WOUND HEALING IN THE DENTAL PULP: A CLINICAL STUDY

AND AN EXPERIMENTAL INVESTIGATION USING

POTENTIOMETRIC TECHNIQUES

by

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Abstract

A review of the literature dealing with wound healing in general, and pulp wound healing in particular, is presented in the first section of the thesis. It is noted that current knowledge of wound healing has not advanced far beyond the descriptive, and a large speculative literature exists relating to the mediators of both the inflammatory and proliferative phases. Wound healing in the pulp follows the general pattern of wound healing in other connective tissues. Normal function of the capillary-fibroblast system is essential and an optimal relationship seems to exist between the inflammatory and proliferative phases. Undoubtedly the most important recent advance is the understanding of oxygen requirements in wound healing. Tissue oxygen tensions are believed to govern cellular differentiation, proliferation, mitochondrial calcium loading and cell function. The use of micro-electrodes for the measurement of pO_2 in pulp wounds may support the contention that pulp dressing materials create an appropriate environment rather than actively stimulate healing.

In the second section, clinical trials are described, the object of which was to assess wound healing in the human pulp following pulpotomy and to assess the efficacy of three different pulp capping agents. All teeth were treated during the routine provision of dental care in general practice by one dentist. Large numbers of patients were assessed over periods ranging from six months to five years using two direct inspection criteria not previously used in combination. The results were statistically analysed. Following pulpotomy, attempts at healing occur in all

age groups and in conjunction with all three dressing materials. Very young teeth, erupted less than 18 months, responded less well as assessed by the two direct inspection criteria at a six month post-operative period. The addition of a proprietary cortico-steroid preparation to Calnex improves healing, especially in the very young age group. Bridging was noted to continue beyond a six month period. A reduction in vitality was observed in spite of complete bridge formation in many cases. Neither complete bridge formation or lack of symptoms can be taken as a guarantee of mainten-ance of pulp vitality and emphasises the need for long term monitoring following pulpotomy. The investigation suggests that pulpotomy is especially useful in the treatment of the young apprehensive patient who presents for the first time with a painful carious lesion.

In the third section, a series of potentiometric experiments are described which allowed the quantitative investigation of the permselectivity of human dentine to ionic transport. The results are presented and interpreted in the light of ion-exchange theory. They suggest that dentine acts as an ion-exchange membrane. Potentiometric measurements at different pH values indicate that dentine is amphoteric and possesses a net negative charge at physiological pH. An approximate value for the iso-electric point of dentine was calculated and was found to correspond with the pH of the base of deep carious lesions. A correlation of fixed negative charges, as determined electrometrically, with values calculated from chemical analysis, suggests that the glycoaminoglycan content of dentine may be responsible for conferring the major part

of the active net negative charge.   The clinical implications following from these results are discussed and it is suggested that there is a physiological closing of dentine tubules by an organic barrier in functional teeth, prior to the onset of caries and that this barrier is located in the zone about 1mm from the pulpo-dentinal interface.

Statement of the problem

The treatment of the deep carious lesion is one of the most common and difficult problems encountered in general dental practice. Two important parameters need to be considered in relationship to the carious lesion and its treatment, i.e. the dentinal reactions and the pulpal reactions. The permeability and protective properties of dentine at the base of the deep carious cavity will influence the extent of pulp pathology. The treatment procedures used should depend on the proper diagnosis of the existing pulp conditions.

The present investigations were undertaken to assess the disputed efficacy of the pulpotomy technique in the treatment of chronic partial pulpitis and to conduct preliminary quantitative potentiometric investigations into dentine permeability in unerupted, intact and carious teeth.

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SECTION I

SURVEY OF THE LITERATURE

A.   WOUND HEALING: GENERAL CONSIDERATIONS

It has long been observed that tissues are restored to their
anatomical and functional state through a biological sequence of
events.   Arey (1936) gave a comprehensive survey of early investi-
gations.   Edwards & Dunphy (1958) reviewed the mechanisms of healing.
Numerous papers detail the sequential events of healing as shown by
both light and electron microscopic studies (Johnson & McMinn, 1960;
Washburn, 1960;   Ross & Benditt, 1961, 1962a,b;   Ross, 1964, 1968,
1969).

Schilling (1968) presented a review of information on wound
healing that had occurred since Arey's classic review.   He stated
that it was important, ultimately, to know all the precise mechanisms
of wound healing.   He defined a wound as a disruption of the
anatomical and functional continuity of living tissue.   Wound
healing is the restoration of functional continuity of living
tissue.

Hunt (1970) and Forrester (1976) summarised the latest develop-
ments to date in wound healing research.   Hunt (1970, 1973) stated
injured tissues are restored through a biological sequence of
definable components.   These components are considered, by him, to
be set in a series analogous to the clotting mechanism.   To exploit
the possibility for control which such a sequence offers, each
component should ideally be studied in relation to the completed
series.   Forrester (1976), in outlining the pattern of processes in
wound healing, acknowledged that our present understanding is still
not far beyond the descriptive phase.

Briefly, the general pattern of the processes involved in wound healing of surface membranes is: an initial inflammatory response followed, within a variable period of time, by the mobilization of underlying cells which migrate into the defect. In the skin lesion this includes the centripetal spread of surface cells as well as cells of the underlying connective tissue including vascular endothelial elements. In most sites the migration of cells is accompanied by an increase in mitotic activity.

Ross (1968) considered wound healing to be one dynamic event commencing with the infliction of the wound and ending with the restoration of tissue architecture and cellular function. For ease of discussion, he subdivided the one dynamic continuum into two phases: the inflammatory phase and the proliferative phase. It is well documented in the literature that a definite relationship exists between the initial inflammatory response and the proliferative phase. Schilling (1968) listed the following mechanisms involved in wound healing: platelet aggregation and blood clotting; formation of fibrin; an inflammatory response to bacteria, foreign bodies, cellular injury and necrotic tissue; alteration in ground substance; endothelial and capillary proliferation; fibroblastic proliferation and surface covering; variable regeneration of certain cell types; variable contracture and remodelling. He was of the opinion that healing is not complete until the disrupted (skin) surfaces are firmly knit by collagen; until there is obliteration of dead space and a restoration of a surface covering. Lasting function must be restored. He also suggested that the mechanisms of wound repair are merely an extension of the same

processes governing growth, multiplication, regeneration, function, death and turnover of the cells in the body day in and day out.

The inflammatory phase

Inflammation is a dynamic continuum. It has been defined by Ebert (1965) as "a process beginning with a sublethal injury to tissue and ending with complete healing". The acute inflammatory reaction is normally subdivided into:

1. Vascular response: a) changes in vessel calibre

    b) changes in the vessel walls and blood flow

2. Swelling and exudation: a) the fluid exudate

    b) the cellular exudate

3. Changes in other tissue components, including alteration in ground substance and the concomitant redistribution of electro-lytes and alteration in pH; loss of mast cell granules is one of the early signs of acute inflammation.

It is not proposed to repeat Cohnheim's careful description of the changes which constitute the vascular response (Cohnheim, 1882) nor to detail the morphological changes that more modern techniques have added (relatively little) to the accounts of Cohnheim (Clark et al., 1936).

The most characteristic feature of acute inflammation is the formation of a fluid exudate. This is produced by (1) an increased vascular permeability to proteins; (2) an increased capillary blood pressure; (3) the breakdown of large molecular tissue proteins; (4) the increase in fluidity of tissue ground substance. The most

important of these is the increased vascular permeability. Within
the last two decades it has been possible to correlate the increased
permeability produced by different types of injury with ultra-
structural changes in vascular structure occurring at the time of
the fluid exudate.

The combined technique of vascular labelling and electron
microscopic observation has dramatically increased the knowledge of
the degree and time course of fluid and protein leakage in many types
of inflammation. Majno & Palade (1961) were the first to report on
gaps occurring at the junction of adjacent endothelial cells
subsequent to the application of histamine, serotonin or bradykinin.
The gaps occurred in venules and not capillaries under their
specific experimental conditions. Reformation of normal junctional
morphology occurred within a few minutes. In further descriptions
of the ultrastructure of vascular leakage, Majno et al. (1969)
reported on an alteration in shape of endothelial cell nuclei.
This type of nuclear alteration was considered to be similar in
nature to alterations occurring during cellular contraction. Becker
& Murphy (1969), using immunofluorescent staining, demonstrated a
protein within endothelial cells which they considered identical
with smooth muscle actomyosin.

