he named tubular invagination of the enamel capsule (or T-hypoplasia). This condition is due to an invagination of the enamel capsule in an approximately tubular form and is often sufficiently deep to reach the dentino-enamel junction. Such invaginations occur primarily in the line of anatomical grooves and are filled with cells of the enamel organ which have degenerated to necrotic organic material. He believed that such developmental faults play apart in the initiation of carious lesions in the areas in which they occur.

The effect of fluorine upon forming enamel is quite marked, resulting in the appearance of irregular patches of white or brownish pigmentation of the teeth. The microscopic appearance has been quite extensively investigated and it would appear that the chief part of the enamel to be affected is the outer zone of the interprismatic substance which may be defective or completely absent. Williams (1928b) showed that in slightly malformed conditions it is possible to get a penetration of silver nitrate to some considerable depth, the silver being deposited between the prisms and the interprismatic substance. In the more marked conditions, the staining by the silver nitrate penetrates all structures. He stated that there are three phases: firstly, there is an abnormal condition of the sheaths which causes them to be more readily stained; secondly, the interprismatic substance appears to be more permeable to stain; thirdly, the substance of the enamel prisms is not
homogeneously fused together but exists in the form of numerous granules and spherical bodies.

It has also been noted that the transverse striations of enamel prisms appear more marked in enamel which is insufficiently calcified (Orban, 1944). It is also well known that they become far more prominent in sections treated with dilute acid (Noyes, Schour and Noyes, 1933). The same authors also stated that "in perfectly calcified enamel the rods appear without cross-striation. The appearance of cross-striation is a sign of either incomplete calcification or decalcification by external factors ... Carefully decalcified sections of enamel show the cross-striations through their difference in staining reactions. The cross-striation together with the interprismatic substance represent a system of lesser calcified areas which are richer in organic substance than the rest of the enamel".

Certain points emerge from this short discussion of abnormalities within enamel. These are: (i) In such conditions, certain structures which contain organic material (namely, the striae of Retzius and the transverse striations of the prisms) become more apparent.

(ii) This organic material is more apparent in such situations, partly because it is present in greater concentration and partly because it is less calcified.

(iii) These organic tissues appear to be more permeable to dyes than the same tissues in normal enamel.

(iv) Hypocalcified areas, or areas in which there is an abnormal concentration of organic material, when viewed either macroscopically or microscopically, appear to be pigmented.

The importance of these observations will be discussed after I have reviewed the various theories concerning the aetiology of dental caries.
Chapter 6.

THE PATHOGENESIS OF DENTAL CARIES.

This problem has excited interest for many centuries but little would be gained in reciting the various suggestions that have been made through the years concerning the cause of caries. Suffice it to say that Miller in 1830, before describing his own work, listed the following theories as current at that time: (i) disturbance of nutrition, (ii) inflammation, (iii) the action of worms, (iv) putrefaction, (v) chemical action, (vi) parasitic agents and (vii) the electrical theory. He then proceeded to enunciate his own views in the words that I have already used but wish to repeat: "Dental decay is a chemico-parasitical process consisting of two distinctly marked stages: decalcification, or softening of the tissue, and dissolution of the softened residue. In the case of enamel, however, the second stage is practically wanting, the decalcification of the enamel practically signifying its total destruction".

If we examine this description carefully in relation to his remarks on enamel, it is seen that whilst Miller emphasises the part played by acid he did not eliminate the possibility that a proteolytic process was involved. However, it may be assumed to be of secondary importance. He described the action thus: "The bacteria directly participating in the process, inasmuch as they invade the broken-down enamel, perhaps drive the prisms farther apart, and destroy the remnant of organic matter". He then described the clinical appearance of an early lesion in the enamel in the following manner:

"As the first indication that the process of destruction has begun on the external surface of the enamel, we notice that it has lost its normal polish and transparency; then a white (not black) irregular spot of chalky color appears; a sharp instrument (e.g., the point of a needle) will not easily glide over the surface, but will readily detect the presence of a slight roughness caused by a softening or disintegration of the enamel, by which it is gradually changed into a soft cheesy powder. This dissolution of the enamel may be best observed when decay advances from the dentine upon the inner surface of the enamel (secondary decay); here the broken-down enamel-prisms cannot be washed away, so
that quite a thick layer of a perfectly white cheesy substance may often be found...

"A discoloration of intact smooth enamel does not occur; some change or other must have taken place in the enamel before a discoloration can take place, and this change is nothing else than a softening or decalcification of it.

"This discoloration appears in very different grades; when the decay proceeds very rapidly (caries acutissima), it is slight or wholly wanting (white decay). In other cases, only the margin of the enamel is colored brown to black while the center of the cavity remains white. When the decay proceeds very slowly, that is, when it is of long standing (caries chronica), the greater part of the affected tissue is deep brown or black."

He followed this with a description of the microscopic changes in which he firstly emphasised the "distinct appearance of the transverse striation of the enamel prisms". He further stated: "The transverse striation is distinctly marked. In sections the margin appears indented, and the enamel-prisms more or less dislocated. The whole looks as if the interprismatic substance were dissolved, i.e., the connection between the prisms destroyed. Sections of enamel ... have shown the bacteria forcing their way between the loosened prisms". He then linked these observations with experiments carried out in vitro. On this matter he stated: "About the same result is obtained where normal enamel is treated with diluted acids".

