PROBLEMS ASSOCIATED WITH THE TREATMENT OF THE FLOOR OF THE DEEP CARIOUS LESION.

A CRITICAL REVIEW.

SUBMITTED IN SUPPORT OF CANDIDATURE FOR THE DEGREE OF MASTER OF DENTAL SURGERY.

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INTRODUCTION

"Sir Roger told them, with the air of a man who would not give his judgment rashly, that there was much to be said on both sides".

ADDISON: The Spectator.

The uncertainty of our present state of knowledge regarding cavity sterilization is emphasized by two articles appearing in a recent publication each taking completely opposite points of view on this topic, both being supported by convincing evidence.

It would appear that the problem of cavity sterilization involves many questions which are fundamental in the practice of restorative dentistry - for example, the mechanism of the carious lesion in dentine, the need or otherwise to remove the last layers of softened dentine, the marginal leakage of restorations, and the relation of all of these to the condition of the pulp beneath restorations and to recurrent caries.

It is averred by some research workers in this field that bacteria penetrate the dentinal tubules ahead of the carious lesion, and therefore an attempt should be made to inactivate
them before inserting a restoration.

Others believe that acid decalcification precedes bacterial invasion of the dentine, and that the last softened layers are sterile.

Another view is that even if bacteria are to be found in advance of the main lesion there is no satisfactory method available of destroying them.

Bradford \textsuperscript{25} has the following comment to make: "I would like to see the stage reached where one could, with a clear conscience, clean out a cavity as far as the walls of the cavity were concerned and treat the floor with medicaments so that any caries present just ceased. Papers have been presented over the years insisting that this is quite a reasonable thing to do, but unfortunately there is no scientific evidence to support it ... but in my view it will not be long before there is the necessary evidence. So that, provided it is possible to insert a watertight filling - which is difficult - and providing that the margins of the cavities and the walls are clean, the situation should be reached where the floor could be ignored. In doing so it might well be that the number of extractions which have to be performed would be considerably reduced".
It would of course be preferable for the subject of cavity sterilization to become redundant. That is to say, for there to exist an ideal situation in which Preventive Dentistry and Dental Health education ensured that patients, particularly children and adolescents, no longer presented themselves with cavities so deep that the integrity of the pulp or the preservation of the tooth itself becomes problematical. Until such time however, the subject dealt with in this review may reasonably be considered to be of some interest to the practising dentist.
SECTION I

DENTINE.

Introductory.

Before proceeding to a discussion on cavity sterilization, it is first necessary to give a brief outline of some of the more modern concepts of the normal structure of human dentine. Widdowson defines dentine simply as "An organic matrix impregnated with lime salts and permeated by tubules which radiate outwards from a simple pulp chamber". This description is adequate as far as it goes, but is not sufficiently inclusive to be acceptable at the present day.

No description of dentine is satisfactory which does not recognise that it is a substance which results from the activity of the pulp, and that divorced from the pulp it is incapable of growth or reaction. A preferable definition of dentine, therefore, is that of Bradford: "A protective cap produced and maintained by the pulp". Massler has said: "Dentine and pulp must be considered together as a single organ".
The cells of the pulp which are directly connected to the dentine are the odontoblasts, and they may be regarded as being to the dentine what the osteocytes are to bone. Orban\textsuperscript{103} states: "The odontoblasts are associated with the formation of dentine, and mediate its nutrition. Histogenetically and biologically they have to be regarded as cells of the dentine".

This conception of dentine/pulp as a reactive unit, and not merely an inert inorganic substance leads to two speculations in regard to cavity sterilization.

Firstly, that it is not logical to treat a vital substance in intimate contact with living cells with protoplastic poisons and caustic substances.

Secondly, it is known that the pulp has a reparative potential, and is able, under propitious circumstances, to produce secondary dentine, and in some cases new primary dentine; therefore the aim of any treatment of the floor of the cavity should be to stimulate this protective reaction by making the environment as favourable to the pulp as possible.
The Anatomy of the Pulp.

It is not proposed to deal with the anatomy of the pulp in great detail, or in any critical sense, since adequate accounts of this can be found in textbooks. Nevertheless, a reminder of the salient points does not seem out of place in view of its indivisibility from dentine.

The pulp is a highly vascular connective tissue enclosed within the pulp cavity. This connective tissue consists of ground substance in which cells are supported, and through which runs usually a single artery entering via the apical foramen and breaking up into arterioles, which ramify in the pulp cavity and divide into capillaries, which in turn empty into a network of veins draining the pulp (Grossman 61). It is generally stated that there is no organized lymphatic system, but that there are intercellular spaces through which the lymph drains. Orbàn 104, however, says that there are in fact lymph vessels, but that special methods are needed to demonstrate them.

The ground substance is an amorphous gelatin-like material composed to a great extent of mucopolysaccharides: hyaluronic acid, chondroitin sulphuric acid, and others. These are polymers which give the pulp its viscosity. Embedded in this substance are collagen and reticular fibres (Seltzer 122).
The Cells of the Pulp.

a. The connective tissue cells.

b. The defence cells.

a. There are three kinds of connective tissue cells:

(1) The odontoblasts which are a compact layer of columnar cells lining the periphery of the pulp.

(2) The fibroblasts, spindle-shaped or stellate cells which have a more embryonic character in the pulp than in other parts of the body.

(3) Undifferentiated mesenchyme cells, which are spindle-shaped and may differentiate into connective tissue cells or defence cells.

The connective tissue of the pulp has a potential for the production and maintenance of dentine. It is a reservoir for the continuous supply of dentine matrix forming cells throughout the life cycle of the dental pulp (Seltzer 122). This aspect will be considered later in dealing with the formation of dentine and the reparative function of the pulp.

b. The defence cells comprise:

(1) Large mononuclear cells which are phagocytic for bacteria and cellular debris. During inflammation of the pulp other defence cells such as lymphocytes and polymorphs derived from the blood may be found.
(2) Histiocyes or 'resting wandering cells'.
These are usually round or pleomorphic, they may be spindle-shaped. They may act as fixed phagocytes, or they can mobilise and become large, amoeboid phagocytes.

The formation of dentine begins approximately in the fifth month in embryo. The basic facts of its formation can be summarized as follows:

(1) The thickening of the basement membrane at the amelo-dentinal junction.

(2) Production of silver staining fibres: argyrophil or Korff's fibres from within the pulp. They thicken at the periphery of the pulp to form relatively thick bundles which pass between the newly differentiated odontoblasts.

(3) The differentiation of the spindle-shaped mesenchymal cells closest to the basement membrane to assume a columnar shape and become arranged in a continuous layer, the odontoblasts. They are linked to one another by intercellular bridges. As the odontoblasts develop they form a fine process which extends in length as the matrix grows and thus always permeates the entire thickness of the dentine while the odontoblast remains alive (Jenkins).
(4) The formation of a reticular matrix comprised of coarse argyrophil fibres surrounding polygonal spaces which are at first filled in with undifferentiated tissue, which at this stage is almost indistinguishable from the cytoplasm of the odontoblast cell. This may be regarded as the original framework upon which dentine is built and is called 'predentine'.

(5) The calcification of predentine which can be observed histologically as the formation of spheres of inorganic material beginning in that part of the matrix which occupies the tip of the cusp, and extending in conical increments throughout the crown and into the roots (Jenkins).

It is important to realize that dentine is not formed by the odontoblasts, but by the connective tissue in the pulp.

The Calcification of Dentine.

The exact mechanism of this process is not yet fully understood. Of the process of calcification in general Sognnaes has written: "Basically, calcifying tissues share three principal types of ingredients, at least under normal conditions:
(1) a fibrillar, highly oriented framework dispersed in
(2) a more amorphous organic ground substance (which is not
too well understood but has properties of a carbohydrate protein
complex) and infiltrated with (3) inorganic crystals which in
higher forms (and in occasional lower forms) are composed principally of calcium phosphate with crystallographic properties widely
interpreted to be those of hydroxyapatite". It has been suggested
that the initiation of calcification is due to the stereochemical
configuration of collagen fibrils inducing apatite deposition, but
this theory does not explain why collagen does not calcify in all
parts of the body instead of only in bone, dentine and cementum.

Of the calcification of dentine in particular, Orban\textsuperscript{102}
is of the opinion that the odontoblasts are associated with matrix
formation and nutrition only, and that calcification of the pre-
dentine occurs as a result of chemical changes taking place in the
cementing substance, which provides for the automatic combination
of calcium salts in the tissue fluid with molecules of cementing
substance.

Bevelander and Johnson\textsuperscript{17} disagree with this view and they
postulate that during the differentiation of the odontoblasts,
alcaline phosphatase comes to be localized within the nucleus,
cytoplasm and dentinal fibril just before and during early
calcification. They drew these conclusions from work on freshly
slaughtered pig embryos. They conclude with certainty that the function of the odontoblasts and its fibril is to produce and mediate phosphatase transfer within the predentine.

Jenkins agrees that the odontoblasts have been shown to contain alkaline phosphatase, though he does not commit himself with regard to their processes. He suggests that the odontoblast process may be a means of transfer of calcium phosphate to the predentine. This is a different view from the one held by Bevelander and Johnson, who have not considered that calcium phosphate is actually produced in the odontoblast.

Glasstone experimented with tooth germs by immersing them in solutions containing organic and inorganic phosphorus and calcium salts, having first removed the pulp and enamel organ. The uncalcified dentine and enamel became calcified "even in the absence of cells in which phosphatase is present". Her work therefore tends to support the theories of Orban, rather than those of Bevelander and Johnson.

It would appear that the subject of calcification, not only of dentine, but of other tissues, is one on which there is so far no general agreement, and since it is not germane to the main topic of this review it is not proposed to carry the discussion of it further.
The Structure of Dentine.

A great deal of controversy has always existed regarding the microscopic structure of dentine, but since the advent of the electron microscope there has been a radical change in thought concerning the size and nature of the dentinal tubules, and the structure of the matrix immediately surrounding them. Interpretations of these electronmicroscopic studies are still inchoate, but nevertheless, the evidence available, whilst by no means being final or definitive, indicates that the older ideas regarding the histology of dentine can no longer be considered wholly valid.

The older concept of dentine was that it was a more or less homogenous calcified substance within which tubules ran between the odontoblast layer of the pulp and the dentino-enamel or dentino-cemental junction. These tubules housed the protoplasmic extensions of the odontoblasts, which were called 'Tomes Fibres' Bradford. It was also suggested by Neumann in 1862 that the pipe-stem appearance of ground transverse sections of dentine was due to a sheath around the odontoblast process. This structure was accordingly named "The Sheath of Neumann". There was also disagreement regarding the actual diameter of the Tomes Fibre in relation to the diameter of the dentinal tubule. Fleischman in 1905 suggested that in vivo the odontoblast
process occupied the entire lumen of the dentinal tubule, and that the apparent space seen between the tubule wall and the cytoplasmic process was an artifact produced by routine histological preparation. Other workers thought that a peripheral space did in fact exist round the odontoblast process in vivo. Credence was given to the latter belief in 1933, when Fish published the results of his experiments on the permeability of dentine and enamel. He introduced Indian ink into the pulp chamber via the tip of the crown in teeth of dogs and monkeys. He did the same with freshly extracted teeth. It was found that minute particles of pigment held in suspension did in fact find their way into and along the dentinal tubules. From this, Fish drew the following conclusion: "... the precise situation in the dentine in which the ink particles were observed, is the space between the fibril of Tomes and the wall of the dentinal tubule". This apparent space became known as "The Perifibrillar Space of Fish". He also concluded that this space in vivo was normally filled with some form of tissue fluid which might possibly be secreted by the odontoblast process. However, recent electronmicroscopic studies by, amongst others, Scott and Wyckoff, Shroff, Williamson and Bertaud, Yamamoto, Takuma and Frank, indicate that no such space exists, and that the odontoblast process is immediately surrounded by a highly calcified matrix.

In the light of this modern knowledge it is difficult to
imagine how the Indian ink particles penetrated to the precise situation in which they were observed by Fish. 49

The more recent concepts regarding the structure of dentine may be summarized as follows: The dentinal tubule is of the same diameter as the odontoblast process, and in vivo is entirely occupied by the process. Immediately surrounding the odontoblast process is a highly calcified matrix which occupies the area (formerly regarded as a space) between the wall of the dentinal tubule (older concept) and the odontoblast process. This highly calcified matrix has been variously referred to as the 'peritubular matrix' (Takuma 135), 'translucent area' (Bradford 22), 'calcified sheath' (Shroff 113) and 'peritubular translucent zone' (Blake 19). In a later journal however 25, Bradford refers to this area as 'peritubular dentine' and considers it to be a different structure from the rest of the dentine, which he refers to as 'interstitial dentine', and from which he considers the peritubular dentine to be separated by thin layers of uncalcified or hypocalcified structure.
A diagram to illustrate the structure of dentine as seen in decalcified transverse sections. A.D., Afibrous dentine matrix; T.A., Matrix of the translucent area; C., Central layer of the translucent area matrix; D., Dentine process of the odontoblast; P., Peripheral layer of the translucent area matrix; R.D., Reticulum of fibrous dentine matrix; R.F., Radiating filaments.

(After Bradford.)
The Electronmicroscopy of Dentine.

There are two methods by which tooth structure may be studied under the electron microscope:

(1) Replication.

(2) Thin sectioning.

As it is difficult to cut sections of dentine of a satisfactory thinness, the former method is the one more commonly used (Kennedy, Teuscher and Fosdick^72).

Briefly, a section of enamel and dentine is cut, the surfaces etched and covered with a thin film of collodion in ether. When the collodion has dried the sections are floated, collodion side up, in HCl. until the enamel and dentine are dissolved, leaving the replica. In the replica, elevations on the surface of the section appear as depressions, and vice versa.

Scott and Wyckoff^116 sectioned five hundred teeth and made approximately two thousand collodion screens and took three thousand microphotographs. They found that the dentinal tubules exhibited a central fibre with a thin, wall-like structure at the periphery. They did not feel certain enough to say whether this
was a sheath or an artifact produced by the collodion flowing into a space. They found the actual fibre resistant to strong acid and pepsin solutions.

They found that the matrix contained fibres which appeared to be collagen. They also observed other finer fibres but were not prepared to say whether these were in fact part of the matrix or part of the tubule system which had been torn out.

Kennedy, Teuscher and Fosdick, using ultra thin sections of decalcified dentine did not make any definite statements with regard to the structure round the central fibre. They do however remark that if, as seems likely, the central fibre or 'Tomes fibre' is an extension from the odontoblast cell, then any clinical procedure in which the dentine is destroyed is liable to affect the pulp of which the odontoblast process is an intimate part.

Shroff, Williamson and Bertaud agree with the previous authors that the central fibre is an extension of the odontoblast. They also make the definite statement that the space (so called) around the odontoblast process is in fact an area of material appreciably harder than the rest of the dentine, and not a patent canal as was previously supposed.

They think that this surrounding structure is not only harder but contains less organic matter than the rest of the
dentine. The external diameter of this area is roughly three microns and the internal diameter equals that of the odontoblast process, i.e. approximately 1.5 microns. They also observe: "It is significant that the external diameter of this calcified cylinder is of the same order of magnitude as that usually given for the classically accepted canal as seen under the optical microscope".

In view of these findings they draw the following logical conclusions: "If this is true, any diffusion of fluid or particulate matter through the dentine must take place only via the organic constituents of the matrix or the protoplasm of the odontoblastic process".

Two years later, in 1956, Shroff, Williamson and Bertaud published a further paper on subsequent electronmicroscopic studies. In this they amplified the conclusions reached in the earlier study and postulated a complex structure for the dentinal tubule and its contained odontoblast process. Their description of these structures is worth noting even though some parts of it may be regarded as hypothetical, inasmuch as no other workers in the same field are prepared to commit themselves to the same extent. Nevertheless, should the work of Shroff and his associates be confirmed in future research, then dentine will be shown to be a more complex structure than was hitherto supposed.
Their description of the odontoblast process and its immediate surrounding structure is as follows: "There is an outer sheath (a) consisting of collagenous fibrils arranged in a helical manner. This organic sheath separates the intertubular matrix from the calcified sheath (b), but is intimately connected with these structures by interlacing collagen fibres. The calcified sheath (b), encloses on its inner aspect what appears to be a similar organic sheath (c), and this may be the outer layer of the odontoblast process. Within this is a further thicker sheath (d), which has some of the properties of myelin. There is a further organic sheath (e), which surrounds a central core (f)". (See diagram).

In a section on terminology they suggest that the old term 'Tomes Fibre' should be superseded by 'odontoblast process', which occupies the 'dental canal' which is surrounded by a 'calcified canalicular sheath'. However, the term 'peritubular dentine' seems to have become more generally accepted together with 'intertubular dentine', and is simpler and more descriptive than Shroff's terms.

Frank, investigating young molar teeth by the direct sectioning method, agrees with Shroff et al that the odontoblast process is surrounded by a highly calcified zone, opaque to electrons and easily destroyed by laboratory procedures. He
Diagrammatic representation of the structure of the dentinal "tubule" and the odontoblast process. The figure shows a block of dentine containing one "tubule" exposed in such a way as to show the various structural layers seen.