Ryan & Majno (1977) suggested the following mechanisms may
contribute to vascular leakage in addition to cell contraction:
endothelial destruction, formation of gaps by 'unzipping' of
endothelial junctions, transcellular leakage, increased pinocytotic
fluid transport.

Simonescu et al. (1975) stated that there was no apparent reason for the predominantly venular localization of these endothelial gaps. However, reporting on microcirculatory patterns in the mouse diaphragm, Simonescu et al. (1976) detected trans-endothelial channels in all capillary sections. These channels were more frequent at the venular end. More important for the understanding of venular leakage in inflammation was the observation that capillary endothelial junctions were generally 'tight' and impermeable to horseradish peroxidase whereas in the venules the junctions were readily permeable.

Since Majno & Palade's initial findings, the technique of combined vascular labelling and electron microscopic observation of vessels has been used to study acute inflammation in several tissues.

The exudative response can be divided into phases and these differ both with respect to the type and severity of the injury (Burke & Miles, 1958; Spector & Willoughby, 1959; Wilhelm & Mason, 1960). A monophasic response occurs after mild injury and is immediate and transient. Characteristically, it occurs after 1-2 minutes of injury and persists for approximately 10 minutes. This response appears to be mediated by histamine.

A biphasic response is elicited after more severe injuries. A transient, immediate phase takes place first, followed by a delayed phase. The delayed phase occurs after a period of several minutes to an hour and can persist for hours or even days. In the severest of injuries, an immediate sustained response occurs. Increased permeability occurs immediately and remains high. This is probably due to the overlapping of immediate and delayed responses

(Ryan & Majno, 1977).

This type of analysis can establish patterns for each type of inflammation. The pattern of labelling will depend on the anatomy of the microcirculation in the injured area (Zweifach, 1964). Within any one type of tissue, the variability and reversibility of the vascular response depends more on the severity of the injury than any other single factor.

Inflammation is controlled by the presence of a group of substances called chemical mediators. It would appear that each chemical mediator has a specific role at some definite state of the inflammatory reaction (Spector & Willoughby, 1959; Hurley & Spector, 1965; Miles, 1966; Ryan & Majno, 1977). It is important to note that the exact sequence of the appearance of the various mediators, their dependence on or independence of each other, is still largely speculative.

Selye (1953) recognised the individuality of the inflammatory reaction and that this was a reflection of the individuality of the causative agent. Zweifach (1964) stressed that the terminal vascular bed in different tissues is organised differently and has, in many tissues, peculiar and specific characteristics. He contended that it was difficult to relegate particular phases of the inflammatory process to any one substance or combination of substances. Hurley (1972) repeated this observation. He found that there was a different response of vessels in skin and diaphragm to a variety of agents, showing that vessels of similar structure may react dissimilarly to the same stimulus in different locations. Hence, drawing conclusions about features of increased permeability

may be misleading in particular situations.

Hurley (1972) pointed out that the difficulty is not to discover endogenous permeability factors but to determine whether endogenous chemical factors play a part in the response to a particular type of injury and, if so, which mediators are involved.

Walter & Israel (1974) tabulated the substances which have been claimed as chemical mediators. The substances were divided into the following groups:

1.   Vasoactive amines: Histamine, 5-Hydroxytrytamine.

2.   The kinins.

3.   Kinin-forming enzymes.

4.   Biologically active products of the complement system.

5.   Biologically active components of polymorphs.

6.   Prostaglandins.

7.   Nucleic acids and their breakdown products.

It is not within the scope of the present work to enter into detailed discussion of these permeability factors. It is, however, helpful to understand that the vasoactive amines serve to initiate events. In order to be effective, they are present in the tissues before injury. On the basis of the findings of Sheldon & Bauer (1960); Smith & Miles (1960); Wilhelm & Mason (1960) and Hurley & Spector (1965), it is generally agreed that the initial transient phase of increased permeability is mediated by histamine. Serotonin is now known to play an accessory role in rats but not in humans or other species.

The other chemical mediators are not normally present in the tissues. They are produced in response to an injury. Of the

established permeability factors, the kinins appear to be mediators of delayed permeability. It is also possible that the prostaglandins play a role in the delayed response as they are released during cell death and are present during the later stages of inflammation.

It should be noted that much of our knowledge of inflammation is based on studies conducted in skin, muscle and the pleural cavity. There is good evidence that there are important organ differences with regard to the vascular phenomena.

Though the delayed permeability response has been attributed to the action of chemical mediators, Hurley et al. (1967) concluded that in certain circumstances (e.g. after mild thermal burn) the increased vascular permeability of the delayed phase is due to direct damage to the vascular wall. As yet, the relative roles of direct vascular injury and endogenous chemical mediators in increased vascular permeability have not been defined.

The proliferative phase

The proliferative phase of injury or wounding follows the inflammatory phase. It includes the reformation of blood supply, the formation of a surface covering where the wound involves an external or internal lumenal surface, and the production of collagen to bind the wound margins together. Though for descriptive purposes it is stated that the proliferative phase 'follows' the inflammatory phase, it should never be forgotten that one continuous dynamic process is being considered.

Following the infliction of a simple wound, a certain proportion of tissue cells is destroyed. The inflammatory process is initiated in the closely adjacent cells. There follows a proliferation of cells responsible for repair.

Three essential processes can be distinguished in most wounded areas:

1. The active migration of sheets of cells into the defect.

2. The increased mitotic rate by which cell loss is made good.

3. The production of new connective tissue.

Johnson & McMinn (1960) state that these three processes are found in all types of tissue capable of developing mitotic activity. Troll & Lindsley (1955) and Udupa et al. (1956) observed that it is more common for the processes to occur in combination than in isolation.

The proliferative phase is a remarkable phenomenon. Cells receive information from the wounded area and new processes of gene activity follow, leading to proliferation and differentiation. The initiating stimuli or mediators of the proliferative phase are as yet unknown. Wound hormones and loss of mitotic inhibitors have been postulated.

Edwards & Dunphy (1958) stated that injury usually involves tissues of several classes. The reaction of one may modify the other. Weiss (1962) summed up this view by stating: "No cell lives by itself. It always depends on the environment which surrounds it, part of which is other cells."

Many authors referred to the importance of specific recognition mechanisms in growth control and morphology. There is an increasing awareness that in the last resort cell recognition and control of cell

growth has an immunological basis (Tyler, 1947; Weiss, 1947, 1955, 1962; Green, 1954; Burnet, 1961).

The mode of repair after cellular destruction in each tissue class depends mainly on its ability to regenerate its own kind. The form that repair or regeneration takes may depend to an extent on the condition of related tissues, such as biliary architecture in the liver or an intact periosteum in bone.

An extensive review of the cytology of wound healing of mammalian body surfaces was undertaken by Johnson & McMinn (1960). The skin, however, remains the most studied organ and the following account of the processes involved in the proliferative phase, together with the possible mechanisms involved, relates mainly to this organ.

Schilling (1976) stated that what was required was a knowledge of all the precise mechanisms of wound healing. Such knowledge would be applied not so much to accelerate healing but more to control the variables which retard or complicate wound healing. In accord with this viewpoint, there follows a brief review of the known processes involved in the proliferative phase.

(a) Migration of cells. In a healing wound, cell migration is especially obvious in the epidermis. Fibroblasts, histiocytes, capillary spouts, nerve fibres and leucocytes all take part. Migration of these cells is to a large extent initiated by the infliction of a wound (Abercrombie, 1964). The mechanisms governing this movement are largely unknown but two points should be made. In general, cells in uninjured tissue are static. Subsequent to injury and the production of a discontinuity in tissue, cells move from the free edge into the gap.

Cells start to move from a static position and do not merely change direction. Moreover, migration ceases when the gap is filled.