The work of Miller had a profound influence upon research workers from that period onwards. The majority of investigators have accepted his premises as correct and at times one is inclined to feel that their interpretations of their results might have been coloured by attempts to prove, or at least to remain in agreement with, Miller's theory. Certain minor objections were raised from time to time such as those listed in a paper presented by Enright and others (1952) who, however, virtually eliminated to their own satisfaction the majority of objections which they raised.
A different approach to the problem was developed by Mellanby (1922, 1920, 1924) who laid great emphasis upon nutritional factors and asserted that they could so influence tooth structure that they indeed were the most important factors. She claimed that her work showed that there was a close correlation between the structure of enamel and its susceptibility to caries. She also claimed that by variations in the diet one could control the development of normal teeth or of teeth with various degrees of dental defect (hypoplasia).

Her second hypothesis was that the resistance of a tooth to external stimuli could be changed by variations in the diet. In this regard, she took pains to make it quite clear that she used the word "diet" in the sense of the effect of food constituents after digestion and absorption. She emphasised, therefore, the importance of the predisposing, as opposed to the local, causes of the disease.

The work of these two, Miller and Mellanby, gave rise to two conflicting schools of thought, respectively called the environmental school and the nutritional school. On examining the claims of these two schools, however, it would appear that there are few points upon which they disagree to any great extent. Mellanby made no attempt to link her findings with the bacteriological aspects nor did she study the histopathology of early carious lesions within the enamel to any great degree; likewise, Miller limited his researches: primarily to a study of minutaie of the exciting cause. It is incorrect, therefore, to say that these two schools of thought (whilst they caused much dissension amongst workers) are at variance to any great extent concerning the actual development of the lesions within the enamel.

After Bodecker demonstrated the presence of the organic sheaths within the enamel, another theory of causation gradually developed which placed the emphasis upon the breakdown of protein material within the enamel as the prime factor in the formation of carious lesions. Some authors have stressed this factor almost to the exclusion of any breakdown due to acid, whilst others look upon it as a process preceding the ultimate dissolution of the inorganic enamel prisms.
As I have already indicated, the proponents of the nutritional theory have not concerned themselves particularly with the histopathology of lesions within the enamel but more with the structure of the enamel prior to the development of any carious lesion. We have to look more to the proponents of the "acid" theory and the "proteolytic" theory for detailed descriptions of early carious lesions. It is my intention, therefore, to review the literature concerned with the microscopical appearance of early carious lesions, broadly subdividing it to correspond with the schools of thought which have just been discussed.
Figure 20.

A transverse section of an early carious lesion. The majority of the features usually described in such a lesion can be seen, namely, segmentation of enamel rods, penetration in the earlier stages between the prisms and the relationship in some areas of the lesion to the striae of Retzius. This ground section was unstained and the apparent presence of pigment or darkened areas can be observed. (X 120. Transmitted light).
Chapter 7.

THE HISTOPATHOLOGY OF EARLY CARIOUS LESIONS WITHIN THE ENAMEL.

It is not my intention to examine the literature concerning the histopathology of carious lesions beyond the time of Miller. I have already given extracts from his writings which are pertinent to this discussion. These writings were accepted as a basis for most of the work that was done in the earlier part of the twentieth century. In brief, he stated that enamel caries is due primarily to disintegration by acid and at a later stage the scanty organic constituent is also destroyed. The first change seen microscopically is usually an intensification of the transverse striations of the enamel prisms. The cementing substance between the prisms is then dissolved out and bacteria can be seen lying within the space between the prisms which are then loosened and eventually broken away. (Figure 20).

The work of Miller was supported and extended by another American whose work was equally brilliant. In a series of very lengthy papers published over a period from 1897 to 1923, J. Leon Williams examined the structure of enamel in human beings and animals and correlated the pathological conditions of mottled enamel and dental caries with his findings in the normal structures. He remarked (1897, 1898) on the fact that penetration by a lesion could occur without any apparent loss of substance and he agreed with Miller that the cement substance is firstly dissolved out "until canals of considerable size have been formed between the rods". Using magnifications up to 2,000 diameters, he demonstrated how, after the solution of the cement substance, the original organic matrix becomes obvious. "There are many places where the organic connections, the radiating processes which unite the globular bodies in each rod and often those of contiguous rods are plainly seen". At this stage Williams believed that the enamel was completely inert and that decalcification was the only factor involved in dental caries. He stated: "It is purely a chemical process and consists in the removal by an acid of the veil of cement-substance which always conceals the true structure of the enamel. There is no vital reaction because there is nothing vital to react. There is no physiological or anatomical change". He differentiated (1898) between
the way in which structural changes occurred in rapidly or slowly developing lesions. In rapid carious lesions he stated that the acid penetrates quickly and deeply along the lines of cement substance and, as a result, the prisms themselves are isolated and break down before any great change occurs in the internal structure. In slowly developing lesions he believed that the entire substance of the prism is gradually permeated and a resultant sponge-like structure is produced which gives the effect of discolouration.