A, Outer collagenous sheath; B, calcified canalicular sheath; C, inner organic sheath; D, thick acid- and alkali-resistant sheath which is soluble in hot alcohol and stains well with osmic acid and thionin; E, thin organic sheath surrounding the central core F.

(After Shroff et al.)
does not make any comment on the presence of the various organic sheaths, but does agree that there appear to be fibrils transversing the peritubular zone from the intertubular zone. Yamamoto, studying the dentine of horses, elephants and giraffes, also reports the presence of a highly mineralized zone round the odontoblast process, and is prepared to assume that there is a bordering structure between this and the intertubular matrix.

Takuma examined eighty-five teeth of varying ages, some of them unerupted, and he also found evidence of the highly calcified peritubular matrix. He too considers it to be of a different structure from the intertubular dentine. He agrees that there is a continuous fibrillar organic matrix between the two types of dentine.

Miller, and Atkinson and Harcourt also confirm the existence of this highly calcified sheath.

The Permeability of Intact Dentine.

Fish demonstrated in 1932 that it was possible for substances introduced into the bodies of cats and dogs to find their way into the pulp and dentine. He injected trypan blue over a period of two or three weeks, then killed the animals and
sectioned the teeth. He found that the dye had penetrated the
dentine and enamel. He repeated the experiment using methylene
blue, a diffusible dye, and found that it penetrated the dentine
right up to the dentino-enamel junction. In both cases he was
dealing with intact non-carious teeth.

Lefkowitz introduced argyrol into the pulp of dogs' teeth and measured the rate at which the stain reached the enamel. He found that the stain reached the enamel in just over seventeen minutes. He concluded that dentinal tubules were able to transport argyrol particles, the largest of which is approximately 6 to 11 by 4 to 8 microns in diameter. This conclusion seems questionable in view of Shroff's estimate that the actual area of the dentinal tubule within the calcified canalicular sheath (or peritubular dentine) is approximately 1.5 microns in diameter and occupied by the odontoblast process. However, Shroff's figures are based on human teeth and Fish and Lefkowitz experimented on dogs, and it may be that human teeth in vivo would not be so readily permeated by dyes.

In recent years radioactive isotopes have been used in permeability experiments. Wainwright and Lemoine used Carbon\textsuperscript{14} labelled urea to demonstrate permeability of intact enamel and dentine. They found that intact enamel was penetrated irregularly but freely. A diffuse penetration of dentine was
found similar to that of caries. G.C. Blake\textsuperscript{19} impregnated sound, young teeth, in vitro, with mercuric chloride, and found the enamel and dentine permeable. He observed that although there was no perifibrillar space - ".... it is likely that there is free diffusion within the fibril itself". Even if this is the case, it is still hard to imagine how these comparatively large molecules diffuse through a process whose diameter has been estimated as 1.5 microns, which, furthermore, tapers towards the periphery, so that at the point of entry at the dentino-enamel junction, the odontoblast process would present an even smaller diameter. However, there is general agreement that molecules can and do diffuse freely through intact enamel and dentine.

The Defence Mechanism of the Dentino-Pulpal Unit.

The body's reaction to a stimulus depends on the intensity of that stimulus. This is as true of the pulp as of any other part. A persistent stimulus below a certain threshold gives rise to a protective mechanism; the skin for example reacts to a mild subthreshold chronic stimulus by keratinization, but should the stimulus become more acute, the result will be not protective keratinization but marked soreness and inflammation with eventual tissue breakdown.
In the case of the dental pulp, a mild chronic stimulus will produce secondary dentine, but an acute rapid stimulus will produce inflammation (James\textsuperscript{66}). The effects of inflammation on the pulp are more destructive than in other parts of the body because of its enclosure within rigid walls which makes for greater difficulty in dissipating toxic products, and also for greater pressure to result from the inflammatory process than would be the case where the area was less confined. Apart from this, inflammation within the pulp follows the classic vascular picture as outlined by Menkin – i.e., Haemostasis, migration of leucocytes, oedema. There is accumulation of small metabolite molecules giving increased osmotic pressure with consequent swelling and disruption of odontoblasts. The pulp may die, or if it recovers, a reparative dentine is formed from the connective tissue of the pulp and new odontoblasts may be formed from the mesenchyme cells present within this tissue (Seltzer and Bender\textsuperscript{123}).

**Secondary Dentine.**

Consideration of secondary dentine is apt to be confused by great variation in nomenclature, and Kuttler\textsuperscript{78} has suggested the following simple classification of dentine into three types according to structure, motivation, time of appearance:
(1) **Primary Dentine.**

The first-formed dentine arising from the thickening of the basic membrane in the mesodermal pulp as previously described. Calcified ground substance and numerous tubules, (15,000 to 70,000 per sq. mm.) almost straight and fairly wide.

(2) **Secondary Dentine.**

Formed post-eruptively and after the tooth has begun to receive the slight aggressive effects of normal function, e.g., occlusal contacts, chemical irritation, thermal changes, all within the threshold of pulp resistance. This dentine is less permeable and has fewer dentinal tubules per sq. mm. and fewer odontoblasts. It is darker in colour than primary dentine, its tubules are more curved, less regular and narrower in diameter. This has been referred to as 'compensating', 'adventitious', 'normal physiologic' dentine.

(3) **Tertiary Dentine.**

This results from more marked irritation of the pulp such as occurs in erosion, caries, exposure, improper medication etc. It differs from primary and secondary
dentine as follows:-

(1) It is localized exclusively in front of the irritated zone.

(2) The dentinal tubules are greatly reduced, even tortuous, and may be absent.

(3) Calcification is deficient.

(4) There are cellular inclusions which convert into spaces.

This has been referred to as 'pathologic', 'protective' 'reparative' and 'secondary' dentine.

There is however, another phenomenon associated with dentine which has not been mentioned by Kuttler, that is, SCLEROSIS of primary dentine. It seems uncertain at present whether sclerosis is a function of age or irritation, since the longer a tooth has been in the mouth, the longer has it been exposed to normal functional aggressions. Furthermore, it has been shown that the enamel is permeable\(^49, 143, 19\), and it is possible that there is diffusion of the mouth fluids which reach the dentine and cause irritation.

Bradford\(^{25}\) is of the opinion that this could be a factor in inducing sclerosis. This process is the blockage of the tubules with calcified material. This material is crystalline and renders the dentine homogenous and therefore more translucent. Sclerosed
dentine is much less permeable than young dentine.

Lefkowitz\(^8\) studied 104 teeth of known age, excluding all teeth which contained factors not pertaining to age. He found the maximum extent of the 'protective metamorphosis', i.e., the formation of secondary dentine, to occur at fifteen to nineteen years after eruption. He also examined three unerupted teeth, and also nine teeth with artificial crowns in all of which the dentine would have been protected from the diffusion of mouth fluids, and found that the ageing process had continued in these teeth. However, he has overlooked the fact that the crown preparation might have caused some form of protective reaction, and he does not say whether the crowns were metal or porcelain. If metal, then there would be quite an appreciable thermal stimulus to the underlying dentine and pulp. He concluded from his studies that the 'protective metamorphosis' of dentine was a function of the age of the pulp.

Bevelander and Benzer\(^{16}\) studied three hundred specimens of teeth, which included intact teeth, untreated carious teeth, and teeth with amalgam restorations. They found that in all mature human teeth there was a marked circumpulpal zone of secondary dentine. This is what Kuttler calls 'Secondary Dentine' and Bevelander and Benzer call 'physiologic secondary dentine'.
They note: "Tissue in this zone appears to be - (A) Dense, in which the matrix appears opaque and the tubules arranged with varying degrees of regularity; and (B) Transparent, in which the matrix appears hyaline and the tubules are irregularly arranged and few or lacking entirely". This appears to correspond with Kuttler's 'Tertiary Dentine'.

They also found sclerosed tracts leading from the abraded incisal surface to the coronal limit of the pulp horns.

In examining the carious teeth, they found the circumpulpal zone of secondary dentine previously described and - "A considerable amount of sclerosed transparent dentine underlying or adjacent to the carious cavity". There was also a "pulpal plug associated with caries of dentine", which they call 'adventitious secondary dentine'. This reaction of dentine was described in detail by Fish, and will be considered shortly.

On examining teeth with amalgam restorations, Bevelander and Benzer found that the structure of dentine was like that which occurred in untreated carious teeth. They felt that this opposed the idea that cavity preparation per se stimulated the production of secondary dentine. They invariably found sclerosed dentine associated with caries in such a way as to appear to wall off the carious lesion, and concluded that this was the first and
sometimes the only step in the protective metamorphosis of dentine.

Their findings can be summarized briefly:

(1) All mature teeth contain a modified variety of dentine which can be called secondary, and appears to vary in structure.

(2) Peripheral irritation produces another modification, namely a 'sclerosed tract'.

(3) Caries of dentine stimulates a further protective reaction of sclerosis.

In a further study, Benzer\textsuperscript{14} postulated that physiologic secondary dentine was present whether the tooth was functional or non-functional, and that there was no evidence that function was necessary for its production.

The Response of the Pulp to Peripheral Injury.

This was investigated by Fish\textsuperscript{49} in 1932 and his work is still quoted in recent articles. He cut cavities in the teeth of dogs and allowed them to remain open to the mouth fluids for several months. The dog was subsequently killed and the pulp injected with dye. Fish found two distinct types of reaction:
(1) The **DEAD TRACT** reaction:

The contents of the injured tubules had died and were sealed off from the pulp by secondary dentine. This had two parts: (A) A hyaline mass in an organic matrix. This sealed off the central ends of the injured tubules. Such odontoblasts as survived began to lay down tubules, and (B) the second tubular part of secondary dentine formed. Furthermore, irritation via the lateral anastomoses of the tubules irritated adjacent tubules and they also precipitated calcific material, i.e., necrosis of dentine took place, but instead of being exfoliated, it was encapsulated.

(2) The **TRANSLUCENT ZONE** reaction:

There is no secondary dentine, but a plug of calcific material is deposited at the peripheral ends of the tubules to prevent fluid communication between the lesion and the living contents of the tubules. In some cases additional primary dentine had been added at the pulp margin in response to the stimulus of the injury. Sometimes a mixture of the two types of reaction is found. Fish is adamant that secondary dentine production exhibits these two phases. It is, in his view, a specific reaction to injury to the odontoblast process. Bradford\textsuperscript{25} is in agreement with this view, so also are James, Schour and Spence\textsuperscript{66} ".... irritation of the pulp gives rise to secondary dentine formation". Also Weider, Schour and Mohammed\textsuperscript{145} ".... a common response of the pulp to injury.
is the formation of reparative dentine". Gottlieb\textsuperscript{57} however, has an entirely novel theory regarding the formation of secondary dentine. In his view, since odontoblasts do not form dentine, if the peripheral irritation of the odontoblast process is the causative factor, then it must transmit the stimulus to the surrounding connective tissue, which is the source of new dentine. Once a new layer of atubular, or reduced tubular dentine has been laid down, then the stimulus can no longer be transmitted, and, therefore, no more secondary dentine can be formed. Since secondary dentine does in fact continue to be formed, then peripheral irritation is, of itself, not the cause.

He therefore postulates the following theory:

The odontoblast layer acts as a separating layer, keeping the connective tissue of the pulp from the dentine, and slowing down the dentine-forming capacity of the pulp. Once an odontoblast has been damaged, its separating function is diminished, and in that region the connective tissue comes into better contact with dentine, and the inhibiting effect of the odontoblast is lost. Thus, "Secondary dentine is not formed by creating a stimulus for, but by removing a barrier against, increased deposition of new dentine matrix". This is an interesting idea, but one that does not appear to be widely held in the literature. Nevertheless, the fact remains that Gottlieb's theory still includes damage to the odontoblast
as a causative factor, and in considering secondary dentine formation from a clinical rather than a theoretical point of view, it is generally agreed that irritation of the odontoblasts is a major factor.

Where there has been marked inflammation of the odontoblastic layer of the pulp, and the death of some of the odontoblasts, the mesenchymal cells of the pulp differentiate into new odontoblasts. In the opinion of Seltzer and Bender\textsuperscript{123}, when odontoblasts are damaged, they liberate alkaline phosphatase, and this acts as a stimulus not only to the differentiation of cells, but also to the conversion of Korff's fibres to collagen fibres, and the formation of dentinoid. They also think that spicules of dentine which are pushed into the pulp as a result of injury may act as focal centres for the production of new dentinoid. This view is supported by Svjeda\textsuperscript{131}, who investigated the teeth of children and young dogs in which the pulp had been exposed and capped with a variety of medicaments. In successful cases he found the formation of an amorphous calcified layer on the surface of the pulp. (The 'tertiary dentine' of Kuttler's classification). He noted the almost invariable presence of dentine spicules, and considers that they should be regarded as microtransplantations. They become a calcifying centre and are slowly absorbed.
It seems to be generally accepted that secondary dentine and sclerotic dentine are produced in the normal tooth as a result of age and peripheral irritation of the odontoblastic process. The question now arises whether these barriers are produced in response to the carious process, and if so to what extent they are effective in limiting the attack.

W.D. Miller\textsuperscript{94} said in 1904: "Whether the vitality of the tooth offers any obstruction to the advance of decay over and above that manifested by transparent and secondary dentine is a problem still to be solved at the expense of much patience and labour".

Gottleib\textsuperscript{58,151} states categorically that the deposition of calcium salts into the tubules (sclerosis) as a result of the carious process, forms a hyper-calcified layer which is a reliable barrier to the progress of dental caries. He thinks that a cavity may be limited indefinitely by the sclerotic process.

Fish\textsuperscript{49}, although admitting that his observations were based on irritation of non-carious teeth, says that the reaction to caries is essentially the same. This is a reasonable assumption since the advance of caries is associated with a drop in pH. Jolly and Sullivan\textsuperscript{69} found the vanguard of the carious lesion to be definitely within the acid scale of Universal Indicator,
and this was confirmed with micro-electrode measurements. This whole question will be gone into later, but at present it is relevant to point out that either an increase in acidity, or the presence of toxic breakdown products from proteolysis, or both, would have the same irritating effect on the terminal end of the odontoblast process that exposure to mouth fluids does, and therefore, the same reaction could be expected.

Fish^{49,48} thinks that the basic principle underlying all reactions to peripheral injury of the odontoblasts is the production of a barrier between the lesion and the pulp. He says: "The acid produced by the caries had destroyed the enamel and re-dissolved the first-formed deposit of lime salts in the dentinal tubules at the enamel border, so that the fibrils became irritated afresh and deposited more calcium, cutting off the affected area of dentine at a deeper level. Not only are the pulp and living dentine thus still preserved from contamination, but the carious dentine lies outside the pale of nutrition and is completely at the mercy of the invading organism .... After secondary dentine has sealed off a group of infected tubules, it generally follows that the carious process spreads along a 'dead tract' and, decalcifying the calcific barrier, invades the secondary dentine". He says that if the carious attack is a virulent one, the remaining odontoblasts
will be killed and the dentine laid down will be a 'tertiary' homogenous, atubular layer. When this in turn is invaded the ultimate result will be pulp exposure.

Although he maintains that secondary dentine acts as a seal in preventing the entry of toxic materials into the pulp, at the same time he points out that it can readily be destroyed by caries.

Boyle\textsuperscript{21} is of the opinion that the pulp reaction will vary with the rapidity of the attack, and that in slowly progressing caries there will be extensive sclerosis of primary dentine and large amounts of secondary dentine. The reaction will be the same as with abrasion or attrition.

Pribil and Plackova\textsuperscript{109} in roentgenograph studies of dentine beneath restorations observed layers of hypercalcified dentine between the restoration and the pulp. They also observed hypo-calcified dentine between the former layer and the restoration. They postulate that hypercalcification may result from acid formed during the carious attack liberating calcium salts which re-precipitate nearer to the pulp.

Bradford\textsuperscript{25, 24} holds the following view: The approach of caries in dentine results in:
(1) Destruction.
(2) Reaction.

He thinks that complete sclerosis of dentine beneath a carious lesion is rare, but depends on the amount of sclerosis already present - since the narrower the tubule, the more readily it can be blocked. Hence, the advance of caries will be slower in an older tooth. He thinks that a tubule which is blocked at an early stage of the carious attack will form an excellent barrier. He finds the 'dead tract' reaction more common beneath caries than sclerosis, and this can form a pathway for the ingress of bacteria.

There may however, be no reaction at all, and in the same tooth there can be occlusion of tubules or no reaction, and these two states may or may not show a relation to the approaching carious lesion. Tubules which remain unoccluded enlarge at the expense of the peritubular dentine and become pathways for bacterial invasion at a late stage of the disease.

If extensive sclerosis of the dentine has in fact occurred, then the lesion will spread along the line of least resistance, i.e. the amelodentinal junction, and the result will be a large saucer-shaped cavity with undermined enamel.
If there has been no sclerosis, or very little, the caries will advance rapidly towards the pulp and the enamel will not be greatly undermined; the result in this case will be that commonly seen in the teeth of children, in which a comparatively small fissure lesion is found to have progressed to the pulp.

Summary.

Mild stimulation of the odontoblasts such as abrasion, attrition, slowly progressing caries etc., and also age, will result in sclerosis of primary dentine, and the production of secondary dentine.