Abercrombie & Heaysman (1954) observed chick embryo heart fibroblasts in tissue culture. Cells from two explants invaded the intervening area and cell movement ceased when the cells collided. This seemed to be the result of mutual adhesions. The phrase 'contact inhibition' was used to denote this cessation of movement. Abercrombie (1961) and Abercrombie & Ambrose (1962) presented evidence which suggested that cell movement is correlated in some way with mitotic activity. The degree of emigration from cultured tissue fragments depended on the condition of the tissue at the time of explantation. When wounded tissue was cultured, the degree of emigration was elevated. Abercrombie (1964) concluded that cells are 'mobilized' as a result of injury. Wounded tissue, regenerating tissue, tissue cultures, regenerating blastoma, all have increased mitotic activity. All such tissues show an increased mobilization compared to normal adult tissue. There appears to be a close physio-logical link between proliferation and mobilization. Abercrombie presumed that both these phenomena were in response to the same stimuli.

Weiss (1941) demonstrated that cells in tissue culture are sensitive to any orientated structure in the substrate. Fibrin or persistent collagen fibres may direct cell movement into wound gaps in vivo.

A review of the role of chemotaxis in inflammation was presented by Harris (1954). Polymorphonuclear leucocytes and monocytes appear to respond to chemical gradients. Lymphocytes have not been shown to do this. Abercrombie (1966) stated that no such chemotaxis has

been demonstrated or even indicated for adult tissue cells. He considered that tissue cells may respond in certain circumstances to chemotactic stimuli but that this type of mechanism, at best, serves a subsidiary role in wound filling. Observations of injured areas in transparent tissues give conflicting evidence as to whether or not damaged tissue is chemotactic to leucocytes. Allison et al. (1955) failed to observe directional movement of leucocytes towards areas of aseptic injured tissue, whereas Buckley (1963) reported cell movement towards aseptic injured areas in rabbit ear chambers. Florey (1970) found that, once external to vessels, cells moved in no particular direction.

The review so far has been directed towards the movement of cells into or towards a wound defect and the possible mechanisms subserving this movement. The following is a brief discussion of that type of movement which brings about a rearranging of cell types and the achievement of spatial pattern within tissues.

Morphogenesis is the process by which the form of an organism and the arrangement of its tissues are generated. The mechanisms bringing about the rearrangement of embryonic tissue at gastrulation may be similar if not identical to those mechanisms bringing about the redistribution of cell types during wound healing. Tissue culture is used extensively to investigate these problems and the tooth primordium is a model embryonic organ for developmental enquiry in this field.

Tissues can be dispersed into their constituent cells by trypsin. When a random mixture of cells from two organs is prepared and suitably cultured, the cells will clump together in a random mix.

However, on further culturing, the cells will rearrange themselves into a pure association of constituent cells. Holtfreter (1944) was one of the first to demonstrate this power of rearrangement of cellular spatial pattern.

Fell (1951), Moscona & Moscona (1952) and Grover (1962) showed that mouse embryonic tissue aged 7-18 days could be dissociated and reaggregated in vitro. The cells were capable of complex interactions with the production of true histotypical structures within 48 hours. The ability to do this was lost with age. It is apparent that cells are capable of mutual recognition. They seem to respond specifically to messages and stimuli conveyed by neighbouring homotypic and hetero-typic juxtaposed cells and are capable of recombination into aggregates, often with fine precision. For this to occur, some type of inter-cellular and extracellular communication must exist. It is beyond the scope of the present work to consider these mechanisms in detail.

(b) Mitosis. Following the initial inflammatory response to injury or wounding, there is a proliferation of cells which makes good cell loss. The process of mitotic activity and the synthesis of protein are both seen in normal tissue. In wounded tissue they are more prominent. It is therefore perhaps more exact to consider these events of wound healing as an acceleration rather than an initiation.

Schilling (1968) stated that a knowledge of growth characteristics of cell populations was essential in gaining insight into the total process. A knowledge of conditions in the undamaged tissue aids the understanding of conditions during wound healing. In the undamaged, non-growing adult tissue, cell loss is balanced by cell production. In the skin, this is seen by the division of basal cells and the migration

of cells from the basal layer.   Within the epidermal organ a gradation

of events can be observed.   The basal cells are mitotically active;

the more distal cells are more functionally active.   The cells can be

grouped into four distinct classes.   From the basal layer outwards

the four classes of cell are: those involved in mitosis, the immature

cell preparing for tissue function, the mature functional cell, and

the cell at the end of its life span.   The great majority of normal

tissues exhibit these four groups, though the proportions vary

considerably from tissue to tissue, e.g. in the duodenal mucosa,

mitotic cells form a high proportion of the total cell mass and are

involved in division about every 12 hours.   Epidermal cells have a

smaller proportion of mitotic cells and division occurs about once a

month.   The liver is a mitotically inert tissue in the stable state.

Liver cells are almost all functional and may not be replaced during

the life time of an animal.   Odontoblasts may be of a similar

biological status to liver cells.

In summary, each tissue seemingly has a characteristic mitotic

rate which is in balance with its average cell death rate.   This

average mitotic rate is increased following wounding and only presents

at the increased rate during the period of repair.   Chemical mediators

and inhibitors have been postulated to account for the control of these

events but the precise mechanisms remain unknown.

The wound hormone theory, as mentioned in the reviews by

Abercrombie (1957) and Swann (1957) suggests that damaged tissue

produces a hormone which stimulates the activity of cells.   In the

main, this theory has fallen out of favour. Only one paper suggesting

the existence of a wound hormone can be traced in the literature of the

past ten years.   Joseph & Dyson (1970) stated that they observed

enhanced regeneration of epidermal ear tissue in rabbits concomitant with abdominal wound healing. They suggested that the stress produced by wounding increased blood level AĈTH and an increased output of glucocorticosteroids. This effect was considered a possible basis for a wound hormone.

Considerable evidence has been accumulated to support the theory that a negative feedback system operates. It is suggested that mitosis is inhibited by a diffusible substance called a chalone.

Saetren (1956, 1963) reported his experiments which suggested that there was a tissue specific growth inhibitor found in kidney. He speculated that this growth inhibitor substance may be cell-type specific, though this could not be substantiated by his experiments. The inhibitory activity of epidermal extracts on the mitotic activity of mouse ear epidermis was described by Bullough & Laurence (1964a,b). They concluded that a diffusible inhibitor controlled normal epidermis mitosis. They noted that, in the absence of adrenalin, the inhibitor or chalone lost its full power and it was concluded that the actual mitotic inhibitor substance may be an unstable chalone-adrenalin complex.

Bullough & Rytomaa (1965) emphasised that, in most adult tissues, cells have only two specialised programmes of synthesis open to them. The first allows synthesis of enzymes necessary for the mitotic cycle and the second allows synthesis of enzymes essential for specialised tissue function (differentiation). The effect of a chalone is to reduce the number of cells preparing for mitosis and a chalone's main force must therefore be exerted prior to prophase. In the absence of sufficient chalones, the cell will embark on a series of enzyme synthesis characteristic of prophase. In the presence of sufficient chalones, it will synthesize enzymes necessary for tissue function.

Any such 'decision' relating to synthetic activity is likely to involve gene activation. The situation is reminiscent of activation or inactivation of particular regions of the genome by the effector substances of Jacob and Monod. The authors concluded that tissue homeostasis may be controlled by the effector-like action of a tissue (or cell) specific chalone. The chalone concentration determines whether the mitotic operon or tissue operon is activated.

Bullough (1972a,b; 1973) stated that tissue specific anti-mitotic chalones have been found in all tissues so far investigated. The epidermal growth control mechanisms are therefore considered typical. Bullough suggested that a further action of chalone is to reduce the speed at which distal post-mitotic cells age and die. Post-mitotic aging and tissue function, though normally associated, are separate processes. Bullough (1975) reviewed chalone control mechanisms including aging in relation to mitotic inhibition.