His next main works were published in 1923 (a, b) and consisted of one paper dealing with the normal and pathological histology of enamel and a second concerned primarily with the structure of mottled enamel. From this work he concluded that, whilst structure is of importance, it is possible for acid to bring about the breakdown of any type of enamel but that when imperfect structure exists the rate of progress of the lesion will be correspondingly more rapid.

The period from 1900 to 1920 produced little work of any note. Mummery (1926) described the existence of translucent zones in ground sections of carious lesions examined by transmitted light. He believed that these zones indicate an attempt by the enamel to bring about a localisation of the lesion. He felt that they are different from somewhat similar zones mentioned by Miller, and Bodecker stated that they are different from his zone of carious degeneration. Mummery stated: "Whatever this alteration in the enamel may be there appears to be a distinct change of structure which is not due to decalcification and is intimately associated with the decayed area."

Leigh (1927) believed that the initial lesion in enamel is brought about by a decalcification of the intercolumnar substance thus producing spaces between the enamel rods. He felt that the microscopic appearance is very similar to that obtained in the laboratory by the use of acid, the main difference being due to the presence of pigments in the carious lesions.

Fish (1930) suggested that the sites of election of caries are primarily the exposed neck of the tooth, a patch of hypoplasia of the enamel or a fissure at the bottom of which is a permeable lamella, and
also any lamellae. He suggested that caries starts in the depth of a permeable lamella or area of hypoplastic enamel and that the tract of dentine below this becomes dead. He seemed to attribute little importance to the initial breakdown of the enamel itself.

Barker (1931) stated that caries passes from the surface of the enamel to the dentino-enamel junction by way of the enamel lamellae. He also considered that the striae of Retzius indicate the borderlines between the different enamel layers of calcification and that caries of the enamel, when passing from a poorly to a well calcified layer, can be stopped by this barrier and it is then inclined to spread parallel to the course of the striae of Retzius.

Mahe (1934) agreed with the suggestion of initial interprismatic breakdown due to acid attack.

Anderson and Rettger (1937) did not concur with the opinion expressed by Leigh (1927) that the cementing substance is the first material to be removed and they stated that often the intercolumnar material is still intact, even although the enamel presents a picture of disintegration.

Applebaum published a series of papers (1932, 1935, 1938, 1940) in which he, too, took the view that caries is not merely a chemical reaction and that, in addition to different reactions being involved, there are different types of dental caries. He stated that decalcification is of great importance but that it seemed possible for a tissue resistance, such as "enamel sclerosis" to develop. He found (1935) that carious lesions, when examined by polarised light, are surrounded by bands of demarcation which differ from the normal enamel. He also (1940) showed that there is usually a radio-opaque surface over most early lesions and that in some cases, although decalcification of the entire thickness of the enamel occurs, the radio-opaque line upon the surface remains. He suggested that this may be due to a hypercalcification of the surface which exists as a normal condition and that the removal of inorganic salts due to a carious lesion may still leave more mineral matter on the surface than in the depths of the lesion. He also suggested the possibility that the decalcified surface layer may be "remineralised" by the saliva.
authors had considered this layer to be evidence either of a vital reaction or a shift of minerals, Yarden considered that it is an application of Liesegang's mineral precipitation.

Chase (1948) drew attention to the considerable difference of opinion as to the order in which various parts of the enamel are destroyed in carious lesions. Some suggested that the actual prisms are broken down before the interprismatic substance whilst others indicated that the interprismatic substance is removed first. He referred to earlier work of his own which would indicate that the interprismatic substance is the first to be destroyed but this question is by no means yet resolved.

Wolf and Neuwirt (1948) used the shadowed colloidion replica method to study the microscopic changes in enamel in early lesions. They were not prepared to accept the ideas of Gottlieb that lamellae and cracks may act as sites of entry for bacteria and said that they considered the appearance of early carious lesions and of enamel that has been etched by acids is identical in that in both the interprismatic substance is resistant and the ends of the prisms are hollowed out.

Hill (1949) gave a description of early carious lesions examined by transmitted light. He stated: "The interprismatic areas appear broader, the lines of Retzius more prominent and the enamel rods less regular in their outline. The irregularity of the outline of the enamel gives the appearance of cross striations and has been referred to as the basket weave of dental caries. The colours vary from light yellow to reddish brown, dark brown and opaque. These pigments which infiltrate the partially decalcified enamel probably arise from colored substances introduced into the mouth or from the products of oral putrefaction". He further stated: "Two general types of enamel caries may be distinguished, namely, deep penetrating and slow superficial disintegrating types. In the former the acids rapidly dissolve the interprismatic substance and penetrate deeply between the enamel rods, often reaching the dentoenamel junction before a single enamel rod is lost ... In the second type of caries the attack is more superficial, slower in rate, the prisms and interprismatic substance being destroyed en masse".
Hill suggested that irregular penetration of carious lesions through the enamel may be influenced by four factors, namely: (i) anatomical differences in the tooth structure, allowing more rapid penetration of acid; (ii) a time factor in that the central portion of the plaque process may be older than in the lateral extensions; (iii) concentration of acid, that is, the acids at the periphery of a plaque may be subject to dilution by saliva, and (iv) osmotic influences exerted by the pulp through the dentine and the enamel.