More acute stimulation such as a rapidly progressing carious lesion may produce an atubular, calcified layer of 'tertiary' dentine.

If the dentine has had time to become sclerosed and for secondary dentine to be laid down, then this will constitute some protection for the pulp against the ravages of the carious lesion.

In young teeth with little or no sclerosis, and patent dentinal tubules, odontoblasts will probably be unable to produce an effective barrier, and caries will tend to proceed
unchecked to the pulp.

The reaction of the tooth to caries seems to vary from tooth to tooth, and even in different parts of the same tooth.
SECTION II

CARIES OF DENTINE.

Introductory.

There does not yet seem to be any general agreement regarding the exact mechanism of the carious process. For the present purpose it is not necessary to examine the wide field of information relating to the inception of caries on the enamel surface. The problem of cavity sterilization arises at the point at which caries begins to involve dentine.

There are three basic elements involved in the carious destruction of dentine:

(1) The presence of micro-organisms.
(2) The decalcification of the inorganic matter which comprises seventy per cent of dentine.
(3) The disintegration of the remaining thirty per cent of organic substance, comprising the collagen fibres and mucopolysaccarides of the ground substance.
The Mechanism of the Carious Process.

Fundamental disagreement occurs on the question of whether decalcification precedes bacterial invasion, or whether the bacteria are to be found ahead of decalcification. Two authorities contradict each other flatly on this point—viz., Kronfeld—"The first step in caries of dentine is the invasion of tubules by micro-organisms. Next follows the decalcification of the surrounding matrix by the action of micro-organisms. There are always a few tubules that contain micro-organisms far ahead of actual decalcification". Whereas Black states: "Careful observation has shown that micro-organisms do not begin to grow into the dentinal tubules until the calcium salts have been dissolved out for some little distance in advance of them".

Bradford considers that the process consists of decalcification followed by proteolysis. Mandel supports this view.

On the question of the part played by bacteria in the process, Bradford considers that there are two methods of identifying bacteria. One is by Gram's stain, the other by culture. Only culturing gives undisputed proof of their presence. Gram's stain is taken up by the breakdown products of
dentine, and under the influence of the carious process the material within the dentinal tubules becomes more granular and more Gram positive. The granules which take up the space normally occupied by the odontoblast process and by the peritubular dentine are much smaller than bacteria, and it is these Gram positive granules which have hitherto been considered to be invading bacteria. He thinks that if the dentine has had time to become sclerosed, it will be resistant to bacterial invasion — a view which is supported by Gottleib\(^{58}\) and Fish\(^{48}\) and says; "... much material hitherto considered to be infected may be sterile, even if partially decalcified and clinically softened".

At this point it might be argued that Scott and Albright\(^{115}\) who examined carious dentine under the electron microscope, found dentinal tubules completely filled with bacteria, both rod-shaped and spherical organisms. However, the point in dispute is not whether bacteria are or are not found in tubules, but at what stage they may be found, and in this connection the findings of Scott and Albright are interesting. In artificially demineralized carious dentine they found that bacteria in the least affected dentine near the pulpal surface of the carious lesion were present in the odontoblast process; slightly external to this region the tubules were completely filled with bacteria; nearer still to the lumen of the cavity the tubules
appeared to be distended; and in the areas of most advanced
destruction the form of the dentine was completely obliterated
and filled with bacteria. However, since these sections were
decalcified these findings would not indicate whether the
bacteria were preceding decalcification or not. In examining
undecalcified sections, Scott and Albright\(^{115}\) found that in
some preparations large areas of the least affected dentine
were completely devoid of organisms. Further, that such areas
resembled partially demineralized non-carious dentine.
Although their conclusions are tentative, they say that their
evidence tends to support the idea that demineralization is the
first step in the carious process. They also think that bacteria
invade first the dentinal fibres, next the tubules proper, and
finally the matrix.

Johansen and Parks\(^{68}\) in an electron microscopic investiga-
tion of soft carious dentine make the following comment:
"... A general and possibly selective demineralization of the
inorganic phase appeared to be the first phase of the carious
process ... . The finding of an abundance of apparently normal
collagenous fibres indicated that collagen breakdown followed a
general tissue demineralization. The final stage in tissue
destruction apparently involves the more or less simultaneous
dissolution of remaining crystallites and depolymerization of
the collagen fibrils".
Blake observed that: "... although there is no peri-fibrillar space, it is likely that there is free diffusion of substances within the fibril itself." If, as seems probable, the bacteria spread from the odontoblast process, to the peri-tubular dentine, which in the normal state is a highly calcified substance, it seems logical to think that they would find a more ready nidus if this had been previously decalcified.

Prophet examined carious dentine with Gram staining, and found that on the surface of the carious lesion, all tubules were filled with bacteria. He agrees with the above supposition, and says: "... the work of Bradford, Miller and Shroff makes it clear that bacteria cannot occupy the tubules until decalcification has taken place. The space occupied by the odontoblast process is therefore the only pathway initially available to the invading bacteria. Acid metabolites result in decalcification of the peritubular dentine, which results in room for more bacteria ...". He thinks that intertubular dentine is invaded and destroyed only at a late stage of caries.

Boyle considers that proteolysis plays an important part in dentine caries, and also remarks that deep caries can be removed leaving hard discoloured dentine, and that no signs of pulp disease may ensue. McGregor, Marsland, and Batty, whose work will be considered later, also remark on the absence of signs of inflammation in very deep cavities.
Boyle\textsuperscript{21} thinks that as long as the pulp has not been exposed to mouth organisms, the cavity can be treated with mechanical removal of caries and replacement with a restoration, with little attention to sterilization.

Chirnside\textsuperscript{35} has considered the question of bacterial invasion of non-vital dentine. He did a small experiment in which he removed pulps from five pre-molar crowns, sealed off the cavity, and embedded them in a lower denture, which was worn for several weeks. He then sectioned the teeth, and used Gram's stain to detect bacteria. He found that there were bacteria present in the tubules, and he concluded that once the odontoblast had succumbed, either through pulp death or peripheral injury, then bacterial invasion could readily occur.

In this article, Chirnside is merely restating what Fish\textsuperscript{48} concluded in 1930. He believed that if caries starts in one of the enamel lamellae, then the dentinal tubule beneath it has already become a 'dead tract', and that micro-organisms can proliferate freely down this tract until they reached the plug of lime salts which seals it off from the pulp.

Jolly and Sullivan\textsuperscript{69} examined both undecalcified and decalcified sections of carious human dentine, and their findings support the authors previously quoted, who maintain that decalcification forms the initial attack, followed by proteolysis
of the collagenous fraction.

They took pH measurements of sound and carious dentine, and found that there was a significant increase in acidity in the carious dentine. They estimated a pH of 4 with Universal Indicator, and 5.5 with a micro electrode, for carious dentine. This is in accordance with the findings of Grossman, who also found carious dentine more acid than sound dentine, although in his measurements he estimated a pH of 6.5 for carious dentine, and 8.16 for sound dentine. The findings of Jolly and Sullivan agree with those of Canby and Bernier, who estimate a pH range of 4.7 to 5.5.

Jolly and Sullivan found that evidence for proteolytic activity was inconclusive, and observed that proteolytic enzymes act optimally at a pH of between 6 and 8, therefore any organisms working in the deeper carious layers, would have to be aciduric and also produce a proteolytic enzyme capable of functioning under more acid conditions than is usual. (However, if Grossman's readings are accepted, then proteolytic enzymes would be able to work in carious dentine).

With regard to the portals of entry via the dentinal tubules, they found that cultures from deeper areas between the pulp and lesion were consistently sterile, but cultures from actual lesions showed growth of bacteria. They gave a definite opinion that the
position of the relatively few intra-tubular organisms was of little importance, and that these few bacteria played a minor role compared with the superficially residing bulk of organisms.

They agreed with Bradford\textsuperscript{23} that it is hard to identify micro-organisms when there is other stained intra-tubular material present. They think that the variation in depth and irregular occurrence of stained intratubular particles deep in the dentine may be due to defensive mechanisms, or to the ageing process.

They conclude that each lesion develops its own individual appearance depending on a multiplicity of factors, but particularly upon the ability of the pulpal tissues to bring about reactions within the tubules.

At this stage it becomes relevant to review three experimental studies which were made on the relation of bacterial invasion to the softening of dentine. Two of these studies were made in England and reported in 1956 and 1960 respectively. The first by McGregor, Marsland and Batty\textsuperscript{25} was on permanent teeth, the second by Whitehead, McGregor and Marsland\textsuperscript{147} concerned permanent and deciduous teeth. The third study was made in the United States by Dorfman, Stephan and Muntz\textsuperscript{42} and was reported in 1943.
In the 1956 study by McGregor et al, one hundred teeth were used, all of which had cavities large enough to involve the dentine, but not large enough to involve the pulp. Sterility of procedure was carefully observed to avoid introducing contaminants into the dentinal scrapings, and a spoon excavator was used as a revolving bur might have generated sufficient heat to kill bacteria and thus give a false negative. The softened dentine was cultured to check the existence of viable organisms in it, and the remaining floor of firm dentine was scraped and the scrapings incubated for three days, and then sub-cultured onto tomato agar for both aerobic and anaerobic growth.

The teeth were then preserved and numbered for later sectioning, so as to observe the histological appearance of the hard dentine. The results were expressed as a table:

**TABLE I — PERMANENT TEETH**

**HISTOLOGICAL FINDINGS**

<table>
<thead>
<tr>
<th></th>
<th>Dentine entirely free of bacteria</th>
<th>1-20 infected tubules in floor of cavity</th>
<th>Over 20 infected tubules in floor of cavity</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative cultures (hard dentine)</td>
<td>36</td>
<td>8</td>
<td>5</td>
<td>49</td>
</tr>
<tr>
<td>Positive cultures (hard dentine)</td>
<td>25</td>
<td>13</td>
<td>13</td>
<td>51</td>
</tr>
<tr>
<td>TOTAL</td>
<td>61</td>
<td>21</td>
<td>18</td>
<td>100</td>
</tr>
</tbody>
</table>
These results show that there was correlation between histologic and bacteriologic findings in 62% of cases.

In 49% of cases negative cultures were obtained, though histologically, of these only 36% were completely free from bacteria.

Histologically, 61% of the total were free from bacteria in the hard dentine. 21% showed between 1 and 20 infected tubules, and only 18% showed more than 20 infected tubules.

In considering these results, McGregor points out that errors in technique would be responsible for a positive rather than a negative error.

From the table given previously it seems reasonable to suppose that if bacterial invasion on a large scale preceded acid decalcification, it would not be possible to obtain such a high proportion of negative results.

In the second experimental study, Whitehead, McGregor and Marsland repeated their previous investigations, using this time two hundred deciduous teeth and two hundred permanent teeth. Again a sterile technique was used, and the teeth were examined histologically and bacteriologically, being incubated with broth and plated out on tomato juice agar.

The results were as follows:
### TABLE II - PERMANENT TEETH

<table>
<thead>
<tr>
<th>Bacteriology of hard dentine</th>
<th>Free of bacteria</th>
<th>1-20 infected tubules</th>
<th>Over 20 infected tubules</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative cultures</td>
<td>50</td>
<td>23</td>
<td>14</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>43.5%</td>
</tr>
<tr>
<td>Positive cultures</td>
<td>53</td>
<td>45</td>
<td>15</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>56.5%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>103</td>
<td>68</td>
<td>29</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>51.5%</td>
<td>34%</td>
<td>14.5%</td>
<td>100%</td>
</tr>
</tbody>
</table>

In this series only 55% showed correlation between histologic and bacteriologic findings.

43.5% yielded negative cultures, though only 25% of these were histologically free from organisms.

34% showed 1-20 infected tubules, and 14.5% showed more than 20 infected tubules.

In this series the hard dentine showed a slightly greater tendency to be infected, but not significantly enough to invalidate the inferences drawn from the previous experiment.
TABLE III - DECIDUOUS TEETH

HISTOLOGY OF HARD DENTINE

<table>
<thead>
<tr>
<th>Bacteriology of hard dentine</th>
<th>Free of bacteria</th>
<th>1-20 infected tubules</th>
<th>Over 20 infected tubules</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative cultures</td>
<td>23</td>
<td>31</td>
<td>25</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39.5%</td>
</tr>
<tr>
<td>Positive cultures</td>
<td>26</td>
<td>70</td>
<td>25</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>60.5%</td>
</tr>
<tr>
<td>TOTAL:</td>
<td>49</td>
<td>101</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>25.4%</td>
<td>50.5%</td>
<td>25%</td>
<td>100%</td>
</tr>
</tbody>
</table>

A marked difference was noted between the findings for deciduous and permanent teeth. There was correlation between histologic and bacteriologic findings in 50% of cases. Only 25.4% were histologically free from bacteria, and only 39.5% gave negative cultures. 50.5% showed 1-20 infected tubules, and 25% showed more than twenty infected tubules. If this is interpreted literally, it means that one patient in four would have infected tubules, but as McGregor et al point out, there is an average of 45,000 dentinal tubules per sq. mm. of dentine, each being approximately 2 microns in diameter, and in this investigation, if only one infected tubule was seen, it was placed in the 1-20 group. Furthermore, the final scrapings of dentine may have removed the last of the infected dentine, leaving a bacteriologically negative cavity, but giving a positive result for the scraping.
McGregor and his colleagues also investigated the histological results in relation to the type of cavity, and gave the following data:

**TABLE IV — HISTOLOGICAL FINDINGS IN RELATION TO TYPE OF CAVITY**

(a) PERMANENT TEETH

<table>
<thead>
<tr>
<th>Type of cavity</th>
<th>Free of bacteria</th>
<th>1–20 infected tubules</th>
<th>Over 20 infected tubules</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl. 1 Occlusal</td>
<td>34</td>
<td>10</td>
<td>3</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23.5%</td>
</tr>
<tr>
<td>Cl. 2 Interstitial</td>
<td>46</td>
<td>40</td>
<td>15</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50.5%</td>
</tr>
<tr>
<td>Cl. 5 Cervical: margin</td>
<td>23</td>
<td>18</td>
<td>11</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26%</td>
</tr>
<tr>
<td>TOTAL:</td>
<td>103</td>
<td>68</td>
<td>29</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>51.5%</td>
<td>34%</td>
<td>14.5%</td>
<td>100%</td>
</tr>
</tbody>
</table>
(b) **DECIDUOUS TEETH**

**HISTOLOGY OF HARD DENTINE**

<table>
<thead>
<tr>
<th>Type of cavity</th>
<th>Free of bacteria</th>
<th>1-20 infected tubules</th>
<th>Over 20 infected tubules</th>
<th>TOTAL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl. 1 Occlusal</td>
<td>17</td>
<td>26</td>
<td>7</td>
<td>50</td>
<td>25%</td>
</tr>
<tr>
<td>Cl. 2 Interstitial</td>
<td>29</td>
<td>69</td>
<td>39</td>
<td>137</td>
<td>68.5%</td>
</tr>
<tr>
<td>Cl. 5 Cervical margin</td>
<td>3</td>
<td>6</td>
<td>4</td>
<td>13</td>
<td>6.5%</td>
</tr>
<tr>
<td><strong>TOTAL:</strong></td>
<td><strong>49</strong></td>
<td><strong>101</strong></td>
<td><strong>50</strong></td>
<td><strong>200</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

Again there is marked difference between the findings for deciduous and permanent teeth, especially in the findings for Class 2 interstitial cavities, where only 20% of permanent teeth showed 1-20 infected tubules, whereas 35% was the corresponding figure for deciduous teeth.

McGregor et al suggest that this may be due in part to the 'protective metamorphosis' of the adult teeth, which results in secondary dentine, which slows down the rate of penetration of caries. The second reason is that the majority of permanent teeth were extracted for reasons other than caries, whereas the deciduous teeth came from patients with neglected mouths. The
conclusion he draws is that in general there is more risk in leaving bacteria in deciduous teeth, especially in interstitial cavities.

The more general conclusions that the authors draw from their experiments is that softening of dentine is due primarily to the action of acids acting at some distance from the organisms responsible for their production. They think that from a clinical viewpoint, disinfecting the cavity may be unnecessary provided that all softened dentine is completely removed.

Dorfman, Stephan and Muntz\textsuperscript{42}, whose work preceded that of the English group, used two approaches. They took samples from the carious lesion into the sound dentine, and from the sound dentine into the carious lesion. They used twenty teeth for the first method and sixty-nine teeth for the second method. They used sterile excavators, and cultured the material. They expressed their results diagrammatically - (see page 51).

These diagrams indicate that of the twenty teeth in which the samples were taken from carious to sound dentine, almost all the superficial and middle layers of carious dentine were infected, in about half the deep layer of carious dentine was sterile, and in all but one the partially decalcified layer was sterile. This could be explained by the greater risk of contamination in going from carious to sound dentine.
1. Extent of infection in carious dentine: samples of dentine taken from carious surface to sound dentine.

   Superficial layer of carious dentine.

   Middle layer of carious dentine.

   Deep layer of carious dentine.

   Partially decalcified layer of carious dentine.

   Sound dentine.