In summary, the mitotic control of adult mammalian tissue depends on a tissue specific mitotic inhibitor. In addition, a mitotic promotor is present. This is the mesenchymal factor as postulated by Renzio & Rutter (1973). The chalone operates in a more complex manner than originally thought. In the epidermis, mitosis is inhibited by epidermal chalone and promoted by a mesenchymal factor produced by the dermis. Cell proliferation is considered to occur in basal cell layers when chalone is counteracted by mesenchymal factor. Aging is related to mitotic inhibition. Where chalone effectiveness is high, cells are non-mitotic or post-mitotic. Aging is equally inhibited and cell loss is reduced. In populations of post-mitotic cells, there is no cell loss.

(c) <u>Connective tissue formation</u>. Stearns (1940a,b) described the sequence of events in connective tissue formation following wounding in rabbits' ears. Though the time lag was dependent on existing conditions, the sequence of events never varied. Stearns reported this to be: (1) fibroblasts appeared six days post-operatively and following the phase of traumatic inflammation and enzymatic demolition; (2) one or two days later blood vessels were observed; (3) two or three days later still, connective tissue fibres were laid down.

Since this early work by Stearns it has been accepted that fibro-blasts are the cells responsible for collagen fibres of new connective tissue. Jackson (1958) reviewed the subject of fibroblast function.

Levenson et al. (1965) showed that fibroblasts migrated more rapidly than capillary endothelial cells. This is but one example of how inherent properties of different cell types may contribute to and result in a basic sequence of events.

The exact origins of the fibroblasts participating in wound repair have been a point of discussion for some time. In an attempt to resolve the issue, Ross et al. (1970) studied the origins of wound fibroblasts in a series of parabiotic rats. The femurs of one parabiont of each pair were shielded whilst both animals were irradiated. After three days the animals were then wounded. Each animal with partially shielded marrows was given tritiated thymidine while the cross circulation was arrested by clamping. After the tritiated thymidine had cleared the blood, the clamp was released. In the wounds of the shielded animals, tritiated thymidine was observed in epidermis, endothelium, leucocytes, fibroblasts and mast cells. Only

neutrophils, monocytes and lymphocytes were labelled in the non-shielded animals. None of the many fibroblasts present in the wounds of the non-shielded parabionts were found to be labelled throughout the six day investigative period.

The cells which demonstrated the largest uptake of thymidine were perivascular cells of the loose connective tissue adjacent to the wound. It was concluded that mesenchymal cells located in tissue areas adjacent to the wound were the source of new fibroblasts. In the opinion of the authors, neutrophils, being short lived cells, are unlikely to serve as a source of new fibroblasts. It was concluded that the experiments support the notion that fibroblasts are not derived from blood borne cells but from adjacent perivascular connective tissue elements.

The role of oxygen in wound healing

Forrester (1976) has stated that the most important recent advance with respect to wound healing is the understanding of oxygen requirements in the healing wound. Benditt & Hunt (1970) concluded that oxygen increases the rate of healing. They pointed out that there was a restriction in wound oxygen supply following the decrease in blood flow. The duration of oxygen deprivation coincides with the elevation of viscosity of whole blood caused by trauma. The authors emphasise that the relationship between the inflammatory response, the concomitant variations in oxygen tensions, and the metabolic activity of cells participating in the proliferative phase needs further clarification.

Three main experimental methods have been used to study oxygen physiology in healing wounds. These are: the use of implanted wire mesh cylinders or synthetic sponge material; the use of Teflon coils; and the use of oxygen electrodes. Hunt et al. (1969b) stated that the three methods give comparable results.

Hunt et al. (1967) used both polyvinyl sponge implants and wire mesh implants to measure the pO_2, pCO_2 and pH in wounds. After injury, oxygen tensions within the wound started to fall. By the third day, they found that the pO_2 in the 'dead space' had fallen below 10mm Hg. There was an accompanying rise in pCO_2 and fall in pH. (The fall in pH reflects the rise in pCO_2.)

Oxygen tension within a tissue represents a balance between the supply from the vascular system and tissue utilization. The availability of oxygen to produce measurable pO_2 tensions is dependent on capillary oxygen tensions, which is a function of blood flow. The variations from normal of wound pO_2, pCO_2 and pH arise because of the inability of the damaged vasculature to carry out its transport functions.

Hunt et al. (1969a), Niinikoski (1969), Hunt (1970) and Stephens & Hunt (1971) reported on the arterial pO_2 effect on epithelialization, collagen formation and neovascular proliferation. Essentially, the results indicate that the healing process is oxygen dependent and confirm the well known clinical fact that healthy vascular tissue is essential for normal healing and that wounds in ischaemic tissue heal poorly.

Goodson & Hunt (1977) stated that poor wound healing in diabetes may be related to 'small vessel disease', i.e. due to the delay of passage of nutrients into the wound milieu due to endothelial and basement membrane thickening.

Hunt et al. (1969b) proposed that the distance a fibroblast can migrate from the nearest capillary is limited by the oxygen tension in the extracellular fluid. The pO_2 near budding capillaries was found to be 40-50mm Hg whereas, at the wound edge, the value was between 10-12mm Hg. Hunt suggested that, when the fibroblast's environmental oxygen tension fell below 10mm Hg, the cell could probably no longer enter mitosis, synthesise collagen or migrate. Oxygen must diffuse up to 100μm from the last functional capillary to the furthest functional cell. This involves diffusion through the following biological barriers: the endothelial cell membrane; the extracellular matrix; the cell membrane; the cell cytoplasm and the mitochondrial membrane.

Hunt et al. (1969a) and Niinikoski et al. (1972a) illustrated that regenerating tissue exists in an environment which is oxygen poor. Tissue oxygen tensions were inversely proportional to the distance from the nearest functional capillary. In their experiments, the pO_2 gradient existing within the wound was measured with the inspired air containing different percentages of oxygen. It was observed that the gradient became steeper when the inspired air had higher percentages of oxygen, i.e. the oxygen tension in the immediate vicinity of the capillaries rose substantially compared to the pO_2 at more distal parts. This was interpreted as showing that cells near the capillary will utilize more oxygen when this is made

available.

Ehrlich et al. (1972) estimated the mean capillary to wound edge oxygen tensions by injecting Antimycin A, an inhibitor of mitochondrial electron transfer, into wounds and measuring the oxygen tension in wound fluids. Gradients were estimated when the animals breathed normal air, and then air where the oxygen was 45% and 10% of the total gases. The oxygen gradient was almost flat in hypoxic animals and increased as the arterial pO_2 rose.

The sharp oxygen gradient between the extracellular fluid at the growing edge of granulation tissue and the extracellular fluid nearest the capillary blood supply occurs because oxygen is used more rapidly than it can be supplied. The limitation of supply is a result of diffusion resistance within the granulation tissue and/ or from inadequacy of the injured capillary bed.

Hunt et al. (1972) concurred with the opinions expressed in the above paper. They stated that vital portions of the repair process take place in severely hypoxic environments. They considered that diffusion seems to be the limiting factor supplying oxygen to the advancing healing tissue edge.

Niinikoski et al. (1972b) monitored pO_2, pCO_2 and pH in experimentally induced wounds. Subcutaneous implanted cellulose sponges were used to collect wound tissue samples for analysis. Between the seventh and fifteenth day post-operatively, the pO_2 was 8 - 15mm Hg. Ahonen (1968) has shown that cell division in wounded skin tissue is greatest during this period, the fibroblasts being the most active cell type. Wound healing appears therefore to occur in unfavourable oxygen environments. Niinikoski et al.

acknowledged that the metabolic response of tissues to injury places great demands on the mechanisms whereby substrates, particularly oxygen, are transported to the cells concerned with inflammation and repair.

Heughan et al. (1972) measured tissue pO_2 in experimental wounds in normovolemic rats. These were lowered significantly by the intravenous administration of normal saline. The suggested cause of this effect was the resultant oedema. The authors concluded that oedema, from whatever cause, may inhibit oxygen transport sufficiently to impair healing. Reporting on the clinical aspects, Heughan et al. (1974) stated that healing is impaired in conditions which are often associated with anaemia, e.g. malnutrition, abnormalities of circulating blood volume and increased blood viscosity following trauma. Mild or moderately uncomplicated normo-volemic anaemia in otherwise healthy individuals does not impair delivery of oxygen to wounds and is of no consequence to wound healing.