Kronfeld (1949) described the various depths of lesions in enamel. On the enamel surface he described the beginning of disintegration of the decalcified enamel prisms. Below this is a zone in which the rod structure is partly lost. Next is a zone of transverse striation ("an expression of decalcification that has just started") which is followed by normal, intact enamel. He also stated that the striae of Retzius can be clearly seen in carious enamel.

Fargin-Fayolle and Fries (Chompret, 1950) noted that lesions may end abruptly without any zone of transition, or they may gradually fade into the intact enamel.

Bibby has contributed much to assist in the understanding of our problem. In 1932 he examined the evidence that the organic structure in enamel may act as a passive defence against caries. He concluded that "the keratin of the enamel shares this property of resistance to bacterial destruction. In histological preparations the keratinous rod sheaths have been shown to persist long after the rod substance itself has been destroyed, and have been shown to limit the lateral spread of enamel destruction". He believed that decalcification goes on within the organic tube but that a point is reached when the bacteria are deprived of their food supply and thus the decalcification is halted. However, it could be possible for proteolytic organisms to persist in the deeper parts of the lesion but the end-products of their metabolism would probably accumulate and eventually inhibit their growth.

In a later paper (1935) he remarked that the nature of the pathological process is still not understood but he drew attention to certain factors which he considered had been overlooked. He referred again to the statement that rod sheaths are resistant to acids and to
certain proteolytic enzymes which he tested and he therefore believed that the sheaths serve to limit the lateral spread of caries and so determine the shape of the lesion. He agreed with the earlier investigators that the breakdown of the inorganic portions precedes that of the organic.

In 1940 he stated that a study of the relative solubility of enamel surfaces and of different areas of enamel surfaces reveal a complete lack of correlation between those parts of the teeth most susceptible to decay in the mouth and those areas which are most rapidly dissolved by acids in vitro. He again suggested the possibility of organic structures having some protective role in the initiation of carious lesions.

The Proteolytic Theory of Dental Caries.

Baumgartner (1911), after discussing the structure and composition of enamel, gave his interpretation of the various stages of the process of caries. He described firstly the destruction of the enamel cuticle followed by the destruction of the surface layer of the enamel prisms. He stated that microorganisms (primarily cocci) are present only in those parts which are richest in organic substance. He then claimed that those portions of the enamel which are least calcified and possess the greatest concentration of organic substance are destroyed completely by microorganisms. This process, he said, shows the directly opposite series of appearances to those which are observed under the microscope in ground sections of enamel after attack by acid. He concluded by saying that caries is a parasitic process and is caused by animal and plant protista. Harmoniously constructed tooth enamel and not necessarily calcium content is the best protection against the initiation of dental caries.

Fleischmann (1914) used the double decalcifying method of Bodecker to study lesions within the enamel. He stated that he found microorganisms entering the tissue between the enamel rods, thus reducing the interprismatic substance whilst the prisms remained intact. He formulated the view that the organisms enter the organic constituents on the surface of the enamel and destroy it and that they then produce acid which dissolves the calcium salts in that area. The organisms then penetrate
the decalcified prisms, destroying them. The main innovation in this view is that Fleischmann believed that the acid is produced within the tooth itself (and possibly from the organic material within the tooth structure) and not by elements outside the tooth. This theory might well be compared with that developed recently by Pincus.

Malleson (1925) followed on his work on the investigation of the structure of enamel by examining a series of decalcified carious lesions of the enamel. He traced the sequence of changes which occur in enamel during the primary stages of caries, commencing from the formation of a plaque in which he assumed the presence of "the specific organisms of caries". He noted firstly that in the areas which clinically appear opaque (which seems to be the earliest sign of a carious lesion) the prism cortex stains quite strongly. (The term "prism cortex" was used by Malleson to indicate the prism sheath). The deeper infection of the lesion occurs either by the microorganisms penetrating by way of the interprismatic substance or the prism cortex or else through a pre-existent fissure. He concluded by stating: "... there being no doubt that decalcification plays an important role in all stages of dental caries. It cannot, however, be the sole cause of these changes ... and it would seem as if the organic content of the affected enamel were in some way increased".

Bodecker was one of the first people to prepare any satisfactory decalcified sections of enamel. Indeed, it was primarily through sections prepared by his technique that the presence of organic structure within the enamel became accepted.

In 1921, H.W.C. Bodecker stated that is was physically and chemically impossible for acid formed by fermentation in the mouth to reach the zone of advancing caries in a sufficiently active state to decalcify the tissue and that it must be formed at the point where decalcification is taking place. He further stated that microorganisms always invade tissues along certain lines and that they follow the path which offers the most suitable nourishment. He suggested that they advance most readily in the organic structure containing protoplasm. He stated: "Not before the destruction of the uncalcified organic matter in the outer zone (of enamel) has been definitely determined can the initial
lesion of caries and with it the aetiology of this disease be explained".