2. Extent of infection in carious dentine: samples of dentine from sound dentine to carious surface.

   Superficial layer of carious dentine.

   Middle layer of carious dentine.

   Deep layer of carious dentine.

   Partially decalcified layer of carious dentine.

   Sound dentine.
In the second group of sixty-nine teeth, in which the samples were taken from sound dentine to carious surface, in all cases the sound dentine was found to be sterile, in all but five cases the partially decalcified layer was sterile, and in forty-five cases, that is, in 75% of cases, the deep layer of carious dentine was sterile.

Dorfman, Stephan and Muntz\textsuperscript{42} make the following comment: "It is clear that decalcification has preceded bacterial invasion, and there is usually a . . layer of sterile decalcified dentine overlying the sound dentine".

Their conclusions are in substantial agreement with those of McGregor and his colleagues, and they also maintain the opinion that removal of all softened dentine should eliminate all infection, except that due to contamination from operative procedures. They also think that if the decalcification process has just reached the pulp, it may be more desirable to leave it in place, rather than risk exposing the pulp by removing it.

At this stage the evidence seems to favour those who believe that decalcification precedes invasion by bacteria, nevertheless the opinions of eminent authorities who hold the opposite view cannot be summarily dismissed.

Appleton\textsuperscript{4} states categorically that pulpal infection occurs
before actual gross pulp exposure, and that bacteria penetrate further into the tubules than is indicated by any gross change in the dentine. He does not cite any great weight of evidence for his assumption, and appears to base it on the belief that the tubular nature of the dentine provides a ready pathway for the organisms. However, since the discovery of the peritubular layer of dentine, it is now realized that the actual lumen available to bacteria is only about 1 - 1.5 microns (Shroff et al.\textsuperscript{113}) which is still wide enough to admit organisms, but not quite the broad highway that was hitherto supposed, when it was believed that a perifibrillar space existed.

Bartels\textsuperscript{10}, in an article published in 1949, says: "Whether bacteria can penetrate the dental tubules ahead of calcification is still a controversial matter ...." and he quotes the opinion of Appleton cited above. Eleven years later, in 1960\textsuperscript{11}, he published a further article in which he quoted his earlier opinion in an entirely unchanged form. In both articles he refers to Kronfeld's standard textbook on dental pathology\textsuperscript{77}, a quotation from which will be found earlier in this chapter.

The evidence presented in Kronfeld's book appears to be dependent on histological sectioning and staining, which is not, as Bradford\textsuperscript{23} pointed out, as positive a proof as culturing. Electronmicro-photographs of sections by Scott and Albright\textsuperscript{115} are also published, but, as was noted earlier in this section,
the findings of these two workers tend to support the opposite view to that cited in the text.

Bartels reports two experiments which are concerned with the clinical aspects of cavity sterilization. The first, by Zander concerns bacteria in the dentine after cavity preparation. He studied ten teeth in which there were caries, but the pulp was not involved. Having removed all obvious caries under aseptic conditions, he extracted the teeth, decalcified them, and prepared sections. He found micro-organisms in the hard dentine of four teeth at a distance of 0.08 to 1.2 mm. beneath the cavity floor, while the distance from the pulp at which micro-organisms were observed varied from 0.24 to 1.9 mm. He concluded from this that it was theoretically possible for bacteria to survive and multiply in dentine beneath restorations, and that if this happened, one could expect four failures out of ten in restored teeth. (Which Bartels admits is not supported by clinical experience).

This experiment is open to the same criticism which Bradford levelled at all histological techniques, in regard to this special subject, namely that it is hard to differentiate between breakdown products and bacteria. Furthermore, the very small number of teeth used does not justify any general conclusions being drawn from this work.

Seltzer prepared cavities under sterile conditions and
cultured the material obtained from the cavity floors. He then immersed the cavity floors in various sterilizing agents, filled the cavities with gutta percha, and covered them with a layer of filling material. He made cultures again after intervals of two weeks and one year. He came to the conclusions that:

(1) The chances for mechanically eliminating all bacteria from a shallow cavity are greater than 50%.

(2) Once the bacteria get into the dentine the chances for eliminating them by proper cavity preparation become progressively less the larger the cavity.

(3) In deep cavities the inability to remove all bacteria by careful excavation emphasises the need for sterilization of dentine prior to inserting the restoration.

The question of whether micro-organisms can enter the dentinal tubules prior to decalcification has been considered by Bender, Seltzer and Kaufman\(^{13}\) in connection with impression taking during operative procedures. They used thirty-nine teeth, from five dogs and one monkey. Their experimental technique seems adequate, and they took the precaution of culturing both blood and saliva before applying the preparation of *S. faecalis*, to ensure that it was not already present. They found, that in dogs' teeth at least, bacteria can in fact penetrate the tubules and deposit in the pulp. They also found more bacteria where pressure had been used, as in forcing inlay wax against the surface.
Thus far the evidence would seem to indicate that bacteria are able to penetrate the dentine ahead of the softening process, but in fact they do not do so in overwhelming numbers.

The Bacteriology of the Carious Process.

Some of the more specifically bacteriological work will now be considered, and in doing so, it may be that further opinions will be offered regarding the bacterial invasion/decalcification controversy.

Again, it must be emphasised that none of the opinions claim to be definitive. "There is no general agreement regarding the nature of micro-organisms involved in caries ..." Pigman et al. "The exact mechanism of caries is still unknown ..." Roth.

As mentioned earlier in this section, it is thought that acid decalcification is one factor in the disintegration of dentine, and that proteolysis of the organic component is another.

Evans and Prophet consider that collagenase, an enzyme originally discovered to be produced by clostridium welchii, may play a part in disintegrating the organic component of dentine. They found that collagenase could in fact disintegrate ground dentine, but only after it had been decalcified. They showed
that the enzyme was active over a pH range of 5.6 - 8.5 and that
the optimum pH for simultaneous decalcification and disintegration
was pH 5.9 - 6.5. They said that it was not known whether the
organisms involved in dental caries were able to elaborate this
enzyme. However, Lucas and Thonard showed that many of the
organisms recoverable from the mouth were capable of disintegrat-
ing collagen.

Engel also thought that collagenase might be involved in
dentine destruction. He also thought that the 'spreading factor'
yaluronidase might be implicated as well. Steinman has also
made this suggestion. He thought that the degree of polymeriz-
ation of ground substance might be a factor in resistance to
attack by these enzymes, and suggested this as a reason for the
more rapid spread of caries in young and in deciduous teeth, i.e.,
a lower degree of polymerization of ground substance. He sub-
stantiates the opinion of Evans and Prophet (vide supra) that
some initial alteration of dentine is required before collagenase
can act on it, but found that this was not provided wholly by acid
action alone. Burnett and Scherp also find that proteolytic
bacteria make little impression on intact dentine.

Roth studied the micro-organisms associated with caries
in connection with collagen chemistry. As it was not possible
to obtain calcified collagen in its unaltered state, she used
hide powder, which has the disadvantage of being:-
(1) possibly not as like tooth collagen as is desirable, and
(2) has to be denatured before being available for experiment.

She reached the conclusion that bacteria are present in the carious lesion which are capable of breaking down a denatured protein in a bacteriologic medium. The organisms were present in all areas of the carious region. She found 102 organisms capable of lysing hide powder, and listed them in five groups:

(1) Nocardia dentocariosus.
(2) Gaffkya dentocariosus.
(3) Micrococcus pyogenese.
(4) Mixed group of anaerobic streptococci.
(5) Anaerobic form of actinomyces.

Burnett and Scherp, investigating the distribution of proteolytic and aciduric bacteria in the carious lesion found that proteolytic bacteria were absent from the initial enamel lesion, but that lactobacilli and other aciduric and acidogenic organisms were present in that situation. In the superficial layers of deep dentinal caries they found caseinolytic, ovolytic and dentinolytic bacteria. In this area they found the highest total concentration of cultivatable bacteria. In the advanced areas of deep caries they found a relatively low concentration of these bacteria, but they regularly isolated aciduric and acidogenic forms from this area. They concluded from their observations that proteolytic bacteria do not advance the carious lesion, but destroy the organic matrix after decalcification has taken place.
In a further investigation of the area between intact and
decalcified dentine, Burnett and Scherp\textsuperscript{30} cultured scrapings
from this area. They found Gram + cocci regularly, both aero-
bically and anaerobically. Lactobacilli only constituted 5\%.
They presumed that in the deep dentinal lesion the micro-
organisms can in some way obtain nutriment from the organic
matrix. They admit that "the reactions obtained from the or-
ganisms in culture do not necessarily correspond to those taking
place within the tooth. They said that a large number of the
cocci seemed to be neither aciduric nor proteolytic, but may
function in the carious process over the longer period during
which the reactions take place in vivo.

Canby and Bernier\textsuperscript{32} cultured scrapings from the deep layers
of carious dentine, using thirty-six teeth. They found that no
bacteria could be cultured in 36\% of cases, but, in contrast to
Burnett and Scherp\textsuperscript{30}, they found that in those cases where cultures
could be obtained, \textit{L. acidophilus} was found in over 90\%. In addi-
tion to \textit{L. acidophilus}, they isolated Strep.\textit{viridans}, Neissera
Catarrhalis, Micrococcus gazogenes, \textit{Staph.} alb\textit{us}.

They think that the hard unsoftened area between the carious
lesion and the pulp chamber is often sterile, in fact they often
cultured this dentine and found it to be so. They suggest as a
reason for this that a low pH is not the best environment for
even acidophilus organisms, for which the optimum pH is about
6.2 (see also Evans and Prophet). However, the deeper layers of decalcified dentine are at a pH of 5.5 to 4. In the more superficial layers of caries, the acids are buffered by saliva, which results in a pH more favourable for the growth of ordinary mouth organisms. (This argument would not be valid for those types of carious lesion in which there is only a small fissure lesion in the enamel, and gross dentine caries beneath, as these lesions are fairly effectively protected from the saliva). Canby and Bernier think that the marked acidity of deep carious dentine may prevent the ingress of organisms into the pulp via the dentinal tubules, by precipitation of calcium salts into them. This idea has also been put forward by Crawford and Gottleib.

Sognaes and Wislocki in their investigations on carious dentine state that "... In advance of the pervading microorganisms the tooth substance undergoes so much demineralization that histologic sections can be prepared without preliminary decalcification". They were not able to isolate the enzymes capable of depolymerizing mucopolysaccharides. They found that in advanced caries the dentine was invaded by a variety of organisms, including rods, cocci, and threadlike forms. They find that the presence of these bacteria indicates an advanced stage of carious breakdown. They think that the carious process is initiated by demineralization, followed by depolymerization of the ground substance, that there is a widening of the dentine tubules at the expense of the surrounding ground substance, and finally, complete disintegration with the tubules merging into
Pigman, Gilman, Powell and Muntz\textsuperscript{107} investigated the action of individual bacterial strains on teeth, under 'in vitro' conditions. They used an artificial mouth, which consists of an extracted human tooth mounted in stone on an acrylic box. This is placed in a cylindrical funnel and a nutrient solution is allowed to drip continuously for the experimental period, in this case, twenty-five weeks, at a temperature of 35 - 36 deg.C. Their results were summarized as follows:

<table>
<thead>
<tr>
<th>ORGANISMS</th>
<th>DECALCIFIER</th>
<th>Destroyer of decalcified dentine matrix</th>
<th>Colour produced in dentine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enamel surface</td>
<td>Dentine &amp; subsurface enamel</td>
<td></td>
</tr>
<tr>
<td>L. Casei (Hadley)</td>
<td>Strong</td>
<td>Strong</td>
<td>Medium</td>
</tr>
<tr>
<td>L. Casei (Mahler)</td>
<td>Weak</td>
<td>Strong</td>
<td>Weak</td>
</tr>
<tr>
<td>S. Salivarius (Miven)</td>
<td>Medium</td>
<td>Strong</td>
<td>Medium</td>
</tr>
<tr>
<td>S. Salivarius (Kraus)</td>
<td>Medium</td>
<td>Strong</td>
<td>Strong</td>
</tr>
<tr>
<td>S. Faecalis</td>
<td>Weak</td>
<td>Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>C. Perfringens</td>
<td>Negative</td>
<td>Negative</td>
<td>Medium</td>
</tr>
<tr>
<td>M. Albus</td>
<td>Medium</td>
<td>Medium</td>
<td>Strong</td>
</tr>
<tr>
<td>M. Aureus</td>
<td>Weak</td>
<td>Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>H. Catarrhalis</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Their work indicates that many types of organisms have the ability to attack tooth structure, though whether their action in the mouth is represented by these experiments is not known. Furthermore, dentine is more susceptible to more of these bacteria than is enamel, at least, in vitro.

Pigman et al, think the pigmentation of carious dentine is possibly associated with a rate of dentinal decalcification greater than matrix destruction.

Deakins suggested that the pigmentation of carious dentine could be due to a melanin, which might be produced from a change in the organic matrix as a result of the carious process, giving rise on acid hyrolysis to an insoluble pigment.

Armstrong thought that the brown pigmentation might be the result of reaction between the dentine matrix and carbohydrate degradation products. He accordingly investigated the possibility of the presence of carbohydrate material in carious dentine and found results varying from 1.8% to 4%, but said that he could not be sure that this was not merely a contaminant from the mouth.

Onisi and Nuckolls in a report on the organisms that they isolated from the carious lesion, point out that it is very difficult to avoid surface contamination in taking samples of
carious dentine, and they think that in some of the work done in this field, there have been insufficient precautions taken in this regard. They think that to isolate the teeth with rubber dam and paint them with iodine is probably not sufficient to sterilize the outer surface, and that such contamination can greatly alter the picture.

In their particular sampling they treated the teeth with ultra violet light and with paraffin at 200 deg.C. They found that the ultra violet light did not sterilize the tooth surface, but in some cases the paraffin did, and they used only those teeth from which they could not cultivate any colonies from the external surface. They took samples from the sound but pigmented area underlying the obvious carious lesion.

They took samples from 75 lesions, and of these, 33 failed to develop colonies. From the remaining 42 lesions they obtained 97 strains of bacteria. 11 of these they tentatively classified as staphylococci, Gram + rods, and Gaffkya. The remaining 86 strains were pleomorphic. They admit that the method of sterilization might possibly have killed off thermosensitive bacteria. They think that the pleomorphic forms are truly representative of the carious lesion. In a later study, they isolated 86 strains from carious teeth, and found amongst them Nocardia, Actinomycetes, Corynebacteria, and Bacillia.
Jores, Cohen and Calisti in a study of infected primary molars report that they isolated Strep. salivarius, Strep. mitis, Microaerophilic streptococci, Beta haemolytic streptococci, Lactobacilli and Staph. albus from the carious lesions.

A novel suggestion by Turner, Crowell and Dexter is that possibly a viral attack on the organic matrix of the tooth may lead to a release of calcium salts. This theory does not appear to have been followed up by any other workers, and does not warrant serious consideration.

It becomes clear from even a brief consideration of a small part of the work done in dental bacteriology that there is no clear evidence as to which bacteria are responsible for the destruction of tooth structure. All that can be said is, that it does seem probable that acid decalcification precedes bacterial invasion, but possibly not in all cases. In a Consultant Symposium 'Our empiric cavity sterilization' published in 1951, opinions were sharply divided on the question; Stephan, supporting the 'Decalcification first' theory, and Seltzer the 'Bacterial Invasion First' theory. However, work which has been done since tends to support the former; especially the work of MacGregor and his colleagues. Keil in an article published in 1958, is prepared on the evidence offered, to support the belief that carious destruction is dependent on preliminary decalcification. This is not to say however, that in every case
of deep dentinal caries the remaining layers after excavation of gross caries can be regarded as sterile and ignored. As recently as 1960, Schmidt, Crowley et al.\textsuperscript{112} speak of "the undeterminable location of micro-organisms".

Bodecker\textsuperscript{20} has suggested that there may be two types of caries, the acute type, in which decalcification and proteolysis occur at such a rate as to preclude the deep invasion of the tubes by bacteria, and a chronic type which is characterized by a slower disorganization of the dentine, and the bacteria have the opportunity to invade some of the tubules beyond the line of destroyed dentine.

A reasonable view is that of Young\textsuperscript{150} who, after reviewing the literature, reached the conclusion that until it is possible to be certain that the cavity is sterile after preparation, it is better to use some form of medication which will destroy bacteria without harming the pulp. Whether such a form of medication exists is another question, and an attempt to answer it will occupy a large part of the remainder of this review.

The evidence regarding the identity of the organisms responsible for the carious breakdown is so conflicting that no opinion of any worth can be offered. All that can be said is that a vast variety of organisms appear to be recoverable from the carious lesion. It would seem that some of the techniques
used are such as to cast doubt on the value of the results obtained. However, what can be said is that in trying to determine areas of sterility, the errors are likely to be as a result of contamination — therefore, if an experiment reports a negative result, the chances are that the area concerned actually is negative.

The Fate of Bacteria Sealed into the Cavity.

If, as..seems probable, it is not possible to eliminate all bacteria from the floor of the cavity by mechanical preparation alone, the question arises as to the subsequent activity or otherwise of such organisms as are sealed in beneath the restoration.