Hunt (1972) was in general agreement with these remarks. He made the following observations. Any factor which increases the arterial pO_2 within safe limits while the patient is normovolemic and without causing vasoconstriction will increase collagen synthesis and reduce infection. Hypovolaemia causes vasoconstriction, and an increased arterial pO_2 cannot restore normal wound oxygen tensions. Within normal wounds, due to the fact that injury damages the vascularity and increases diffusion resistance, the oxygen supply is governed by arterial pO_2 and not the haemoglobin content of the blood.

Kenny & Fink (1966) established that the optimum pO_2 for fibro-blast growth in tissue culture is 60mm Hg. Niinikoski (1969) indicated that a high ambient oxygen concentration impaired fibroblast proliferation. It may be implied from these papers that there is an optimum pO_2 for fibroblast proliferation as shown in tissue culture and in vivo experiments. Niinikoski (1972b) reported that the pO_2 in normal skin wounds is 8 - 15mm Hg during this period.

Hunt & Hutchison (1966) used stainless steel wire mesh cylinders and Teflon coils to study oxygen tensions in wounds with particular reference to bacterial pathogenicity. They summarised their results thus: oxygen tensions ranged from 5-28mm Hg which, in their opinion, probably represented the oxygen tension in the advancing edge of granulation tissue. Oxygen tensions would be expected to be higher in granulation tissue. They found that the dead space has a natural resistance to infection which, however, can be overwhelmed by large numbers of bacteria.

Hunt (1972b) reported on the relationship between the infecti-bility of soft tissue wounds and chronic hypoxia and hyperoxia. Three sets of rabbits were kept in normoxic, hyperoxic and hypoxic conditions. Steel mesh cylinders were implanted subcutaneously and inoculated with Pseudomonas aerogenosa. The results indicated that the 'normal' hypoxic conditions existing in wounds are a major factor in the susceptibility of wounds to infection. Hunt was of the opinion that changes in host resistance probably accounted for this. White cells can ingest but not kill bacteria in anaerobic conditions.

A burst of oxygen consumption is measurable concomitant with the death of phagocytosed bacteria. Hohn & Hunt (1975) reported on the function of polymorphs and monocytes within wounds. The function of these cells is known to be largely dependent on their oxidative metabolic capacity. The authors concluded that the metabolic and bactericidal function of these cells is not impaired within 'normal' wound tissue.

Calcification and oxygen tensions

Calcification and bone growth occur in vivo in areas of low oxygen tensions. Kelly et al. (1959) demonstrated that puppies with tibial arterio-venous fistulae consistently showed lengthening of the experimental tibia compared with the normal contra-lateral tibia. Brighton et al. (1969) studied the relationship of oxygen tension to bone formation. The costochondral junctions of 21 day old rats were used as an in vitro model, and explants were grown in various oxygen concentrations. Optimum bone formation occurred at the lowest (5%) oxygen concentration. It was concluded that low oxygen concentrations in the gaseous environment of the explants favoured bone growth. The epiphyseal plate is seen to be extremely sensitive to the prevailing oxygen tensions and it would appear from these two papers that osteogenesis occurs at a different oxygen tension from chondrogenesis.

Brighton & Heppenstall (1971a), using puppies with femoral arterio-venous fistulae, suggested that oxygen tensions in the epiphyseal plate may regulate plate function. They postulated that a low pO_2 was

associated with anaerobic metabolism and increased plate growth, whereas high pO_2 was associated with aerobic metabolism and decreased plate growth. They demonstrated this in vivo, showing that, as the oxygen supply to explants increased, the epiphyseal plate growth decreased (Brighton & Heppenstall, 1971b). In this study, explants exhibited a growth lag phase for 1-2 days post-operatively. Active growth commenced by day 3. The recorded oxygen tensions in various zones remained constant at day 0, 3 and 7. Little oxygen was consumed despite active growth. The authors concluded that the low oxygen tension is therefore not a result of oxygen consumption. Papers by Kuhlman (1960) and Kuhlman & McNamee (1970) supported the notion that low oxygen tensions within the epiphyseal plate are not due to cellular utilization. Brighton & Friedenberg (1975) reviewed the literature relating bone growth with low pO_2. It was concluded that new bone formation under hypoxic conditions was not due to an increased oxygen consumption but to a decrease in oxygen supply at the cellular level. One reason for this is that new vascularity requires support. Ground substance must precede the blood vessel growth and therefore the new improved vascularity is delivering oxygen to an increased, proliferating and actively synthesising cell population. The results of the more important investigations are summarized:

1. Epiphyseal chondrocytes in all zones have the necessary enzymes to effect aerobic and anaerobic metabolism (Kuhlman, 1960). The zone of hypertrophic cells follow a predominantly anaerobic pathway (Kuhlman & McNamee, 1970).

2. Glycogen is present in the cytoplasm of chondrocytes in the upper four-fifths of the epiphyseal plate and ends abruptly in

the middle of the hypertrophic zone (Brighton et al., 1969).
Electron microscopic studies by Holtrop (1972) and Brighton et
al. (1973) confirm these findings.

3. There is an abundant blood supply to the proliferating zone of
the cartilagenous growth plate (epiphyseal plate) and a poor
blood supply at the bone/cartilage junction (Rhinelander &
Baragry, 1962; Rhinelander, 1965, 1968; Rhinelander et al.,
1968; Lauren & Kelly, 1969).

4. Measurements of tissue oxygen tensions indicate that pO_2 is low
at the bone/cartilage junction. The consensus opinions are
that low oxygen tensions found at the bone/cartilage junction
are due to a lack of oxygen supply and not to an increase in
consumption. In the zone of proliferation, pO_2 is high,
aerobic metabolism occurs and glycogen is stored. In the
hypertrophic cell zone, pO_2 is low, anaerobic metabolism occurs
and glycogen is utilized (Hunt, 1964; Hunt et al., 1969a;
Hunt & Pal, 1972).

5. At the base of the hypertrophic zone a release of calcium from
the mitochondria occurs into the extracellular matrix. This
is believed to be an energy linked process (Chance, 1963;
Chance & Azzi, 1967; Azzi & Chance, 1969).

Brighton et al. (1969) and Heppenstall et al. (1975) discussed
the possible physiological role played by the low oxygen tensions in
the healing osseous wound. Both authors proposed the following
notions which they considered required further investigation. An
abnormally large pO_2 gradient exists between the blood vessels and
the wound margin in soft tissue wounds and between the proliferating
cell zone and the calcification front within the epiphyseal plate.

In both situations the oxygen tension gradient is proposed as a
possible stimulus, in the first case for healing and, in the second,
as a stimulus for calcification.

Cortisone and wound healing

Granulation tissue formation is low, fibroblasts remain small
and little collagen is formed under conditions of excess systemic
glucocorticosteroid administration (Walter & Israel, 1974).   Howe
et al. (1950) indicated that very large doses of cortisone, however,
are required to delay wound healing in man.

Fibroblasts are extremely sensitive to corticosteroids both in
vitro and in vivo.   Corticosteroid-induced morphological changes
include the loss of spindle shape and the assumption of a globular
form (Ragan et al., 1949;   Grossfeld & Ragan, 1954;   Dougherty et al.,
1956;   Holden & Adams, 1957;   Wellings & Moon, 1961;   Rozen &
Chernin, 1965;   Ruhmann & Berliner, 1965;   Berliner et al., 1967).

Dougherty et al. (1956) suggested that the characteristic
rounding of cortisone-treated fibroblasts is a protective mechanism
enabling the cell to resist a chain of destructive events initiated
by inflammatory agents.

Castor (1962) and Castor & Muirden (1964) reported that corti-
costeroids inhibit both mucopolysaccharide and collagen production
by fibroblasts in vitro.   Berliner et al. (1967) and Ruhman &
Berliner (1967) stated that the sensitivity of tissue culture fibro-
blasts to naturally occurring and synthetic steroids is a consistently
measurable phenomenon.   They stated that the inflammatory responses
and the fibroblast responses parallel each other;   moreover, the

action of fibroblasts in tissue culture can be correlated with inflammatory events.