Although Bodecker was working with his brother (C.F. Bodecker), these views were changed quite considerably by the time C.F. Bodecker published their next paper.

In a "preliminary communication" in 1927 he stated that he considered caries of the enamel not to be a simple dissolution of its inorganic salts "but a more complicated process somewhat analogous to caries of the dentine". He believed that the acid penetrates the enamel in all directions but that the various structures are not affected quickly and that in the earliest stages the enamel rods are more easily disintegrated than the rod sheaths or the cement substance. On this observation he suggested that the bacteria penetrated not through the rod sheaths but through the actual enamel rods. He also described an area lying immediately adjacent to the bacterial focus which, in decalcified sections, showed a great affinity with staining agents. This, he termed the "zone of carious degeneration".

Bodecker did not state definitely in this paper what he considered to be the causative agents but he stated that the appearance of the sections examined by him seemed to indicate that there was an added amount of organic matter in the rods themselves which was not made up by the bacteria. He also stated that it was primarily the rods which underwent this change leaving the sheaths intact. He was not prepared to presuppose such a high degree of vitality in the enamel as to suggest that it was an attempt on the part of the enamel to segregate the carious from the healthy area but he did agree with the observations of Mummery (1919) that in ground sections of carious enamel a transparent zone can be seen beneath the lesions. He also qualified his remarks, however, by stating that the transparent zones described by Mummery are much larger than the zones of carious degeneration.

In 1929 Bodecker and Bodecker put forward the suggestion that enamel is destroyed by two mechanisms, namely acid decalcification (by such organisms as L. acidophilus) and the proteolytic action of some streptococci or diplococci. They stated that the organic content of the enamel rod is destroyed more rapidly than the organic sheath. They believed that these two structures differ chemically, the sheath being
composed of a keratin and the rod of "calcified protoplasm". It will be noted from this that they presuppose an organic struma within the rod structure itself, a suggestion which will be again referred to later in this dissertation.

Bodecker again in 1948 put forward his ideas which had not changed fundamentally in the intervening nineteen years. He did elaborate his ideas to the extent of suggesting that the two destructive mechanisms (that is, decalcification and proteolysis) are not active in identical areas. He suggested that incipient, penetrating enamel caries seems to be caused by proteolytic organisms which destroy the permeable organic channels, particularly lamellae.

The writings of Gottlieb have been voluminous but the majority of his opinions which were apparently unchanged at his death are to be found in his book. Gottlieb considered that the breakdown of the enamel protein was the most important occurrence in the initiation of carious lesions. His arguments can best be illustrated by quoting directly from his book ("Dental Caries", 1947).

"... caries spreads in enamel very similarly to the way silver nitrate stains it. The carious process spreads along and affects the organic components of the enamel, which are finally digested. Later the more calcified prisms are affected and necrotized. The carious cavity is the end result of the necrotizing action. We call it proteolytic ..."

"... it (caries of enamel) is apparently produced through the invasion by a specific group of microorganisms along non-obstructed organic roads in the clinical crown ... In rare cases the proteolytic action may appear without acid and the result is still caries. But if the acid acts without the yellow pigment component, no caries can develop; only a chalky enamel appears.

"... It is quite a temptation to assume that acid paves the way for the proteolytic action by decalcification of the tissue. However, it has been shown that moderate acid action on enamel is, in most cases, self limiting because it erects a barrier against further progress of microorganisms by producing a zone of transparent enamel. But where no strip
of transparent enamel is erected the acid action spearheads the yellow pigmentation in invasion through the prism sheaths, and the idea of its preparing the way cannot be discarded.

"In the case of invasion of a lamella where the yellow pigmentation forms the spearhead, the possibility of any preparing action by acid is out of the question. Only when invading along the prism sheaths does acid form the spearhead ... Although exceptions may be found, acid alone rarely penetrates to the dentin through thick enamel without any trace of yellow pigmentation. It appears more probable that the proteolytic action, in the already invaded enamel parts, facilitates the progressing penetration of additional acid producers".

When referring to a decalcified section, he stated: "... the ladder-like arrangement of organic framework is thickened, apparently by invasion of microorganisms. This organic framework is dark and the spaces between appear light, that is, empty. The calcified part of the enamel was apparently not affected and dissolved by the acid of the histologic technique".

In one of his papers (1944) he stated that this ladder-like framework seen in carious lesions is far thicker than it would be if it were merely the organic framework freed from mineral salts. He suggested that this thickening is due to the invasion of and enrichment by microorganisms. He summed up his view on the process of caries thus:

"First the organic framework is thickened; Second the process invades the prisms making them more acid resistant whilst the prism sheaths turn black; Third the prism sheaths disappear while the isolated prisms are still present; Fourth a necrotic mass replaces the enamel structure".