Gabel\textsuperscript{51} states that the chief reason for the death of pulps beneath large restorations is bacterial invasion of the pulp. This implies that the remaining organisms are still viable even after being cut off from the oral cavity by insertion of the restoration. Theoretically this is quite a reasonable assumption, firstly, because some of the bacteria concerned are anaerobic, secondly, because there are probably few restorations which exhibit perfect marginal seal.

In practice, however, opinion is divided as to whether organisms do remain viable after being sealed in. Orban\textsuperscript{101}
admits that under a properly placed filling, bacteria remain for years with no further signs of growth. He thinks that this is probably because of poor environment, or, if a sterilizing agent has been used, due to this. He thinks that the few bacteria left in tubules can be dealt with - they or their toxins - by the pulp, and that formation of secondary dentine will hinder further penetration.

Bacic prepared cavities in ten carious teeth, cultured the dentinal surfaces and then sealed the teeth with sterile cotton, gutta percha and zinc phosphate cement. He found gamma streptococci present in 6 teeth, strep. viridans present in 2 teeth, lactobacillus acidophilus present in 5 teeth, and staphylococci present in 2 teeth. He then cultured the cavities periodically to determine the survival time of the bacteria. He did not use any disinfecting agents. He found that lactobacilli died within a period of 2 - 10 months, but that streptococci persisted for more than a year in 30% of cases. In no instance did he find recurrence of gross caries. He concluded that if it is possible to use a disinfecting agent to eliminate a potential focus of bacterial growth, it would be better to do so.

Seltzer maintains on the contrary that it is possible to find recurrent decay even under carefully executed restorations where there has been an attempt to sterilize the cavity.
In this respect a case report by Gale \textsuperscript{52} is interesting. He treated fifty cases with grossly carious teeth. He cleared the cavity peripherally, but left soft decay at the base over the pulp. In some cases he inserted zinc oxide and in some cases zinc oxide and copper cement. He took radiographs of thirty cases. In only one case did pain develop. A year later he retook radiographs, and in no case was there an increase in the caries beneath the filling. A year later again, thirty-two of the original patients re-attended, and in only one case was extraction considered necessary.

Two years is hardly a sufficiently long period of time in which to judge the success or otherwise of this treatment. If it is claimed that such a measure is a palliative such as might tide a child over a period of two or three years to give the second molars time to come into position, then it certainly has merit. But whether it is successful as a long term measure has not yet been experimentally demonstrated.

Advocates of the practice of sealing soft caries beneath a restoration claim, on empiric grounds, that it is a successful long-term measure. Kraus \textsuperscript{75, 74} apparently has had great success with the "Baldwin Technique", whereby a layer of soft cement is placed over the entire cavity, and an amalgam restoration is packed on top of this, thus squeezing the cement to the margins, where the amalgam is burnished over it.
Amongst others who are in favour of leaving soft caries rather than risk an exposure are MacGregor, Massler, Herbert, and Prader.

Bartels, in considering the fate of bacteria sealed into cavities, considers the point that moisture is necessary for bacterial activity, and remarks on the suspension of such activity in dried fruit and dried meat. He thinks that hermetically sealed-in micro-organisms are in a similar predicament, but that seepage of saliva at the margins of a restoration could re-activate them.

Nuckolls also thinks that micro-organisms become inactive under a hermetic seal. He thinks that their metabolism is reduced to a point where they cause no further changes in dentine, but that they probably remain viable for some time— as Besic indicated. Nevertheless, the production of secondary dentine beneath the lesion tends to wall off the bacteria from the pulp.

Both Boyle and MacGregor et al. have commented on the absence of signs of inflammation in pulps beneath deep cavities. Boyle suggests the possibility that micro-organisms destructive to dentine may be non-irritating to living cells. He says that lactobacilli are essentially harmless to living cells. This theory was cited a few years earlier by Kraus who says that acidogenic bacteria are not pathogenic and do not cause inflammation. He thinks that under anaerobic conditions these bacteria
produce more acid than under aerobic conditions, and that this makes the environment more hostile for true pathogens. (See also Canby and Bernier\textsuperscript{32}).
SECTION III

THE STERILIZATION OF DENTINE.

General Remarks.

If, as seems reasonable in the light of the previous discussion, it is desirable to insert a restoration on to a sterile base on the one hand, or to inactivate bacteria in a deep-seated near-exposure on the other, the next question to be considered is by what means, if any, it is possible to accomplish this end.

Some of the medicaments which have been advocated for the purpose will be considered in the following pages.

As long ago as 1891, Miller was reporting on the efficacy of sterilizing agents. Some of the chemicals he used have long since fallen into desuetude, for example, Iodine trichloride, and Mercury Bichloride. Others still have a place—whether justified or not—in modern practice, such as phenol, which Miller found effective if sealed in overnight, hydrogen peroxide, and the essential oils which did not meet with his approval.
It must be remembered that Miller did not have at his disposal modern histological techniques with which to examine the effects of these drugs on the pulp and odontoblasts, and indeed, it seems that only in comparatively recent years has there been an interest in the biological aspects of cavity sterilization.

In this field there are still many disadvantages to be overcome, not the least of which is availability of material. If histological studies are carried out on carious teeth which are sufficiently decayed to warrant extraction, then it becomes debatable whether the histological picture presented is a reflection of the drug under test, or the carious process. To carry out studies on sound human teeth it is necessary to find large numbers of patients who need orthodontic extractions. Even if such patients are forthcoming in sufficient numbers, there is still the problem of ensuring re-attendance over a period of time, which makes long term surveys a difficult proposition.

The logical outcome of these difficulties therefore, is to use experimental animals, and this is what is most frequently done. The pitfalls in this procedure have been discussed by Seelig who makes these observations:

(1) The dental pulps of different animals react differently to irritating substances being placed in freshly cut cavities. Dogs have the most permeable dentine, and present the most
marked pulpal changes. The monkey's dentine is not as permeable as the dog's, and human dentine is less permeable than either; therefore, a human tooth does not react as severely to a given irritant as a dog's or monkey's tooth.

(2) A preparation cut into fresh dentine, with a medicament placed, may give a more severe reaction than if the same medicament is placed in a cavity cut into carious dentine in which the pulp has had time to produce a defence barrier of secondary or sclerosed dentine, in which the permeability is less than in primary dentine. Older pulps will not react as markedly as younger pulps, but there is always a great individual difference in reaction.

(3) The cavity preparation. If the cavity is cut slowly and with a coolant, the reaction will be less than if a wide deep cavity is cut with an overheated fast revolving bur.

These factors, Seelig points out, should be born in mind when considering the results of histological investigations. He says - "The work on animals does not tell us what reaction to expect in any specific case. It tells us the relative irritative qualities of various materials. At best, the experiment informs us with which materials we must be careful. If a material causes no reaction in the pulp of an experimental animal, then it will
cause no reaction in a human tooth ....". It is with these comments in mind that the work on cavity sterilizing agents could be evaluated.

Sterilizing Agents.

SILVER NITRATE. \((\text{AgNO}_3)\). This is perhaps the best known of all sterilizing agents, and certainly the one most hallowed by custom. It is still common practice for dentists to impregnate cavities (especially those of the deciduous dentition) with it, in order to avoid removing the last layers of caries. The drug is commonly used in the form of an ammoniacal solution, as first advocated by Howe in 1917, which is applied to the surface of the dentine, and then precipitated with eugenol. The theory behind its use is that the precipitated silver penetrates the dentinal tubules and blocks the ingress of further bacteria. Also, silver nitrate is itself a protein coagulant, and will block the tubules by coagulation of the proteins found within them, as well as coagulating the proteins of such bacteria as may be present in the tubules.

Barker\(^7\), \(^8\) in 1937 laid down the following requirements for an ideal cavity sterilizing agent:

\(\text{(1) It must destroy bacteria.}\)
(2) It must penetrate dentine.
(3) It should be as non-irritating to the pulp as possible.
(4) Must not discolour the teeth.
(5) Should not require more than five minutes to act.

Although this last requirement is frequently mentioned, it is not a vital one, since there is no reason why a drug should not be sealed into the cavity beneath the lining, or else, sealed in for several days beneath a temporary filling, prior to the insertion of a permanent restoration.

Barker investigated the sterilizing properties of seven drugs by using cubes of dentine, and he subsequently verified his findings under 'in vivo' conditions. He found that silver nitrate and thymol were the only effective ones, being the only two that sterilized the dentine within two minutes. He found that silver nitrate, together with oil of cloves, creosote and phenol, when applied to cavities for periods of up to a month, do not appear to affect the vitality of the pulp "... as far as can be judged by clinical observations and histological preparations".

Zander\textsuperscript{158} in 1941, was of the opinion that silver nitrate would check caries as far as it penetrated, but remarked that it did not necessarily penetrate as far as the bacteria extended.
He observed three zones of penetration, an outer zone with a heavy deposit of silver, a middle zone with a lighter deposit, and a heavy deposit on the inner zone. He thought it was possible that in those cases where it had been claimed that silver nitrate had halted the carious process, the cause might well be the absence of organisms rather than the presence of the drug. At this time he advocated the routine use of silver nitrate in all cavities where appearance was not important - an opinion that he was to revise twelve years later.¹⁵⁴

Seltzer¹¹⁹, ¹²¹ in the same year (1941) found silver nitrate ineffective in comparison with other drugs. He found it to be ineffective even after being sealed into the cavity over a period of time. His findings directly contradict those of Barker, as the latter found thymol and silver nitrate the most effective agents, whilst Seltzer found them the least effective. It must be noted that Seltzer is unique in finding silver nitrate ineffective. He postulates that protoplasmic poisons such as silver nitrate do not sterilize dentine because they are self-limiting, and do not come into contact with bacteria in the tubules. Later evidence to be mentioned does not substantiate that silver nitrate is self-limiting. It might also be noted that his finding that phenol is a good sterilizing agent is in direct contradiction to Hardwick⁶², whose work will be considered later on.
Nevertheless, despite these findings in regard to silver nitrate, in a further article published in the same year, Seltzer and Werther advocate ".... the judicious use of Howe's silver nitrate in cases of deep-seated caries bordering on pulpal exposure in the case of young and of deciduous teeth ....". They sealed the silver nitrate into a total of 204 teeth, but only 93 teeth were subsequently examined owing to failure in re-attendance. They found that this treatment led to bacteriostasis and laying down of secondary dentine. The question to be considered here is whether this desirable result was achieved by the silver nitrate or to the change in environment subsequent to sealing, which other workers (e.g. MacGregor, Kraus, Prader, Lefkowitz, Massler, and Lorinczy-Landgraf) maintain, will lead to bacteriostasis. In fact, Seltzer himself admits that under a properly placed filling, bacteria will remain for years with no further signs of growth.

Zander and Smith and Zander and Burrill in studies of the penetration of silver nitrate into dentine, made the following observations:

1. Silver nitrate penetrates sound dentine.

2. It can also penetrate secondary dentine.

3. The penetration is a purely mechanical phenomenon.

4. Depth of penetration increases with time and eventually reaches the pulp.
(5) Penetration through sound dentine did not severely injure the pulp.

Zander and Burrill\textsuperscript{155} also noted that variations in duration of application, type of precipitant, and order of application, produced no changes in the character of the penetration.

In 1943, Dorfman, Muntz and Stephan made their studies on the evaluation of germicides\textsuperscript{43}, and the effective penetration of germicides\textsuperscript{150}. These were 'in vitro' studies and must be regarded as comparative studies rather than as a representation of a process within the actual cavity. They found that saturated silver nitrate proved to be the best germicide, and that only silver nitrate and hydrogen peroxide were able to sterilize pieces of dentine 0.8 to 1.4 mm thick. A three minute application of silver nitrate did not consistently sterilize to a depth greater than 0.25 mm. of dentine. After ten minutes most of the samples were rendered sterile to a depth of 0.75 mm.

They found that if applied for a sufficient length of time, silver nitrate was an effective penetrating germicide. However, in contrast to Zander and Smith\textsuperscript{158} and to Zander and Burrill\textsuperscript{155} (see previous page), and also to Weiss\textsuperscript{146}, they contended that it was unable to penetrate either sound or secondary dentine.

In 1949, Hardwick\textsuperscript{62} did an extensive survey on the steriliz-
ation of carious dentine, and as a result found the clinical use of silver nitrate justifiable. He listed the following properties as desirable in the salt of a heavy metal to be used in cavity sterilization:

(1) The basic radicle should be highly bactericidal.
(2) The acidic radicle should be highly bactericidal.
(3) The salt should be highly soluble in water.
(4) The acidic radicle should not react with calcium ions present in the dentine to form an insoluble salt.
(5) The basic radicle should not react with phosphate ions to form an insoluble salt.
(6) If self-limiting action is required the chloride of the basic radicle should be insoluble in order that it may be precipitated by tissue fluid chlorides.
(7) If the salt is easily reduced it will act more quickly as a germicide and at the same time be precipitated more rapidly by the tissue fluids making it more self-limiting.

He found that silver nitrate fulfilled nearly all these conditions. Turning his attention to the histological effects on the pulp and the depth of penetration he says:— "Silver nitrate solution will quickly diffuse into that space containing tissue fluid lying between the dentinal fibril and the tubule wall ....". However, it is now generally accepted
that no such space exists, and it would be more correct to say that the solution diffuses into the protoplasm of the odontoblast processes themselves, where it is precipitated by the proteins present.

He notes three zones of staining similar to those observed by Zander.

In speaking of the pulp reactions (he used human teeth) he says:— "These vary from complete disorganization of the odontoblast layer, round cell infiltration and pulpal haemorrhage to very slight reaction .... there might be penetration nearly to the pulp". In view of these remarks it is hard to see the logic of his conclusion which is that ".... silver nitrate applied to exposed vital teeth produces remarkably slight reaction to pulpal tissues. Due to precipitation by tissue fluids the action is self-limiting".

The pulp reactions he describes are by no means 'remarkably slight' and if, as he observes, the penetration reaches nearly to the pulp, the drug can scarcely be regarded as 'self-limiting'.

So far most of the discussion has been concerned with the efficacy or otherwise of silver nitrate as a sterilizing agent with little consideration of its effect on the pulp. In 1956,
Perreault, Massler and Schour\textsuperscript{105} investigated the reaction of the odontoblasts of rat's incisors to various chemicals. Their purpose was to describe changes in function rather than to record static histological description.

The changes can occur in:-

(1) Post-operative dentine calcification and formation.
(2) Degree of calcification.
(3) Amount of post-operative dentine.
(4) Pulpal changes.

Although the rat incisor is more permeable than the human tooth, it is also quicker growing and therefore the effects can be more readily observed. Perreault, Massler and Schour\textsuperscript{105} reported very adversely on silver nitrate. They found that it produced extensive pulpal injury in the form of haemorrhage and abscesses when applied for ten minutes, even in shallow cavities. Ammoniacal silver nitrate they found to be more destructive than the saturated solution.

In all specimens, silver nitrate quickly penetrated the entire thickness of sound dentine, and injury extended well below the line of precipitated silver. The caustic effect was not self-limiting. It was the only agent to penetrate in depth. Their conclusion was that: "Harmless substances are
ineffective, and the effective agent is harmful”.

Massler remarks that silver nitrate is not as effective as was first supposed because the silver ion is precipitated by the protein in the tubule, and this combines with the depolymerised protein present, and is thus rendered unavailable for sterilization. However, it must be remarked that in previous studies concerned with germicidal capacity (Seltzer’s being the sole exception), silver nitrate appears to be effective compared with other agents.

Englander, James and Massler examined and reported on 26 carious human teeth, aged from 17 - 21 years. They also agreed that silver nitrate was not self-limiting and caused pulp damage, though they thought that the pulp was likely to localize the injury. They found that it penetrated sound and secondary dentine, and that it was more likely to cause pulp damage under these conditions than when applied to carious dentine. They postulate that blood in the haemorrhagic regions underlying carious dentine may precipitate the silver, thus preventing further damage. They question the continued use of the drug in view of their findings.

Weiss in 1960 published an article which reviewed the effects of silver nitrate on older teeth. He used eight bicuspid
teeth from patients aged 40 - 60. He found that in every case the ammoniacal silver nitrate penetrated primary dentinal tubules almost to the pulp. In some cases, owing to the discontinuity of the primary tubules, the secondary dentine proved to be a barrier; in other cases it was penetrated. Eugenol caused only a surface precipitate of colloidal silver, which did not prevent progress of the particles towards the pulp. Effects on the pulp varied from slight disarrangement of the odontoblasts in shallow cavities to severe haemorrhage - though the pulps displayed marked defensive reaction. Weiss considers that the damage done to pulps by silver nitrate solution far exceeds that caused by gross overheating with a bur. He concludes: "The fact that walling off of the injured area occurred in every case examined is a tribute to the defensive and reparative powers of the pulp and is not considered a virtue of silver nitrate itself .... the use of silver nitrate is not conducive to the maintenance of a vital healthy pulp".

Both Weiss and Zander think that loss of tooth sensitivity is probably a result of destruction of sensory elements rather than tubule obliteration. In this article Zander revises his earlier opinion and concludes that - as the extent of bacterial invasion is unknown, as sterilizing agents may be detrimental, and remaining bacteria may be harmless, that cavity sterilization is not necessary, and may be undesirable in some cases.
Further aspects of the action of protein coagulants in general on dentine have been investigated by Burnett\textsuperscript{28}, Amler and Bevelander\textsuperscript{3}, Amler\textsuperscript{2}, and Martin\textsuperscript{87}.