Numerous papers indicate a depression of scar tissue formation accompanied by a depression in wound tensile strength following both local or systemically administered cortisone and hydrocortisone acetate. These include: Spain et al. (1950); Savlov & Dunphy (1954); Sandberg (1963, 1964a,b, 1966); Di Pasquale et al. (1964); Manning (1967).

A very large systemic dose of cortisone is required to depress wound tensile strength (Spain et al., 1950; Savlov & Dunphy, 1954; Sandberg, 1963, 1964a). The inhibitory effect of cortisone, as manifest by a reduction in wound tensile strength, varied with the interval between the day of wound infliction and the initiation of cortisone treatment. Spain et al. (1950) also reported that the marked suppression of granulation tissue formation is not observed when cortisone treatment is started as shortly as two or three days after wounding. Sandberg (1963) reported significant reductions in the tensile strength of wounds and of collagen formation in granulation tissue recovered by the sponge implant technique when cortisone was injected only pre-operatively for three days, or for the last six days of the healing period. Cortisone evidently exerts its influence on healing during the initial days.

The following papers report on the reversal of steroid-induced inhibition or retardation of wound healing.

Prudden & Wolarsky (1967) showed that cartilage extracts reversed steroid-induced inhibition of wound healing. It was shown

experimentally that long chain polymers of acetylglucosamine are potential stimulators of wound healing. Neutrophil lysosomes were reported to break down ground substance which released this acetylglucosamine polymer. Ehrlich & Hunt (1968) reported that Vit. A can reverse the inhibitory effect of cortisone. Vit. A is a known lysosomal labilizer. Hunt suggested that this is evidence of the importance of lysosomal enzymes in wound healing.

Rudolph & Klein (1973) have shown that cortisone diminishes the lysis of ^{3}H labelled collagen as well as inhibiting new collagen formation. Ehrlich et al. (1974) inserted subcutaneously plant protein labelled with ^{14}C. No labelled hydroxyproline was implanted. However, radio activity was detected in recovered collagen hydroxy-proline. The results indicate that cells within the healing wound are able to degrade preformed protein and re-use the liberated amino acids for collagen synthesis. Ehrlich et al. (1974) suggest that the inflammatory process has a nutritional role. Phagocytic cells derive useful amino acids from injured cells. The authors refer to the evidence in the literature indicative of the non-essential nature of polymorphs and lymphocytes for wound healing. On the basis of their own results they implicate macrophages as being important for wound repair. The inhibitory action of cortisone on wound healing is attributed to its interference with the digestive activities of wound phagocytes.

Thorn et al. (1953) and Allison et al. (1955) reported on the suppression of the inflammatory response by systemically administered cortisone. Allison et al. (1955) found that arteriolar dilatation was inhibited as was leucocyte migration. They concluded, however,

that, from their experimental evidence, the anti-inflammatory action of cortisone cannot be explained on the basis of its vaso-constrictive properties alone.

The following is a list of suggested mechanisms by which corticosteroids may exert their effect on the inflammatory process:

1. An influence on the vascular wall leading to decreased perme-ability (Ebert & Barclay, 1952; Allison et al., 1955; Fruhman, 1962; Allison, 1965; Peters et al., 1972).

2. An influence leading to decreased leucocyte motility ascribed to a direct effect of hydrocortisone on the leucocyte cell (Ketchel et al., 1958).

3. A systemic action lowering the available number of circulating leucocytes (Gell & Hind, 1951).

4. A vasoconstrictive effect. The maintenance of arteriolar tone by cortisone suppresses the intense vasodilation characteristic of the inflammatory lesion and consequently reduces haemorrhage and oedema.

5. An action dependent on the depletion of tissue histamine levels (Kovacs, 1965) and on the reduction of histamine formation (Schayer et al., 1954). This may be due to the effect of cortisone on the histamine-forming enzyme, histidine-decarboxylase (Sandberg & Steinhardt, 1964).

6. A stabilizing effect on lysosomal membranes (de Duve et al., 1962; Weissman & Thomas, 1963; Weiss & Dingle, 1964; Weissman, 1965; Hunt et al., 1969b).

7. An action dependent on the depletion of serum complement levels (Gewurz et al., 1965; Atkinson & Frank, 1973).

8. An action inhibiting polymorphonuclear chemotaxis (Ward, 1966).

Synopsis of section

Wound healing can be defined in terms of the restoration of functional continuity following cellular disruption. It is a complex integrated biological process involving cell regeneration, cell proliferation and collagen production. Current knowledge has not advanced far beyond the descriptive phase and there is a large speculative literature on the chemical mediators of the inflammatory and proliferative phases. The repair organ is the capillary-fibroblast system. An optimum relationship appears to exist between the inflammatory phase and the proliferative phase. A minimal inflammatory stimulus is required for normal healing. The variability and reversibility of the inflammatory response depends more on the severity than on the type of injury. The most important recent advance is the understanding of oxygen requirements in wound healing. Tissue oxygen tensions are now believed to govern cellular differentiation, proliferation and mitochondrial calcium loading as well as cell function. Fibroblasts are rarely found more than 50-80μm from the nearest functional capillary but macrophages have a lower oxygen requirement and are found at the free edge of growing granulation tissue. Anti-inflammatory drugs such as Cortisone will delay healing if administered systemically in large amounts during the first few days after wounding. Once healing is established, Cortisone is without effect. The local application of cortico-steroids, by affecting local vaso-motor activity and the inflammatory process, may contribute to the achievement of an optimal relation-ship between the inflammatory and proliferative phases of wound healing.

B. WOUND HEALING IN THE PULP

The inflammatory phase

The microcirculation of the pulp is basically similar to that in other connective tissues. It consists of arterioles, metarterioles, capillaries and venules (Boling & Robinson, 1942; Perint, 1949; Kramer, 1951; Keller & Cohen, 1955; Russell & Kramer, 1956; Matthews et al., 1959; Bernick, 1960; Kramer, 1960; Adams, 1962; Boyers & Neptune, 1962). Arterio-venous shunts have been reported in the human pulp (Saunders, 1957; Provenza, 1958; Provenza & Biddington, 1958; Cheng & Provenza, 1959; Provenza, 1968) and in the rodent pulp (Kindlova & Matena, 1959; Adams, 1959), and in other species (Butcher & Taylor, 1952; Klingsberg et al., 1959; Boyers & Neptune, 1962; Castell, 1962).

The nerve supply to the pulp contains both sensory and motor elements. The sensory supply mediates only the sensation of pain. Reflex arches may exist which influence pulpal circulation. Sensory elements initiate these reflexes within the pulp. The motor leg of the reflex arch is provided by motor fibres which end on the muscular walls of pulpal blood vessels. These motor fibres are predominantly, if not exclusively, sympathetic vasoconstrictors (Edwards & Kitchin, 1938; Christensen, 1940; Taylor, 1950; Ogilvie et al., 1966; Ogilvie, 1967; Edwall & Kindlova, 1971; Scott et al., 1972; Olgart et al., 1972).

The degree of function of the microcirculation (the capillaries are the functional unit) depends on (1) basal myogenic tone; (2) vaso-constrictor influence; (3) humoral factors which antagonise the

vasoconstrictor influence.

Biogenic substances associated with an immediate-type inflamma-
tory vasodilation have been reported as existing in pulp tissue
(Kroeger, 1968; Pohto & Antila, 1970; Del Balso et al., 1976).
Schayer (1965) and Kahlson & Rosengren (1968) have suggested that
histamine has a physiological function in circulatory homeostasis in
both normal and wounded tissues. Kroeger (1968) and Pohto & Antila
(1970) believe histamine and bradykinin play a role in vaso-motor
regulation in pulpal tissue. Edwall et al. (1973) found that
histamine and bradykinin had no effect on pulpal blood flow under
resting conditions. They suggested that their action is limited to
altered states. They found that histamine causes a vasodilation in
the cat pulp tissue if the vasoconstrictor tone is increased. Del
Balso (1976) noted a four fold increase in histamine levels within
the porcine pulp following thermal injury. Mechanisms controlling
vascular inflammatory events appear similar to those existing in
other connective tissues.