Hinds (1942) believed that acid does not produce a lesion in enamel which is similar to a carious lesion. He further stated that resistance of a tissue to acid does not imply that that tissue will be resistant to proteolytic action and he considered lesions to be carious only where there is an invasion of the organic portion by proteolytic organisms. He offered little in the way of evidence for his contentions which are based primarily upon the work of Gottlieb.
Frisbie and others (1944) extended their work on amelogenesis to include observations upon the distribution of the organic matrix of the enamel and its relation to the histopathology of caries. They concluded from their earlier work that the "unit of the matrix is the hyalinized rod together with the inter-rod or interprismatic matrix ... it is possible to distinguish during development, several elements in the structure of the rod matrix and to differentiate different stages in their hyalinization. Each presents a cortex, core and a sheath. The peripheral or cortical portion is the most completely hyalinized element, is first to calcify, and remains the most highly calcified portion of the rod. The centre or rod core is less completely hyalinized, less heavily calcified and may contain areas which are immature and which have failed to undergo complete transformation. Surrounding the hyaline rod and therefore external to the cortex is the rod sheath which can be selectively stained and which appears to be in continuity with the cell membrane ... The rods are connected by an inter-rod matrix which possesses staining reactions similar to, although less intense than, those of the hyaline rod itself. This substance is calcified at a slightly later period and may remain as the least fully calcified portion of the enamel". By the use of carefully decalcified and stained sections, they claimed that the earliest pathological change which can be observed in a superficial carious lesion is an intense acidophilic reaction to stains by the matrix immediately beneath the surface. Closely associated with this change is a "progressive loss of structural detail in enamel elements" which in advanced conditions brings about homogeneity within the matrix.

"Continued proteolysis results in the exposure of individual rods which have not as yet undergone so complete a change. These rods or groups of rods therefore project beyond the area of liquefaction, rendering the invaded surface increasingly irregular. In this manner the rod core and inter-rod intervals are opened up establishing portals of entry for further bacterial invasion".

It can be seen from these excerpts that these investigators have reached conclusions upon both the form of normal enamel and the histopathology of carious lesions which are quite at variance with the opinions of most other present day authors. They state that they have been unable to observe such structures as the striae of Retzius once the
calcium salts have been removed. These structures have usually been considered to be sites of a greater concentration of organic material but they believe them to be purely optical phenomena which disappear with the removal of the calcium salts.

A further paper was presented by Frisbie and Nuckolls in 1947 in which they described in greater detail the histopathology of early enamel lesions. They stated that there are quite distinct types of penetration by microorganisms depending upon the rate of breakdown of the enamel. They described various filamentous and coccal forms of microorganisms which they believed to play some part in the destruction of the enamel. They described the formation of a central focus of infection with "multiple irregular channels spearheading in all directions" which occurred after the initial penetration. They suggested that the same spheroidal organisms cause the breakdown of both the enamel and the dentine and that the greater content of organic material in the dentine is responsible for the greater rapidity of its breakdown. It is somewhat difficult to decide which portion of the enamel they believe is destroyed first but it would seem that the spearheading passes along the organic matrix and that the rods are then finally destroyed. The descriptions of Frisbie and others, whilst far more elaborate, are very similar to those given by Bodecker and both have based their observations primarily upon the interpretation of appearances in decalcified sections of the enamel.

Pincus (1935-1939) carried out investigations into the nature of the organic structure of the enamel. More recently he described (1948) the presence of what he termed "groove protein" which differed from that in Nasmyth's membranes. He then put forward the suggestion (1949) that oral bacteria possessing the enzyme sulphatase may destroy the mucoprotein (which he believes is the type of protein present in the organic portion of the enamel) and thus release the combined sulphuric acid. This freed acid can then in turn react with the inorganic constituents of the enamel and so assist in its breakdown. Pincus stated that the changes in the organic structures of the teeth are thus regarded as primary and those in the inorganic structures as secondary in the process of caries. To support his observations, he stated that he had demonstrated the presence of calcium sulphate within carious lesions which would indicate that
portion of the apatite has been converted into the somewhat less soluble calcium sulphate.

Other Contributions to the Elucidation of the Histopathology of Dental Caries.

Reviewing the work of the great number of writers who have attempted to solve this problem, one is impressed by the number of occasions upon which dental caries is linked with the occurrence of systemic disturbances. In all of these writings there is an implied belief in the "vitality" of enamel. The majority of these writers, however, limit their discussions to generalities, although some indicate the way in which they believe the lesion actually develops.

Broderick (1928) discussed at length the possibility of the vitality of enamel but, despite the fact that he linked the development of caries with systemic disturbances, he stated: "If we accept then, as with reservations I think that we must accept, the doctrine of Miller that the exciting cause of dental caries is the fermentations of carbohydrate foodstuffs in contact with the enamel ..."

Allen (1933), too, held views divergent from those usually held. He assumed enamel to be a living tissue in intimate association with the blood and lymph. He considered the outer layer of enamel to be the least mineralised and then suggested that should the "quality of blood" supplying calcium salts both for nutrition and maintenance vary to any great extent, "the result is immediately felt by the tooth in certain particular areas at the dentino-enamel junction. There an absorption of tissue takes place. Calcium salts are lost without the slightest manifestation of changes being apparent on the surface. This brings about an interruption of the supply of lymph to the overlying enamel and produces a drying of this tissue in the areas suffering interruption and is manifested externally as a whitish opacity. Stress of mastication completes the destruction of the enamel by carving in the overlying structure".