Burnett\textsuperscript{28} made a study of the accessibility of both organic and inorganic components of dentine after treatment with protein coagulants. Burnett and Scherpb\textsuperscript{29} hold the view that acid decalcification liberates the organic components of dentine, making them available for proteolysis. Burnett's study was to try and determine whether the use of protein coagulants decreased the availability of these components. The agents he used were 2% silver nitrate, 2% neutral formalin, 40% zinc chloride with 1% polyoxy-alkaline sorbiton monolaurate, and 20% potassium ferrocyanide with 1% p.s.m. (This substance is a surface reducing agent.) He found that even after long treatment none of the agents used had an "overwhelming or even decisive effect" upon the accessibility of organic or inorganic components of dentine matrix. In fact, in some cases the tyrosine and histidine groups were more accessible after being treated with the chemicals, than with the distilled water control.

In 1951, Martin\textsuperscript{87} published his findings on the measurement of dentine permeability using radioactive phosphorus after treatment with various drugs. Those used were phenol, alcohol, cavity varnish, silver nitrate and eugenol, NaF\textsubscript{2}%, followed by CaCl\textsubscript{2} 2%, ZnCl 40% followed by potassium ferrocyanide 20%, and zinc phosphate
cement. After treatment with these agents, 0.02ml. of P32 was sealed in with amalgam for 24 hours.

Martin found that in only two series of the teeth was penetration of the isotope prevented by the treatments, namely, when zinc cement was used and when NaF. and CaCl₂ was used. He thinks that the latter method, i.e. the formation of a precipitate of calcium fluoride on the floor of the cavity may be an effective way of decreasing dentine permeability.

Amler² and Amler and Bevelander³ also made studies of permeability with radioactive phosphorus, but their studies extended over a longer time period than those of Martin. They also found that the drugs used actually increased permeability, and that the intensity of penetration increased with time. (See diagram on p. 86). Amler makes the following cogent remark:— "From theoretical considerations there is no logical assumption that coagulation and consequent death of living protoplasm in the dentinal tubules will decrease permeability. Considering the behaviour of living cells such as the amoeba and the red blood cells, the opposite appears to be true. Cell membranes which are highly selective during life acquire unlimited permeability upon death ....".

This point is again stressed in the further article by Amler and Bevelander³. Although they are in fact agreed upon
Summary of relationship of intensity of $P_{32}$ penetration into dentine as a function of length of time medicament was in contact with dentine:

Time interval in days.
the desirability of dentine sterilization, they doubt the ability of the commonly used drugs to accomplish this. They say:— "The indiscriminate and irrational use of medicaments is one of the most outstanding examples of mistreatment of the dental organs. Drugs such as phenol, silver nitrate, cavity varnish, alcohol etc. are used in everyday practice, in the futile empirical hope of cavity sterilization and of limiting the dentine permeability without regard to the physiological effect in teeth, as if teeth were truly 'inorganic blocks'.

It is interesting to note from the diagram on p. 86 that in this series of experiments fluoride renders the dentine less permeable over a period of time.

In these experiments with radioactive phosphorus, what is demonstrated is that dentine is rendered either more or less permeable to this particular substance. It does not necessarily follow that it is rendered likewise for other substances such as saliva and bacteria. However, such results are an indication that claims made in the past that silver nitrate and phenol render dentine impermeable, are probably without any scientific foundation.

Recent work by Perreault, Massler and Schour\textsuperscript{105}, and
Englander, James and Massler\textsuperscript{45}, as well as Weiss\textsuperscript{146}, indicates that silver nitrate is not self-limiting, has powers of penetrating both primary and secondary dentine, and of causing inflammatory responses in the pulp.

In vitro, silver nitrate has been shown to exhibit bacteriocidal, or at least, bacteriostatic properties, though the depth of effective penetration is only 0.75 mm. after a ten minute application. (Stephan, Huntz and Dorfman\textsuperscript{130}).

An estimate of pulp damage is to some extent a subjective one, what one worker considers 'severe' another may think 'mild'. Furthermore, pulps show great individuality in their response and ability to recover from injury, and there is evidence that pulps are able to recover from inflammation caused by silver nitrate.

Bearing all these considerations in mind, there does not appear to be a great deal of justification for the use of silver nitrate as a sterilizing agent.
A hydroxybenzene, colourless needle-shaped crystals, melting point 40 deg.C. Soluble in various fluids including glycerine, alcohol, fixed or volatile oils. A protoplasmic poison.

Barker found that phenol would sterilize cubes of carious dentine if applied for at least half an hour. Hardwick found it inefficient but this was after only a two minute application, and Stephan, Muntz and Dorfman also found that it was unable to penetrate carious dentine after a three minute application. Seltzer sealed phenol into teeth for one year, but found that he was still able to cultivate bacteria from the floor of the cavity even after this period. However, he used gutta percha and amalgam as his sealing agents, and there could be some doubt as to whether this would provide sufficient protection against marginal leakage. In estimating the comparative value of various medicaments he found pure liquid phenol to be 69% effective. The results he obtained in this series of experiments are noteworthy, since he reported 47% success with physiologic saline, which was put in as a control, and only 39% success with thymol in alcohol, and 28% with Howe's
silver nitrate, both of which have been recognised as efficacious disinfectants by other workers, whereas so far no one seems to have claimed this property for physiologic saline.

Thomas\textsuperscript{137} found that phenol was able to penetrate young dentine if sealed into the tooth, but was unable to penetrate secondary dentine. He found the pulp reaction to be sub-acute and noted the presence of groups of small mononuclear cells. He says that: "90% phenol will coagulate all organic matter it contacts". However, the findings of Amler\textsuperscript{2} and Martin\textsuperscript{87} regarding protoplasmic poisons have been noted in the previous section on silver nitrate, and apply equally to phenol. Perreault, Massler and Schour\textsuperscript{105} found that the action of phenol seemed limited by the dentine - which is consistent with Thomas's report - and that it had a mild and limited action on the pulp, although the reaction was more severe in deep cavities, and after being sealed in for two or three days.

Zander and Glass\textsuperscript{157} noted that Calcium Hydroxide when applied to pulp exposures produced an initial superficial necrotization prior to subsequent satisfactory healing. In view of the fact that phenol is an escharotic drug, they experimented by phenolizing pulp exposures before treatment with CaOH, but found that this procedure made no difference
one way or the other to the repair of the damaged pulp.

Takigawa in a test devised to find a suitable drug for treating teeth with vital but injured pulps found that phenol gave the greatest sedative effect clinically, but subsequent histological investigation showed that it produced greatest tissue damage, compared with eugenol, creosote and camphorated parachlorophenol. In 17.2% of teeth treated with phenol the inflammation increased. The conclusion to be drawn from these findings would appear to be that the sedative effect of phenol was probably due to the destruction of sensory elements in the pulp.

Phenol is one of the earliest known antiseptics, and in the form of carbolic acid, was used in the first crude attempts to reduce the mortality rate from septicaemia in surgery. Its merits are dubious owing to its destructive effect on living tissues. It appears that it is capable of sterilizing dentine - though to what depth is still unknown - if sealed in for a few hours, and though it does have a deleterious effect on the pulp, it is, in this respect, superior to silver nitrate. There would appear to be no point whatever in swabbing a cavity with phenol before inserting a restoration.
THYMOL.

\[
\text{CH}_3 \quad \text{CH} \quad \text{OH} \\
\text{CH} \quad \text{CH}_3 \quad \text{2}
\]

Thymol is a monohydric phenol which occurs on its own, or with carvacrol, in certain essential oils. It possesses the odour of oil of thyme in which it is found, and forms large colourless crystals. It is a mild antiseptic.

Barker\textsuperscript{7} found a solution of thymol in alcohol was an effective germicide which sterilized cubes of dentine within five minutes. Seltzer\textsuperscript{121} found it to be moderately effective, though his results are open to doubt in view of the success he obtained with his control saline solution. Perreault, Massler and Schour\textsuperscript{105} found that thymol had no significantly deleterious action on the pulp even when sealed in for three days.

Mayer\textsuperscript{90} found that a solution of 50% thymol in alcohol was an effective germicide, but his conclusions are of no value since he merely treated the surface of the dentine, and subsequently cultured a chip of dentine from the surface.
Day carried out an extensive series of tests in which he cultured carious dentine from deep cavities, left carious dentine overlying the pulp, sealed in pure thymol, cultured the centres four weeks later, and also examined some of the teeth several years later.

He also made a comparison of bactericidal efficiency between phenol and thymol. Of this he says: "Our results ... show that thymol is 23.4 times more germicidal than phenol. Phenol then is contraindicated in cavity sterilization because of its low germicidal powers .... A few crystals of thymol left in the dentine cavity will sterilize indefinitely". This is a thoroughly illogical statement. The fact that thymol has been shown to be more bactericidal than phenol does not prove either that phenol is necessarily a poor antiseptic, or that thymol will sterilize 'indefinitely'. The use of such a vague term as 'indefinitely' is unsuitable in a supposedly scientific article.

After re-culturing the carious dentine which had been sealed off for four weeks, he found it gave negative cultures. The teeth that were examined several years later were found to have successfully laid down secondary dentine, and appeared to be vital and functioning normally. Day does not say what he used
as a cavity seal, but it is interesting to note that in one tooth in which the treatment did not halt the carious process, the seal was found to be faulty.

His experiments indicate that if a carious lesion is isolated from the oral cavity, it is probable that the bacteria present will become inactivated, and that the pulp will be able to lay down secondary dentine. Also, they indicate that thymol is not inimical to the pulp. This article does not in any way prove that thymol as such is wholly responsible for the results obtained.

![Eugenol molecule](image)

**Eugenol.** A monohydric phenol.

Barker found that oil of cloves would sterilize cubes of dentine after being sealed in for a minimum of 36 hours. Hardwick gave oil of cloves a bacterial index of 30% and zinc oxide/eugenol dressing of 53%. This was, by his standards, less efficient than the salts of heavy metals, but more so than pure phenol, undiluted metaphen, and hydrogen peroxide. In fact the zinc oxide/eugenol dressing was rated as more effective than penicillin, which had a
bacterial index of 40%.

Bartels\textsuperscript{9} investigated the effect of eugenol and oil of cloves on micro-organisms in 1947. He inoculated agar with various micro-organisms at three different pH levels: 6, 7 and 8. He found that eugenol and oil of cloves showed the same effect, and that the pH levels made no difference.

He found that organisms varied in their sensitivity towards these agents, and that \textit{I. Typhi} and \textit{Monilia Albicans} showed the greatest sensitivity, but that \textit{B. Pyocyaneus} was completely resistant.

Turkheim\textsuperscript{138} did 'in vitro' tests of zinc oxide/eugenol on incubated petri dishes, and noted growth-free zones. He devised a further test to demonstrate the longevity of the bactericidal effect; by transferring the same disc of zinc oxide/eugenol from plate to plate 83 times over a period of nine months he found that, although the disc was disintegrating by this time, it was still able to produce a growth-free zone. Another disc showed a similar result with 130 transfers over a period of fourteen months.

In a further series of experiments\textsuperscript{140} Turkheim impregnated
discs of sterile decalcified dentine with micro-organisms — namely, staphylococci, L. acidophilus, escheridia coli, and monilia albicans. They were sealed into a sterile glass tube with ZnO/Eugenol for periods of 1, 3, 6, 24, 32 and 48 hours respectively. The controls were all positive, but the other samples were found to be sterile after from one to three hours. However, this encouraging result was negated when it was found in a further series of experiments with actual caries extracted from teeth, that ZnO/Eugenol had no germicidal value at all.

Turkheim was unable to account for this discrepancy, though he does suggest that it may be due to, firstly, the attenuation of the bacterial strains used through countless sub-cultures; secondly, culturing on an artificial medium.

Having failed to establish satisfactorily the germicidal potency of plain zinc oxide/eugenol cement, Turkheim then experimented with a reinforced cement. The formula of this was:

- Zinc oxide: 50 parts w/v.
- Resin: 24 "
- Asbestos: 23 "
- Merc. ammon. chloride: 2.5 "

**LIQUID:**
- Eugenol: 95 ml.
- Thymol: .5 gm.
- Cellulose acetate: qu. s.

(For clinical use 0.5% is sufficient).
He found that artificially infected dentine was rendered sterile within three hours of contact with the cement. Under the same conditions natural decay obtained from freshly extracted teeth was not sterilized until it had been in contact for at least ten and in some cases twenty hours. Nevertheless, this result was more encouraging than those of the previous series of experiments. His conclusions are a little vague. He says:

"... it should be possible to disinfect, if not even to sterilize the bottom of the cavity ... provided of course that the pulp is clinically healthy at the beginning of the operation". The bottom of the cavity is either sterile or it is not. To speak of 'disinfection' is meaningless, the word is simply a lay term for sterilization.

Turkheim has used this reinforced cement on teeth 'in vivo', first removing the soft caries, and then sealing in the dressing for two months, after which an X-ray was taken. Although he only reports on two cases, they both showed reactions to vitality tests, and secondary dentine formation two years later.

Massler is an advocate of the use of zinc oxide/eugenol dressing in deep cavities. He thinks that if the caries has destroyed more than half the thickness of dentine, it is probable that the bacteria have already reached the pulp. However, healing
and the formation of secondary dentine are, in his view, inherent capabilities of the pulp. He therefore suggests that in deep cavities, all the soft, necrotic superficial portion should be removed, and a zinc oxide/eugenol dressing sealed in for two to four weeks. This should then be removed, and any further soft dentine, which by this time will be dry and powdery, should be excavated. If a hard floor of secondary dentine has not yet been formed, this process should be repeated until a hard floor is formed in the cavity, by which stage the restoration may be inserted. This method of Massler's has the virtue of meeting the arguments of both sides - those that say that caries must be excavated until a hard floor is reached, and those who say that soft caries should be left rather than risk a pulp exposure. It would certainly seem preferable to re-excavate after a period of weeks, rather than restore over a layer of carious dentine. Apart from any other considerations, if soft dentine is left in the floor of the cavity, there is no way of knowing whether or not an exposure has actually occurred. By using Massler's method, if it should happen that the pulp has become infected beyond recovery, and incapable of laying down secondary dentine, then this fact will become manifest during the second excavation. The sealing properties of zinc oxide/eugenol will be discussed in a later section, but at this stage it may be mentioned that they have been found to be superior to zinc oxyphosphate cement.
The palliative properties of eugenol on the inflamed pulp are well known.

ANTIBIOTICS.

With the realization during recent years of the development of antibiotic-resistant strains of bacteria, and also the possibility of undesirable side-effects resulting from antibiotic treatment, the use of these agents for relatively minor conditions has become debatable.

However, articles have been published claiming that they have value in cavity sterilization, and therefore they must be included in any review on this subject.

It must be admitted that in this role they might have a certain claim to recognition, insofar as they are undeniably bacteriostatic and, provided a wide spectrum antibiotic is used, or a polyantibiotic mixture, they are fatal to a wide range of bacteria, though not to yeasts, which have been found in the carious lesion (Roth, Onisi and Nuckolls). Furthermore, they are non-irritating to tissue.

Conversely, it can be argued that when sealed into a cavity, the molecules can readily reach the pulp chamber and
thus may pass into the general circulation. It has been estimated (Burkman, Schmidt and Crowley\textsuperscript{26}) that a dentinal tubule one micron in diameter could accommodate in a flat plane, 12,500,000 molecules of antibiotic. Since only a very small quantity of a given substance is needed to sensitize a person, the possibility exists that this might lead to an allergic reaction if the patient were to be given a larger dose of antibiotic at a later date.

Burkman, Schmidt and Crowley\textsuperscript{26} made a preliminary report in 1958 of an investigation of a mixture of penicillin and camphorated parachlorphenol. They used 44 cases, the age range was 13 - 30 years, and they sealed in a stiff paste of the two substances with gutta percha. 50,000 units of soluble penicillin were used. At the first visit the carious lesions were cultured. A week later the middle layer of caries was removed and cultured, and at a third visit a week later, a further culture was taken and then the mixture was sealed in permanently.

From a bacteriological viewpoint, 75% of cases gave negative results after the third visit, and 45% gave negative results after the first visit. The implication here is that a quarter of the teeth treated contained organisms that were resistant to both drugs. From a clinical viewpoint, all but
three cases were considered successful, and the periods of observation ranged from 3 months to 1½ years. This means that of the eleven teeth in which negative cultures were not obtained, eight nevertheless survived as successfully as those in which the bacteria had been inactivated.

They made the following observations:

(1) The soft leathery dentine became hard and dehydrated.

(2) There was improved response to vitality tests.

(3) Painful teeth were more comfortable in a matter of minutes.

(4) Later there was repair of periapical pathosis.

This last observation is remarkable. It is hard to believe that a pulp which had degenerated sufficiently to cause periapical pathosis, could recover to give a response to vitality tests.