There are conflicting reports concerning the source of pulp
histamine. Wislocki & Sognnaes (1950) described mast cells in monkey
pulps and also in non-inflamed human pulps. Anneroth & Brannstrom (1964)
reported autofluorescent mast cells in human pulp tissue. Sulzmann
(1966) reported mast cells in normal canine pulp tissue. Pohto & Antila
(1969), using fluorescent microscopy, could not detect mast cells in
rat, cat or human pulp tissue. They did report a non-intracellular
fluorescence suggestive of histamine in all the examined species.
Zachrisson (1971) detected significant numbers of mast cells in inflamed
pulp tissue in human deciduous and young adult teeth. Langeland

(personal communication) stated that the detection of mast cells is dependent on the proper use of histological techniques. Histamine-containing mast cells are normal constituents of human pulpal tissue.

The proliferative phase

The following section relates to the mitotic activity in the dental pulp and the proliferation of cellular elements with special emphasis on the regeneration of odontoblasts.

Autoradiographic studies with labelled thymidine show that the mature, uninjured pulp has a low mitotic activity. Labelling was observed mainly in the cells of the vascular wall or in cells close to the vessels (Paynter & Hunt, 1961; Hwang & Tonne, 1965; Pinzon et al., 1966; Sveen & Hawes, 1968; Zach et al., 1969).

No labelling was observed in the odontoblast layer in maturing or mature teeth (Hunt & Paynter, 1961; Paynter & Hunt, 1961; Stanley, 1962; Cotton, 1964, 1968; Hwang & Tonne, 1965; Robins, 1967).

An increased mitotic activity within the pulp tissue was reported following the application of various irritants to dentine and pulp by Glass & Zander (1949); Zander & Glass (1949); Langeland (1957, 1961a,b); Postie et al. (1959); Zach & Cohen (1965); Harrop & Mackay (1968); Zach et al. (1969); Felt et al. (1970). No mitotic activity was reported in the mature odontoblast layer in any of these studies.

Tonna & Cronkite (1961) defined the labelling index as "the ratio of the number of cells labelled to the total number of cells of the same cytological type". In the pulp, even under severe stimulus from cavity preparation, the labelling indices for the fibroblast-

precursor-odontoblast system are low.   They range from 0.5% four

days post-operatively to 3% after eight days.

Robins (1967) reported mitosis in the preodontoblasts.   Cotton

(1968) considered the odontoblasts to be post-mitotic as they were

not labelled by tritiated thymidine.

The following authors consider mature odontoblasts to be post-

mitotic cells: Glass & Zander (1949);   Zander & Glass (1949);

Langeland (1957, 1961a,b);   Marsland & Shovelton (1957);   Hunt &

Paynter (1961);   Hwang & Tonna (1965);   Zach & Cohen (1965);   Robins

(1967);   Searls (1967);   Harrop & Mackay (1968);   Sveen & Hawes (1968);

Zach et al. (1969);   Luostarinen (1971).

The consensus opinion, as stated in these papers, is that the

odontoblasts are incapable of mitotic division and are post-mitotic

cells.   This statement implies that wound healing in the pulp and

the formation of a reparative dentine bridge following pulp

exposure with the loss of odontoblast cells, occurs by processes

involving differentiation of cells deep in the pulp.   The prolifera-

tive phase of pulpal wound healing is accompanied by cellular

differentiation.   It is not known whether the cells giving rise to

odontoblasts are "determined" preodontoblasts or undifferentiated

mesenchymal cells.   The biological status of the cells has important

clinical implications.   Theoretically, determined preodontoblasts

may require no more stimulus for their subsequent differentiation

than the loss of existing odontoblasts.   Events during wound

healing may represent an acceleration of normal physiological cell

replacement.   If the cells are of true undifferentiated mesenchymal

origin, a more complex induction system (such as that known for tooth development and involving ectodermal-mesenchymal factors) may be required.

Papers referring to mitotic activity within the odontoblast layer have appeared in the literature.   These include papers by Boyle & Irving (1952);   Scheiss et al. (1966);   Gotjamanos (1967) and Luostarinen (1971).

Gotjamanos (1967), using tritiated thymidine, reported mitotic activity in the odontoblast layer of rat teeth.   Boyle & Irving (1952) considered that, where normal differentiation and function is inhibited, cells become post-mitotic.   They concluded that odonto- blasts are "reverting post-mitotic".   Luostarinen (1971) also suggested that under given conditions the odontoblasts may undergo mitosis.   He reported cell proliferation and differentiation following trauma to the rat pulp tissue.   Maximum proliferation occurred about 48 hours post-operatively.   Differentiation gave rise to an odonto- blast type of cell.   Though Luostarinen stated that the study revealed that no mitotic activity occurred in the majority of odontoblast cells, observations made on the incisors suggested that, under certain conditions, mature odontoblasts may undergo mitosis.

Scheiss et al. (1966) cultured odontoblasts obtained from split human teeth.   Mitosis was observed.   This was taken as proof that there may be times when odontoblasts can engage in mitotic activity.

General conclusions as to the biological status of the odontoblast, drawn from studies involving the continuously erupting rat incisor, should be made with care.   Moreover, any mitotic cell seen in the odontoblast layer could be of endothelial origin.   The consensus

opinion is that the odontoblast response to injury is not to engage in mitotic activity.

Prior to discussing the replacement of lost odontoblasts, the response of these cells to non-fatal injury will be presented.

Following dentine trauma, there may occur a displacement of odontoblasts into related dentinal tubules. The term universally used to describe this phenomenon is "aspiration of odontoblasts" and was coined by Kramer & McLean (1952). The exact mechanism is not known. Kramer (1955) was the first to interpret this movement as an indication of an outward flow of pulpal fluid.

Langeland (1957, 1961a); Brannstrom & Lind (1965) and Langeland & Langeland (1968) concluded that the only definite criterion of injury to odontoblasts was in fact aspiration of these cells and their nuclei into the dentinal tubules. Langeland stated that other phenomena such as vacuolisation and cytoplasmic inclusions could be observed in normal uninjured teeth.

Stanley & Swerdlow (1958) considered that a build up of intra-pulp pressure, associated with the inflammatory response, caused the aspiration. They believed that, whenever oedema, hyperaemia and fluid exudate occurs near the pulpal wall, aspiration of cells will be observed. Erythrocytes as well as odontoblasts will be involved.

Brannstrom (1960a,b; 1961; 1962a,b) reported on the dentinal and pulpal responses following the application of heat, pressure and airblasts. Contra-lateral teeth were used to make comparisons. Linden & Brannstrom (1967) and Brannstrom, Linden & Johnson (1968) investigated the effects of dehydrating solutions such as alcohol, sugar-syrup, silicate, phosphoric acid and calcium chloride applied

for 60 seconds to buccal cavities. The heat of drilling at 80,000 rpm, using a diamond without coolant for 12 minutes, was also observed. Aspiration of odontoblasts was observed in every case. This was attributed to the movement of tubule fluid contents.

Several papers have reported on the aspiration of odontoblasts beneath apparently intact dentine and enamel. These include the following: Nygaard-Ostby (1951); Shovelton & Marsland (1958); Stanley & Swerdlow (1958); Brannstrom (1962a) and Brannstrom & Lind (1965).

Brannstrom & Lind (1965) consider, however, that there has been some pulpal damage whenever aspirated odontoblasts are seen. They cite the case of aspiration occurring beneath incipient enamel caries (white spot without cavitation). Both James & Schour (1955) and Seltzer & Bender (1959) suggest that the force of extraction and even post-operative procedures may result in odontoblast displacement.

Vacuolization was considered indicative of odontoblast injury by Seeling & Lefkowitz (1950); Kramer & McLean (1952); Muller & Maeglin (1953) and Seltzer & Bender (1959).

Langeland (1957, 1961a) and Stewart (1961) found vacuolization in normal teeth. Stewart (1965b) considered that vacuoles in coronal odontoblasts are physiologic and not indicative of a cellular response to injury. Seltzer & Bender (1965) state "a number of conditions previously described as indicative of pathologic or regressive changes are now believed to be artifacts. Amongst these are so termed vacuolization of the odontoblast cells."