Maddern (1933) considered that physical factors are by far the most important and claimed that caries is the manifestation of a series of abnormal stresses applied to the tooth after eruption which bring about an alteration in structure and an "interference with the nutrition".
The repetition of the stresses occasions the breaking down of the structure of the enamel commencing at the external region and particles in the region of the resultant stress fail by shear. He stated: "The break would obviously occur in the lines of least resistance, that is, in the interprismatic substance of the enamel".

Forshufvud (1948) described the existence of an "ultra capillary network" and likened caries to an ulceration. He, too, stated that the lesion is due to the deficient healing capacity of the hard tissues of the tooth and therefore claimed that it is a disease primarily of the blood plasma. However, he stated that the local cause "is probably due to the arisal of cracks in the hard tissues of the tooth".

de Vries (1949) placed most emphasis on the relation between endocrine secretions and the autonomous nervous system as causative factors in dental caries.

Lura (1949), too, believed that the metabolism and possibly vitality of hard tissues is of paramount importance and that dental caries bears very direct relationship to metabolic disturbances. He placed considerable importance on the concentration of phosphatases within the enamel.

Aitken (1949), by sealing carbohydrates within prepared cavities in vital teeth, believed that he demonstrated the existence of a carbohydrate metabolism in enamel and dentine which can only occur in vital teeth. He postulated that caries depends primarily upon the ability of carbohydrates to penetrate into the enamel structure. He attempted to demonstrate that bacteria are unable to account for the concentration of end products of carbohydrate metabolism that is to be found in carious enamel.

The Pigmentation of Carious Lesions.

Practically all clinical descriptions of carious lesions have drawn attention to the existence of some staining of the lesion. Earlier investigators (Miller, 1890) believed that the colour of the lesion could be related to the age and type of lesion. Thus new or rapidly advancing lesions were believed to remain opaque and white whilst the more chronic or arrested lesions were believed to be of dark brown colour. Malleson
(1925) noted that the organic matrix becomes more stained in carious lesions and Bibby (1932) also drew attention to this fact. He believed that the pigmentation of the rod sheath precedes the decalcification and seems to be due not to any defensive action but rather to the actual products of the carious lesion.

Gottlieb (1944) stated that yellow pigmentation is the only singular characteristic of dental caries. He did not believe that it penetrates from the surface as it can be found in dentine below intact enamel or in the deeper layers of enamel whilst the superficial layers do not show it. He suggested that it is formed by invading organisms and that it is not transported but formed at the site of occurrence.

Dreizen and Spies (1950) claimed to produce a yellow-brown pigment similar to the naturally occurring pigment by submitting the organic matrix of non-carious teeth to conditions which they believed might occur during the degradation of glucose by oral bacteria. They suggested that the reaction involves some of the carbonyl-containing derivatives of glucose and the protein within the enamel matrix.

The correct interpretation of this phenomenon of pigmentation within lesions may prove of great importance in the final solution of the problem of dental caries and it will be discussed again later.

It will be realised from the preceding review that there remain numerous unsolved problems, not only in relation to the histopathology but also in relation to amelogenesis and the fundamental structure of the enamel. It will be seen that the idea of enamel being a totally inorganic, inert mass has gradually changed and the main problem now concerning investigators is whether the organic constituents of the enamel can provide a channel through which body defences may act, or by means of which ionic interchange may occur. The more recent concept of Hiskells and Frisbie concerning the existence of an organic structure throughout virtually the entire mass of the enamel has again thrown even the question of the structure of formed enamel into a state of flux so
that there exists no firm basis of factual evidence upon which further work can be built.

In the task of correlating the various observations upon the histopathology of carious lesions, it would seem that most workers believe that the rod sheaths or the substance between the rods are those which are first affected in the development of lesions but it will be noted that there are still other workers of high repute who regard the penetration of the rods themselves as the first step in the development of the lesions. Also, there is great difference of opinion concerning the relative importance of the removal of inorganic salts and the breakdown of protein elements (if indeed they are broken down at all) and further whether the tooth substance itself is inert or possesses any potentially vital defensive mechanisms which can affect the development of carious lesions. To attempt to elucidate some of these problems, the following investigations were undertaken (Part II).
From the mass of work which has been examined, I would like to draw attention to the following observations which have a bearing upon my investigations.

**Enamel.**

1. Normal enamel is a highly calcified substance containing only relatively small quantities of protein.

2. Normal enamel is composed of carbonate apatite and \( \alpha \) tricalcium phosphate. The \( \alpha \) tricalcium phosphate is believed to exist in the interprismatic substance (Dallemane and Melan, 1945).

3. The permeability of normal enamel to different solutions depends upon its structure (Pickerill, 1912).

4. Stains are able to penetrate normal enamel from the dentino-enamel junction outwards but they cannot penetrate through the external surface inwards (Leikowitz and Bodeker, 1938). This is particularly true of teeth which have been erupted for some time (Pickerill, 1912; Atkinson, 1948).