In 1960, Schmidt, Crowley, Horner and Burkman made a further study of the parachlorophenol/penicillin mixture. This time they treated 146 teeth, of which 127 or 87% became negative after 1 - 3 treatments. However, they used 15 teeth as controls in which, after removal of soft caries, and sealing for a week, 5 were found to be negative; i.e., a third of the controls became negative, without the use of any medication whatever.
Colton and Erlich remark on the dubious value of the commonly used medicaments such as phenol and silver nitrate; (Schmidt et al.\textsuperscript{112} also comment to this effect). Colton and Erlich experimented with polyantibiotics which they added to dental cements. They also compared the zones of inhibition shown on culture media by zinc cement, silicate cement, silver amalgam, direct filling resin, and cement fortified by antibiotics. They found that all the materials except direct filling resin showed areas of growth inhibition. Experimenting with a few teeth, they found that cements that had been fortified with antibiotics gave negative cultures after two weeks, but unfortified cements did not. In a later series of experiments they obtained substantially the same result.

<table>
<thead>
<tr>
<th>Lithium Cement</th>
<th>Lithium Cement + Polyantibiotic</th>
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<tr>
<td><strong>Before</strong></td>
<td><strong>After</strong></td>
</tr>
<tr>
<td>186 Pos.</td>
<td>159 Pos. (87%)</td>
</tr>
<tr>
<td>24 Neg. (Discarded)</td>
<td>27 Neg. (13%)</td>
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Neither Colton and Erlich nor Schmidt et al report on the histological picture of the pulp after the use of antibiotics (though this is to be the subject of further investigations by Schmidt and his co-workers).
The rationale behind the use of antibiotics as cavity sterilizing agents is as open to question as the use of any other medicament. If the bacteria penetrate the dentinal tubules ahead of the carious lesion, can the antibiotic molecules also penetrate the tubules and inactivate them? It has been calculated theoretically (Burkman et al.26) that they can, but in the experiments quoted, no evidence of depth of penetration was offered. The dentine cultured prior to treatment was from a more superficial part of the cavity than that used in subsequent cultures, and therefore it is arguable that the deeper dentine might have been sterile prior to the use of the antibiotics, since the evidence that deeper layers of carious dentine are sterile is as convincing as the evidence that it is not.

Burkman et al.26 list the following factors as determining success or failure in treatment:

(1) A good blood supply.
(2) The size of the periapical opening.
(3) Freedom from trauma.
(4) Favourable blood picture.

Given that these four factors are present - especially the last one - then with the removal of gross caries and the sealing of the cavity, it seems probable that the pulp will recover, and
that the use of antibiotics is unnecessary, as well as being undesirable for the reasons mentioned earlier on pp. 99-100.

ZINC FERROCYANIDE.

This is not a very commonly used drug, but its use has been advocated for the impregnation of the dentine of deep carious cavities by Müller and Maeglin. 96

They used a solution of 20% $K_4 Fe(CN)_6$ and a solution of 40% $ZnCl_2$.

The reaction is: $2ZnCl_2 + K_4 Fe(CN)_6 = 4KCl + Zn_2Fe(CN)_6$

They say that the zinc ferrocyanide thus formed causes an efficient impregnation of the dentinal tubules which cannot be penetrated by silver nitrate solution. The dentine becomes desensitized shortly afterwards but recovers its sensitivity in a few days' time. "In vitro tests show zinc ferrocyanide to be a precipitate soluble in excess serum, and it is a disinfectant. It is harmless to the pulp and encourages secondary dentine".
The technique of using this substance consists of first removing all discoloured and softened dentine, then introducing the zinc chloride into the cavity and leaving it for about a minute, after which the warmed potassium ferrocyanide is placed and a precipitate is formed.

Barnett\textsuperscript{28} included this drug in investigating the effect of protein coagulants on the organic and inorganic components of dentine. He found that it had no effect on dentine in this regard. Martin\textsuperscript{87} found that it did not prevent the penetration of radioactive phosphorus into dentine. It can only be said therefore, that such work as has been done on this agent does not substantiate the claims made for it by Muller and Maeglin\textsuperscript{96}. From a purely practical viewpoint it might be said that the average practitioner would be unwise to introduce such a highly toxic substance as potassium ferrocyanide into the mouth as routine procedure.

\textbf{CALCIUM FLUORIDE.}

A more satisfactory method of rendering the dentine impermeable appears to be the one advocated by Martin\textsuperscript{87}. The solutions used are 2\% NaF followed by 2\% CaCl\textsubscript{2}. The sodium
fluoride is applied for one minute to the floor of the cavity, followed by the calcium chloride; a precipitate of insoluble calcium fluoride results. Martin, in his experiments with radioactive phosphorus found that this precipitate and zinc phosphate cement were the only two substances that prevented penetration of the $P_{32}$ isotope into dentine. His results are in agreement with those of Amler and Bevelander$^3$. (See diagram p. 136.)

It should be borne in mind however, that Going, Massler and Dute$^{56}$ found $P_{32}$ to have the least penetrating ability of five isotopes tested. (See p. 119).

Burnett$^{27}$, in an investigation of the effects of fluoride on the organic and inorganic components of dentine, found the reactions to be more complex than were hitherto supposed. He found that sodium fluoride was most consistent in depressing the solubility of the inorganic components of dentine, and calcium fluoride the least effective. He found that calcium and potassium monofluorophosphate depressed inorganic solubility significantly.

The evidence reviewed so far suggests that the use of some form of fluoride solution is likely to be far more effective in reducing dentine permeability than any form of protein coagulant.
CALCIUM HYDROXIDE.

The use of calcium hydroxide in direct pulp capping has been common practice for many years. Recently it has been suggested that this compound is valuable in 'indirect' pulp capping. Sowden was of the opinion that calcium hydroxide was able to recalcify carious dentine. His investigations were extensive, he treated 4,000 cases over an observation period of seven years, though he does not make clear in his article whether each case was individually observed over this period, or whether this was the total number of cases observed during the seven years. The teeth studied were in an age range of 2 - 79 years.

The teeth selected appeared to be in need of either pulp capping or vital pulpotomy. The carious area was removed leaving soft caries over the pulp, enabling a layer of calcium hydroxide and an alloy restoration to be placed. Post-operative radiographs were taken, and seven to nine days later a further radiograph was taken. Two or three weeks later a periapical radiograph was taken, the restoration removed together with any remaining soft material. A permanent restoration with a zinc oxide base was then placed.
He had ten failures in the primary dentition which he attributed to fracture of the restoration or pulp exposure. Three failures in the adult dentition he found were due to fracture of the restoration.

His conclusions are non-committal: "How far one can go in this treatment and cause the reconstruction of the carious dentine and still retain a vital pulp is not known ....". There is also a marked inconsistency in his conclusions. He says: "The general health of the patient seems to have a direct bearing on the success or failure of treatment; for example, diabetics seem to be poor risks". But he has made no mention in his report of any failures due to other than fractured restorations, of which there were three. If he has had failures due to poor general health, they were not mentioned in the main part of the article.

Law and Lewis\textsuperscript{79} conducted a similar investigation. They used 66 deciduous and young permanent teeth, and followed up their investigations at periods of seven days to twenty-eight months. The teeth appeared radiographically to be in danger of pulp exposure. Some of the soft caries was removed and a layer of calcium hydroxide paste was placed over the remaining caries followed by soft amalgam. X-rays taken as early as seven days later showed a radio opaque area formed on the pulpal
side of the residual caries. Six months later the filling was removed. The criteria of normality were:

(1) A firm layer of dentine at the base of the cavity after the excavation of remaining caries.

(2) No pulp exposure.

(3) No pathologic condition showing on radiograph of tooth and surrounding tissues. Further radiographs were taken 12 and 24 months later.

The results were as follows:—6 months later, 57 of the original 66 teeth were opened, and of these, 44 (76%) were satisfactory. One year and two years later, clinical and x-ray examination showed no failures in those previously considered successful. 38 teeth were deciduous and 20 permanent.

A histological examination of one tooth not included in the total showed secondary dentine and predentine to be formed on the pulpal surface of the occlusal dentine. There was an active odontoblast layer and a zone of Weil.

Law and Lewis\textsuperscript{79} consider this an indication that the repair process is due to the formation of secondary and new primary dentine rather than to the recalcification of carious dentine.

They considered the failures to be due to pulp degeneration.
already present prior to treatment, and to mechanical failure of
the soft amalgam filling, which might also be due to insufficient
support for it on the calcium hydroxide base.

Klein\textsuperscript{78} made a study of the relationship between the use of
calcium hydroxide as a base material and subsequent sclerosis of
the underlying dentine. The aim of his study was slightly
different from that of Sowden\textsuperscript{127} and Law and Lewis\textsuperscript{79}, since he
was concerned not with the treatment of near-exposures, but with
the possibility of reducing the permeability of dentine beneath
restorations by using a calcium hydroxide/methylcellulose base.

He used this base in 191 deciduous teeth, and restored
160 deciduous teeth with no base beneath the alloy. By X-ray
examination he found that 98\% of the teeth containing the base
showed evidence of dentine sclerosis. Only 1\% of the deciduous
teeth without the base showed evidence of sclerosis.

Klein considers that Sowden\textsuperscript{127} is wrong in thinking that
the carious dentine is recalcified; he thinks that sclerosis
of existing dentine takes place as a response to pulp injury.
In Klein's study, all the carious dentine was removed before
the base was placed, but nevertheless there was a marked
increase in radio opacity observable beneath the restoration.
He used instrumental densinometric evaluation to determine his results, and from this he estimated that the increase of calcification of dentine beneath a calcium hydroxide base may be as high as 125% in some cases. He says: "In this study, the presence of calcium hydroxide material is consistently associated with roentgenographic evidence of sclerotic dentine, about which there is no error .... The data therefore shows sufficient evidence, considering all factors, to support the belief that calcium hydroxide base material clinically affects and enhances the production of sclerotic dentine".

Mjør, Quigley and Finn25 conducted a study to determine the effect of calcium hydroxide and amalgam on non-carious young dentine. They prepared and filled twenty cavities, and they found that the calcium hydroxide-covered dentine showed a significant increase in microhardness as compared with unaffected dentine in the same tooth, and with dentine in unoperated teeth and under amalgam-only fillings. These were micro-radiographic and densinometric studies.

There appears to be a certain amount of evidence available to indicate that calcium hydroxide has the ability of inducing some form of increased calcification in dentine. This property is valuable in a material to be used as a base beneath restorations, and also in those cases where it is desired to induce a layer of secondary dentine to form over the pulp beneath a deep cavity - the so-called 'indirect pulp capping'.
Miscellaneous Agents.

Some other agents which have been used in the treatment of the floor of the carious cavity do not appear to have received the detailed attention of investigators in this field. Seltzer\textsuperscript{120} carried out experiments using the following medicaments: 70\% ethyl alcohol; 10\% aq. sodium carbonate; phenolate; hexylresorcinol; beechwood creosote; zephiran; metaphen. He used 20 controls and 140 test cavities, of which 55\% appeared to remain positive despite treatment. Of all the agents tested, he found that phenolate (isomeric chlorphenolate) was the most effective, and that after a week's treatment the ratio of positive to negative cavities was 3:17. Metaphen was the next most efficient, and then hexylresorcinol. None of the experiments considered the effect of the medicaments on the pulp.

Adolph\textsuperscript{1} made an electronmicroscopic evaluation of the influence of certain drugs on the dentine of vital bicuspid. The drugs used were: Methylene chloride; 95\% alcohol; 3\% $\text{H}_2\text{O}_2$; 2.5\% chloramine; 5\% iodine alcohol; bismuth phenolate; menthol. He found that all these drugs exerted what he termed an 'unfavourable' influence on dentine, especially observable in the dentinal tubules containing the odontoblast fibrils, and in the surrounding matrix.
If the preservation of the vitality of the pulp is one of the objectives in restoring the carious lesion, then the arbitrary use of drugs whose effect on the dentine and odontoblasts is unknown cannot be regarded as a recommended procedure.
SECTION IV

MARGINAL PERCOLATION.

Introductory.

The subject of cavity sterilization would be incomplete without some thought being given to the question of marginal percolation of restorations. Firstly, because it is of little value to sterilize the base of the cavity before restoring it, if, after restoration, bacteria from the oral fluids can seep in. Secondly, because some workers believe that if the bacteria can be sealed away from the environment they will then die out beneath the restoration. The question then arises - to what extent can any restoration be said to provide a hermetic seal?

Experimental Work Using Dyes and Radioactive Isotopes.

There are two main methods of investigation commonly in use. Dyes and radioactive isotopes. The use of these latter substances is more exacting than the use of dyes, and recent investigations tend to show that substances hitherto regarded as having good sealing properties - such as zinc oxide/eugenol cement - are not in fact particularly satisfactory in this respect (Goings & Massler\textsuperscript{54}).
This leads to the question of cavity bases and liners, for should it prove difficult, if not impossible, to manipulate the commonly used restorative materials so that they themselves provide marginal seal, then it becomes desirable to treat the cavity floor in such a way as to decrease the permeability of the dentine, and prevent bacteria from entering and multiplying in the dentinal tubules. Some of the substances which have been used with this objective have been discussed in the previous section. In this section it is proposed to review some of the work that has been done on cavity liners and bases, and on restorative materials.

Some very extensive investigations in this field have been carried out by Going and Massler\textsuperscript{54} and by Going, Massler and Dute\textsuperscript{55, 56}. In their first study\textsuperscript{55} they investigated the marginal penetration of restorations, by using crystal violet dye and radioactive iodine - I\textsuperscript{131}. They used both old and new restorations in 316 extracted teeth, from patients between 26 and 79 years, the average age being 45. They used Class V cavities, because it was easier to get marginal integrity for the restoration, and also because there was less sclerotic dentine beneath these. They found that the presence of sclerosis in dentine definitely lessened permeability.

Their results were as follows: (See diagram p. 116). All restorations showed some degree of penetration with I\textsuperscript{131}, but in the case of gold foil, copper amalgam and red copper
Depths of marginal penetration around different filling materials. (After Going et al).

0 = No marginal penetration.
1 = Superficial penetration.
2 = Penetration of dye or isotope to floor of cavity.
3 = Penetration of dye or isotope all round the filling including the floor of the cavity.
4 = Diffusion of dye or isotope partway into the dentine.
5 = Diffusion of dye or isotope completely through dentine and into the pulp.
cement, the isotope penetrated to less than half the depth of the incisal and gingival margins. With gold inlays, it penetrated to the floor of the cavity, and with silver amalgam, zinc oxide/eugenol cement and temporary stopping (gutta percha + zinc oxide/eugenol) it penetrated into the underlying dentine.

Under zinc phosphate cement and acrylic restorations, both dye and isotope penetrated to the pulp, which was the same result as was obtained with the control open cavities.

In discussing these results, they consider that several factors are involved in the degree of marginal penetration. These are:

1. The condition of the enamel:
   Where there was stainable plaque present permeability was greater, indicating that the integrity of the enamel has been affected making it more porous.

2. Dimensional changes in the materials:
   (a) Changes brought about by temperature variation, due to a difference between the co-efficient of thermal expansion of the tooth structure and the material.
   (b) Setting changes: Gold foils and inlays are not subject to these.
(3) Chemical composition of the material:

It is possible that the fluoride in silicates penetrates adjacent tooth structure, and makes it less permeable over a period of time. The two copper-containing materials (cement and amalgam) showed significantly less marginal percolation. It is suggested that copper forms complexes that bind the $^{131}$I. The negatively charged radioactive iodine accumulated on the surface of all the metallic restorations.

They found that although teeth with very old amalgams showed blackening of the underlying dentine, there was relatively little penetration of the isotope into the dentine. Recurrent caries was always associated with deep penetration.

Old gold inlays showed very little marginal penetration, and silicates a year old showed less than new ones.

It is interesting to note, that in the case of the metal restorations and zinc oxide/eugenol, the dye gives good results, but the isotope shows much greater penetration of the inlay, silver amalgam and zno/eugenol.
In a later study, Going, Massler and Dute investigated the results of using different isotopes to study marginal percolation. As a result they concluded that the ionic charge and chemical reactivity of the ion, as well as the physical and chemical nature of the restorative material, influences the depth of marginal penetration of the isotope.

The isotopes used were:

- $^{35}\text{S}$ as $\text{Na}_2^{35}\text{O}_4$.
- $^{32}\text{P}$ as $\text{Na}_3^{32}\text{O}_4$.
- $^{22}\text{Na}$ as $\text{Na}^{33}\text{Cl}$.
- $^{86}\text{Rb}$ as $\text{Rb}^{86}\text{Cl}$.
- $^{45}\text{Ca}$ as $\text{Ca}^{45}\text{Cl}$.

**Results:** (24 hours immersion).

1. **Unfilled cavities:** $^{35}\text{S}$, $^{22}\text{Na}$, $^{86}\text{Rb}$, $^{45}\text{Ca}$ penetrated freshly cut dentine to pulp chamber.

2. **Silver amalgam:** $^{35}\text{S}$, $^{45}\text{Ca}$, $^{86}\text{Rb}$ showed penetration around margins to pulp chamber. $^{32}\text{P}$ penetration very superficial.

3. **Silicate cement:** $^{22}\text{Na}$ penetrated through dentine and in most cases to pulp. $^{35}\text{S}$ penetrated to floor of cavity but not into underlying dentine. $^{45}\text{Ca}$ showed even less penetration.

4. **Acrylic resin:** $^{22}\text{Na}$ and $^{45}\text{Ca}$ showed penetration through filling margins and dentine to pulp chambers. $^{86}\text{Rb}$ penetrated into dentine but not pulp chamber. $^{35}\text{S}$ penetrated margins but not underlying dentine.
(5) **Zinc oxide/eugenol**: Na$^{22}$ penetrated margins and passed through dentine to pulp chamber. Three teeth filled with a quicker setting mix showed reduced penetration, but even this with Rb$^{86}$ showed penetration to pulp chamber. Ca$^{45}$ reached the pulp chamber but S$^{35}$ only to the dentine.

(6) **Zn PO$_4$ cement**: Penetration by Na$^{22}$ and S$^{35}$ through dentine to pulp chamber. F$^{32}$ very superficial penetration.

(7) **Gold foil and copper amalgam**: Penetration of Na$^{22}$ to pulp. This is in contrast with the good results previously obtained with radioactive iodine$^{55}$.

(8) **Gold inlay**: Much less penetration than with any other restoration. Na$^{22}$ penetrated margins but stopped at floor of cavity.

It can be seen from these results that the only restoration which resisted all isotopes was the gold inlay, and that the penetrating power of radioactive sodium is such that it is able to reach the pulp of all the other restorations. F$^{32}$ was the least effective isotope. S$^{32}$ and Ca$^{45}$ showed deep penetration and also produced the clearest autoradiographs. Rb$^{86}$ gave autoradiographs of insufficient clarity to be of any value.

In an attempt to find a method of preventing this marginal
leakage into the underlying dentine, Going and Massler investigated the influence of cavity liners under amalgam restorations. They listed the qualities of an ideal liner as follows:

1. A thermal insulator.
2. A galvanic insulator.
3. Prevents ingress of mercury into dentine.
4. Reduces marginal penetration and diffusion into pulp.

They used Class V cavities, cut on 234 newly extracted teeth from patients in an age range of 23 - 68 years. They found that radiosodium penetrated deeply and made most liners look poor, but radiocalcium did not penetrate as efficiently and therefore made the liners look efficient. In the unfilled control cavities all the tracers penetrated the dentine and into the pulp chamber within the 24 hour immersion period.

Results:

1. Copalite varnish and Mizzy Poly liner
   (Polystyrene 70%, ethyl cellulose 27%, staybellite ester 3%, betanaphtol in chloroform and xylene).
   These decreased penetration through the margins of amalgam restorations and completely blocked off the penetration of isotopes into dentine.
(2) The Calcium Hydroxide liners (Chembar, Pulpdent paste and Pulpdent liquid) decreased markedly the penetration of radioactive tracers into the underlying dentine but concentrated the isotopes within the liners to a marked degree. Marginal penetration was not affected and may have been increased.

(3) Zinc Oxide/eugenol decreased penetration into dentine slightly but had little or no effect on marginal penetration.

(4) Zinc phosphate cement increased penetration of isotopes into underlying dentine, and slightly increased marginal penetration.

Their conclusions were that the most effective liner under amalgam was copal varnish, but that it was ineffective under silicate. Calcium hydroxide liners were effective under silicates, but ineffective as galvanic insulators. Zinc oxide/eugenol was good as a thermal and galvanic insulator but not as an ionic barrier. Polystyrene ethyl cellulose liners were effective. Zinc phosphate cement was not only ineffective, but irritated the pulp and by etching the under surface of the amalgam increased the susceptibility of the dentine to permeation.

Swartz and Phillips\textsuperscript{132} also tested the permeability of cavity liners, using over 600 teeth. The liners were tested
with phosphoric acid, with sodium fluoride and with acetic acid, the latter representing organic acids such as are found in the mouth. Their results confirmed those of Going and Massler\textsuperscript{54} that cavity varnishes do afford some protection to the dentine.

In carrying out 'in vitro' studies on the marginal leakage of restorative materials, using radioactive calcium, Swartz and Phillips\textsuperscript{133} found considerable variation in the adaptation of amalgam, but again they found that the use of copal varnish improved the sealing properties of this material. They observed penetration all round the margin of silicates, and considerable ionic absorption into the substance of the material itself.

A further series of 'in vivo' tests were performed by Phillips, Gilmore, Swartz and Schenker\textsuperscript{106} in which they used about 200 teeth, both dog and human. The work on human teeth confirmed their studies with dogs, in which they found that the margins of fresh amalgam restorations were completely permeated in 48 hours, but that the permeability was less marked after two months and even less after six months. Again they found that cavity varnish gave improved results.

With silicates they found that there was gross penetration at the margins which did not change with time. They also found that zinc phosphate cement showed less leakage than silicate.
Crawford and Larsen tested gold and amalgam fillings which had been in place for some years and found that \( \text{Ca}^{45} \) penetrated the margins; they concluded from this that ageing does not improve marginal seal. However, as they had no controls or new fillings for comparison, their conclusions lack validity.

Cantwell et al. using \( \text{C}^{14} \) labelled fructose, was able to demonstrate leakage round the margins of amalgam restorations, and Wainwright et al obtained a similar result using \( \text{I}^{131} \) labelled human serum albumin. He found that there was penetration around the restoration but that the leakage was less with the albumin than with the plain radiiodine. These results indicate that although the larger molecules are able to penetrate the margins of restorations, particle size may be a factor in limiting such penetration.

Taylor, Stowell et al. found that gold foil restorations gave better results than amalgam in regard to marginal penetration. With one exception the penetration of gold foil restorations was confined to the margins and to the body of the restoration, but did not penetrate the underlying dentine. It is difficult to see why this should be so. One possible explanation is that under the stress of the packing of the foil, the dentine produces a defence reaction of sclerosis, and it is this, rather than any property of the gold foil itself which limits the penetration.
Another possible explanation is that the method of heavily condensing the foil piece by piece into the cavity ensures very intimate contact between the material and the cavity floor which limits the penetration.

Nelsen, Woolcott and Paffenbarger\(^{97}\) have discussed the mechanism of fluid exchange at the margin. One reason is the difference in the co-efficient of thermal expansion between the tooth and the restorative material. Also, the thermal expansion of the fluid in the minute defects between tooth/restoration.

<table>
<thead>
<tr>
<th>Material</th>
<th>Linear Coefficient of Expansion ((\text{Mm/\text{Mm}} /\text{Deg. Cent. x} 10^{-6}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tooth (Across crown)</td>
<td>11.4</td>
</tr>
<tr>
<td>Silicate cement</td>
<td>7.6</td>
</tr>
<tr>
<td>Dental amalgam (min.)</td>
<td>22.1</td>
</tr>
<tr>
<td>Dental amalgam (max.)</td>
<td>28.0</td>
</tr>
<tr>
<td>Acrylic resin</td>
<td>31.0</td>
</tr>
<tr>
<td>Cast gold</td>
<td>14.0</td>
</tr>
</tbody>
</table>

From this table it can be seen that an amalgam restoration will expand or contract at least twice as much per degree change in temperature as the tooth structure, and\(^{\text{an}}\) acrylic resin restoration nearly eight times. Theoretically, a space 10 microns
wide could develop between the edge of an acrylic restoration and the tooth margin. In comparison with this a lactobacillus is two microns in diameter, and indeed the average diameter of a micro-organism is in the region of 1 - 2 microns. So that even with the less expandable restorations, there is every possibility of bacteria entering between the tooth and the material.

As well as differences in the coefficient of thermal expansion, fluid exchange may also be caused by capillarity, dialysis, diffusion, and changes in hydraulic gas pressure.

Nelsen and his co-workers suggest as one reason for the lesser marginal leakage of old amalgams, that compounds forming under plaques under uncleansed margins may corrode the amalgam, and that these corrosion products could then act as both a mechanical and a bacteriostatic plug.

Menegale, Swartz and Phillips investigated the adaptation of restorative materials as influenced by the roughness of cavity walls. They cut cavities with cavitron, which gave the smoothest finish, a 588 tungsten carbide bur at 225,000 r.p.m. which gave a medium finish, and the same bur at 5,000 r.p.m. which gave the roughest finish. Their results showed that with a given material, leakage was always more with a smooth rather
than a rough cavity. They estimated that leakage around amalgams in smooth cavities was a third more than in rough ones. Silicates produced twice as much leakage and gold foil a quarter as much again in the smooth cavities. They suggest that failure to control cavity surface may be one reason for the deviations found in results in work on marginal percolation.

It seems fairly well established that present day techniques in restorative dentistry do not as yet produce a restoration which hermetically seals the cavity. It has been pointed out (Phillips et al.\textsuperscript{106}) that the exact clinical significance of tracer studies remains unknown, and that work with larger molecules is needed. On the other hand, there are substances present in ionic form in the saliva, as well as in the larger molecular form.

Certainly the radioactive-tracer studies are far more stringent than the older dye studies. Two of these dye studies, one by Grossman\textsuperscript{59} and one by Massler and Ostrovsky\textsuperscript{89} indicated that substances such as zinc oxide/eugenol, a double layer of gutta percha, and amalgam, have good sealing qualities. This has been thoroughly refuted by tracer studies, which show amalgam in particular to have very poor marginal sealing qualities, especially in the fresh state. However, even in the
limited dye studies, self-curing acrylic, zinc phosphate cement and silicate cement were shown to have poor marginal seal. Tracer studies indicate that gold restorations, both foil and inlay, give superior results in this respect to any other material.

Hatt, after investigating the relationship of amalgam to the cavity wall, concluded that complete apposition of the material to the wall cannot be achieved, irrespective of the technique adopted for the manipulation of the material.

A comment by Crawford and Larsen appropriately sums up the situation as it exists at present: "Speculation on these findings would naturally lead one to wonder why fillings perform as well as they do, when it is obvious that solutions penetrate between the fillings and the teeth quite easily".
SUMMARY AND CONCLUSIONS.

At the beginning of this review certain questions were asked regarding the treatment of the floor of the carious cavity. These questions were:

Do bacteria penetrate the dentine ahead of the softening process, or are the last layers of softened dentine sterile?

If bacteria do in fact penetrate ahead of the softened dentine, are there any methods available for inactivating them which will not cause injury to the pulp?

What effect does marginal leakage of restorations have on attempts to achieve cavity sterilization?

An endeavour has been made to review, albeit briefly, some of the work that has been done regarding these subjects. It does not purport to answer these questions, for indeed it would appear that no definite answer exists. All that can be said is that certain probabilities become apparent, that some methods appear to be successful, and that some other methods and techniques do not seem to have much justification.
Firstly, the normal structure of dentine was discussed. It is now well established that the 'perifibrillar space of Fish', said to exist between the dentine matrix and the odontoblast process, is an artifact of staining. Under the electron microscope it becomes apparent that the odontoblast process is surrounded by a highly calcified matrix, the so-called 'peritubular dentine'. The significance of this is simply that it is no longer valid to think of this space as a pathway through which bacteria invade the dentine. Further, it is nowadays realized that dentine cannot be thought of except as intimately connected with the pulp, and it is therefore a much more vital and biological substance than was hitherto supposed.

Secondly, it is now recognized that although dentine is a highly permeable substance, the pulp has an excellent reparative potential, and as a result both of age and of peripheral injury to the odontoblasts, it can produce sclerosis of dentine and secondary dentine, which reduces its permeability and forms an excellent protection for the pulp.

The question of bacterial penetration of the dentine is controversial. The evidence suggests that in a majority of cases acid decalcification precedes proteolysis, and that the odontoblast process becomes a pathway for the advancing bacteria.
by reason of preliminary decalcification of the peritubular dentine. This evidence however is not so incontrovertible as to suggest that bacteria are never found ahead of the main carious lesion, and it is still not possible to say definitely that the last layers of softened dentine are always sterile.

The identity of the organisms involved in dentinal caries is debatable. Many forms have been found by different workers in this field, and there is no clear agreement. One probable reason for this is that it is difficult to isolate the lytic organisms from those that are merely contaminants from the oral cavity. It can only be said that any medicament claiming to be a cavity sterilant needs to be lethal to a very wide range of bacteria, including the actinomycetes.

A number of the medicaments advocated for cavity sterilization were considered, and in general it was found that claims for their efficacy were not substantially supported. In some cases, no proof was offered as to depth of sterilization achieved, and where depth of penetration occurred, as in the case of silver nitrate, damage to the pulp was fairly clearly indicated as a result. Experiments were reviewed which demonstrated that the protoplasmic poisons - used because they were supposed to reduce dentine permeability - in fact tended to increase such permeability.
There was some evidence that fluoride solutions, in particular precipitated calcium fluoride, decreased dentine permeability, and also that calcium hydroxide increased the radio opacity, and therefore presumably the hardness of dentine, quite apart from its use in pulp capping.

A more interesting field of speculation was that of the treatment of deep caries by 'indirect pulp capping', that is, leaving softened dentine over a potential exposure, and sealing the cavity with zinc oxide/eugenol. Advocates of this method are of the opinion that if the bacteria of the carious lesion are isolated from the oral environment, they will die out, or at least become inactive, and the pulp will be able to lay down a protective layer of secondary dentine. There is quite a lot of convincing evidence that this will in fact happen, though whether it will take place once the pulp has been actually exposed by caries is still open to question.

Finally, the problem of marginal percolation of restorations was brought under review. Work in this field has shown clearly that radioactive ions used as tracers are able to penetrate not only the margins of restorations but also the underlying dentine, and sometimes even reach the pulp. It was suggested that there is a need for some kind of protective layer beneath restorations, which is certainly not provided by zinc phosphate cement, and apparently not even by zinc oxide/eugenol compounds. Copal varnish
retarded penetration of the isotopes beneath amalgam restorations.

Despite the evidence of the tracer studies, it is apparent that a large number of restorations, properly executed, are successful, and the significance of these studies from a clinical viewpoint is not yet established. It may be that the viscosity of saliva retards the penetration of ions within the mouth, or it may be that the use of dyes, with their larger molecules, though seemingly less rigorous as a test than radioisotopes, give a truer picture of marginal percolation as it occurs in vivo.

Certain conclusions appear to follow from the foregoing observations. Firstly, nothing is to be gained, whether the cavity is deep or moderate, by swabbing the floor with phenol, silver nitrate, creosote or any such agents.

Secondly, the object of any treatment of the floor of the cavity should be to help the pulp produce its own defence, which it does by laying down reparative dentine. Where the restoration can be based on sound hard dentine, as in a shallow or medium depth cavity, and the carious process has not seriously encroached upon the dentine, some form of sedative layer as a thermal insulation is probably a wise precaution.

The evidence provided by tracer studies suggests that some method of rendering the dentine impermeable before inserting any
restoration would be advisable, and to some extent the use of
copal varnish beneath amalgam restorations can be justified.

The treatment of the very deep carious cavity provides the
most interesting and controversial topic. The question is, whether
or not to remove the last layer of softened dentine, especially
when there is risk of an exposure. There are several authorities
who are of the opinion that it is better to seal in the softened
dentine and give the pulp a chance to lay down secondary dentine,
than to excavate all caries, and expose the pulp. There are good
reasons for thinking they are right, and they are as follows:—

(1) The presumption that every pulp beneath a large
carious lesion is grossly infected is not necessarily
correct.

(2) There is a lot of evidence to suggest that if the
cavity is sealed off with zinc oxide/eugenol, prefer-
ably with a sub-base of calcium hydroxide, the carious
process will cease.

(3) Given that the pulp is uninfected and in a healthy
state, it has been shown to have reparative powers,
and will be able to produce a calcified barrier once
the caries has been inactivated.
(4) In young multi-rooted teeth the alternative to this treatment is generally pulpotomy - pulp capping not being recommended for carious exposures. There is no guarantee that the pulpotomy will be any more successful than the 'indirect pulp capping'.

(5) If pulpotomy is contra indicated, and pulpectomy is not feasible, then the alternative is extraction, and it would appear that nothing is lost by trying to save the tooth by the method advocated.

The disadvantage of the method lies in the fact that until the soft dentine has been fully excavated, it is not possible to see whether an exposure exists, and if so, how large it is. It is of course unrealistic to expect any success from this method where there is a gross carious exposure of long standing, and a dead or dying pulp. However, X-rays are of assistance here, and these plus clinical experience must guide the operator.

There is one practice which is open to severe criticism, that is, of excavating the caries down to a thin layer of dentine overlying the pulp, and then deliberately piercing this with the tip of a probe to get an exposure, especially if the sequel to this is to condemn the tooth to extraction. The procedure in these cases is to place a layer of calcium hydroxide, fill the
cavity with quick setting zinc oxide/eugenol, and, provided no
pain is experienced, leave the tooth for a few months and then
X-ray it. If its condition appears satisfactory both radio-
graphically and in response to vitality tests, it may then be
restored.

Finally, it may be reasonably argued that, as tracer
studies show that all restorations are highly permeable, it is
impossible, with the restorative materials at present available,
to seal off the carious lesion, and that any attempts to do so
are doomed to failure.

It can only be repeated that well executed restorations
function far better than they have any right to do according to
the radioactive ion method of investigation, and that the sig-
nificance of these studies is still uncertain.
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