5. Such stains penetrate through the enamel along the prism sheaths.

6. The existence of a hypercalcified area of enamel at the external surface has been demonstrated (Hodge and McKay, 1933; Thawlis, 1934).

7. Certain anatomical features of enamel, such as the transverse striations of the enamel prisms and the striae of Retzius are due to the existence at these sites of greater concentrations of organic material (Schour and Massler, 1940; Orban, 1944).

8. When the enamel is malformed, it becomes more permeable (Williams, 1923b) and greater quantities of organic material are present (Diamond and Weinman, 1940). Clinically, such teeth in which there is a greater concentration of organic material often appear to be pigmented (Williams, 1923a).
Caries lesions.

1. The occurrence of decalcification in carious lesions has been demonstrated by soft X-ray examinations of early lesions (Gordon, 1938).

2. The possibility of proteolysis occurring in carious lesions has been suggested by observations made upon decalcified sections of carious lesions (Frisbie and others, 1944).

3. The observation has been made that the advance of caries of the enamel may be halted at striae of Retzius and that spread occurs in a direction parallel to the course of the striae of Retzius (Barker, 1931).

4. The observation has been made that prism sheaths are resistant to acids and certain proteolytic enzymes and that they serve to limit the lateral spread of caries and so determine the shape of a carious lesion (Sibby, 1935).
## PART II.

**Experimental Studies.**

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Chapter 3.

OUTLINE OF ORIGINAL INVESTIGATIONS.

An examination of the reviews set out in the previous pages indicates that it is extremely difficult to establish a foundation of indisputable fact upon which further investigations might be built. Undoubtedly, it is still necessary for much work to be done even in relation to amelogenesis and the composition and structure of formed enamel.

It is possible, however, with the knowledge at our disposal, to find a reasonable basis for an attempt to solve some of the many problems associated with the histopathology of early carious lesions. When this work was commenced, it was found that the majority of the previous investigations had been carried out by examining ground sections by transmitted, reflected or polarised light. Some work had been done using decalcified sections, but the techniques involved had proved so delicate and uncertain that they had been little used. As so much of this work was completely dependent upon the powers of observation of the worker, it was felt that the findings often reflected the personal inclinations of the investigator and the possibility of the false interpretation of features which are in reality artefacts and which occur so often in the type of preparation used, could rarely be ruled out.

In the work undertaken it was decided, after having satisfied myself in regard to the general nature of the lesions, to seek methods which would lend themselves to more reliable and objective interpretations. To such a category do chemical and histochemical methods belong.

It was not possible to study all the problems that one wished to study, so it was eventually decided to undertake the investigation of four particular problems. These were:

1. Pigmentation. There are three aspects of pigmentation of lesions. Firstly, most clinical descriptions of carious lesions draw attention to the discolouration of the lesions, which is quite apparent to the naked eye. This discolouration might be designated as "surface
pigmentation. Secondly, numerous investigators have indicated that the organic material within carious lesions increases and becomes pigmented. Thirdly, it has been suggested that the pigmented tissues may act as a barrier to the spread of carious lesions.

It was decided, therefore, to attempt to study the occurrence of pigment in relation to carious lesions, to look for any protective potentialities it may possess and, thirdly, to see if the production of pigmentation were possible in vitro.

2. The outline and appearance of carious lesions. Carious lesions have been examined microscopically by numerous investigators, primarily by the use of ground sections illuminated by transmitted light; others have used stained sections or decalcified sections, whilst others have employed reflected light or polarised light. Because of the unique structure of the enamel and because the very mechanism of the carious process may be linked with calcium removal, it was felt that the less the interference to which the lesions were subjected prior to examination the more accurate the information which the preparations might yield. It was therefore decided to examine lesions firstly by the use of reflected light; after this, to examine lesions which had been subjected to stains which would be selective and specific and at the same time would be unlikely to cause any alteration in the lesion. The use of transmitted light and thinly ground sections were to be used only in a confirmatory manner. After this examination had been carried out, an attempt was to be made to relate the shape of the lesion to the structure of the enamel.

3. The destruction of the enamel constituents. The problem of the destruction of the inorganic and organic components of the enamel is still under discussion, primarily from the aspect of the relative importance of the two processes. It was decided, therefore, to attempt (i) to identify the processes of decalcification and proteolysis within the carious lesions by histochemical methods and by staining methods; (ii) to produce decalcification and proteolysis in enamel in vitro and to compare the results with the conditions seen within lesions; and (iii) to investigate, primarily by histochemical means, the part that the organic material within enamel might play in the formation of lesions.
4. **Protective mechanisms within the enamel.** The belief still exists that enamel does possess, to a slight degree, the ability to initiate defensive mechanisms in order to arrest the progress of carious lesions. Such phenomena as translucent zones beneath lesions and the existence of a hypercalcified surface layer upon all normal teeth are believed to be manifestations of such defensive mechanisms. It was decided, therefore, to investigate the ability of the hypercalcified surface layer to prevent the initiation of decalcification.