Further signs of odontoblast injury are listed as cytoplasmic granules; swelling; pyknosis; karyorrhexis; karolysis; disruption of the pulpo-dentinal membrane; reduced odontoblast numbers; blisters occurring in the odontoblast layer.

The following authors report that aspirated or destroyed odontoblasts are replaced by new odontoblast cells: Glass & Zander (1949); Zander & Glass (1949); Lisanti & Zander (1952); James et al. (1954); Marsland & Shovelton (1957); Seltzer & Bender (1959); Dubner & Stanley (1962); Searls (1967); Sveen & Hawes (1968) and Harrop & Mackay (1968).

Most of these authors suggested or implied that the replacement of lost odontoblasts took place by a process analogous to the development of the original cell from the dental papilla. Stewart (1962, 1965a) considered that the replacement of odontoblasts occurs continuously throughout the life of the tooth. He visualized undifferentiated cells migrating towards the odontoblast layer at the same time undergoing differentiation. Cells then were described as joining up with existing processes. This is a highly unlikely series of events. Odontoblasts are probably post-mitotic. It is more likely that chalone control type mechanisms exist in the pulp connective tissue as described by Bullough (1975) for epidermal tissue. Aging is related to mitotic inhibition. Tissues where chalone effectiveness is high are non-mitotic or post-mitotic. Aging is equally inhibited and so all cell loss is reduced. According to Bullough (1975), in populations of post-mitotic cells there is no cell loss. This appears to be the case with respect to the odontoblast cells.

(a) Healing patterns following pulp trauma.   There now follows a
review of the papers in which healing following pulp trauma was
investigated.   The prime object of this section is to describe
the so-called classical pattern of healing in exposed pulps.
Calcium hydroxide is universally accepted as the dressing of choice
when treating the exposed pulp tissue.

The three essential processes (i.e. active cell migration to fill
the defect, an increased mitotic rate by which cell loss is made good
and the production of new connective tissue) seen in most wounded
tissue are reported to occur within the traumatized pulp.

The processes of wound healing in the pulp appear to be similar
to those in other tissues.   Odontoblast replacement must occur by
cellular differentiation.   Many studies investigated healing
patterns and the intriguing question of the source of regenerated
odontoblasts.   A subsection is included to cover this particular
aspect of pulp wound healing.

(i)   Without frank exposure.   Sveen & Hawes (1968) ground
rat molar cusp tips to produce an area of damage in the pulp horn.
The rats were then sacrificed at various intervals. The sequence of
events such as odontoblast damage, inflammatory responses and healing
corresponded well with previous reports.   An area of necrosis was noted.
Spindle-shaped cells, stated by the authors to be odontoblast precursors,
appeared labelled after 24 hours.   Formation of a dentine bridge began
below the area of necrosis by 48 hours post-operatively.   The first
complete bridge was noted at 4 days;   this became thicker and more
regular with time.   At 8 days well defined tubules were seen.

The authors stated that the remaining vital odontoblasts did not proliferate. The new odontoblasts, responsible for dentine bridge formation, were derived from undifferentiated pulpal cells. Labelled cells which did not migrate towards the site of injury were thought to be newly differentiated fibroblasts or other new cells participating in the overall repair process. Zach et al. (1969) also used tritiated thymidine as a label. Small but deep cavities were cut in the molar teeth of albino rats. Mitotic activity was noted in the pulpal stromal cells as early as 2 days post-operatively and reached a peak by 8 days. Histodifferentiation was not reported to have occurred by this time (cf. Sveen & Hawes, 1968).

(ii) Following exposure. The following papers refer to histological evidence of the processes involved in pulp healing following exposure.

Hopewell Smith (1898) was one of the earliest investigators to use histopathological material. He acknowledged that the injured or infected pulp possessed a hitherto unaccredited healing potential. It was not until Glass & Zander (1949) conducted their experiments that a clear description of pulp healing was recorded. The exposed pulps of human premolars were dressed with either zinc oxide eugenol or calcium hydroxide and examined histologically at 1, 2, 4, 6 and 8 days post-operatively. Unsatisfactory healing was reported following the use of zinc oxide eugenol. An inflammatory reaction persisted at the site of the exposure and at the times of examination the pulp was vital though no bridging was observed. Under a calcium hydroxide dressing a re-establishment of the odontoblast layer and the formation of a calcific dentinal bridge was observed. After healing, the pulp

presented a normal structure and was free from inflammatory cells. Blood vessels were however reported to be dilated.

What is now accepted as the classical sequence of events in pulp wounds dressed with calcium hydroxide is summarised thus.

24 hours post-operatively: a necrotic layer of pulp tissue was seen in contact with the calcium hydroxide. In the underlying tissue haemorrhage was absent and only a few exudate cells were present. A deep basophilic zone (probably calcium proteinates according to the authors) demarcated the necrotic zone from the underlying vital tissue.

14 days post-operatively: the basophilic demarcation zone and necrotic tissue were still present. Against these were laid down new "course fibrous tissue". Deep to this a palisade of odontoblast-like cells formed. Beneath these later cells a normal inflammatory-free pulp was reported to exist.

28 days post-operatively: the necrotic layer was less prominent though the "proteinate zone" was visible. Against the fibrillar material was a calcified barrier with the characteristics of dentine and associated with a palisade of odontoblast cells.

Nyborg (1955, 1958) confirmed the sequence of events described above. These responses were reported to be similar in human and canine teeth. Nyborg observed pulp healing in dogs and human teeth, exposed and dressed with calcium hydroxide and inert material. Histological examination was conducted from 2 days to 16 months post-operatively. Three zones were described peripheral to the zone of hard tissue formation; a superficial layer of debris, a necrotic layer and a blood pigment layer. The last layer was near, if not actually in, the surface of the zone where calcification was

occurring. Nyborg described the healing process as that of blastema formation, his description of events being reminiscent of those occurring during amphibian appendage regeneration; the description of cells with bladder-shaped nuclei is especially similar to so called de-differentiated cells occurring in amphibian appendage regeneration. Nyborg's description was suggestive of a spatial reorganisation of pattern within the pulp and not just a reformation of a lost odonto-blast layer. He stated: "After 2-7 days, the cells have a bladder shaped nuclei with nucleoli, or they are of fibroblast appearance. After 14 days some of the cells resemble odontoblasts and appear along the dense zone. Beneath this zone a hard tissue forms. This was tubular and resembles dentine."

The function of the various cell zones beneath the odontoblast layer in the normal pulp is often overlooked possibly because of lack of knowledge concerning such functions. Nyborg's description of pulp wound healing indicates that pulpal events following trauma are similar to the process of regeneration without growth (i.e. morphallaxis) seen in other organs. The healed pulp, according to Glass & Zander (1949); Nyborg (1955, 1958) and Nyborg & Tullin (1965), should exhibit all the characteristic pattern and spatial organisation of differentiation seen in the normal pulp. When a gross disturbance of pattern occurs, e.g. following pulpotomy, reorganisation should occur both in terms of the reformation of the odontoblast palisade and subodontoblast zones. This is stated to bring to mind the fact that wound healing in the pulp is not solely a reorganisation of the odontoblast palisade. It involves a complex series of events involv-ing several cell types. Short term assessments of healing made

solely in terms of the reorganisation of the odontoblast layer, an associated hard tissue barrier and lack of inflammatory cells, may well not reflect a restoration of pulpal function in the long term.

Nyborg and Nyborg & Tullin outlined their criteria of healing as (1) no signs of inflammatory cells or exudate adjacent to the formed barrier; (2) good staining properties; (3) a normal pulp pattern including an apparently functional vascular system.

Nyborg did not define a satisfactory barrier in any precise terms. He implied, rather than stated, that bridges need not be of a dentinal nature for maintenance of pulp vitality. Pulp recovery was not excluded when there were signs of hyperaemia, vasodilation, reticulation, fibrin, extravasated lymphocytes or blood pigment within the pulp tissues.

Many authors have described pulp wound healing both in humans and in various animal species including Berk & Stanley (1958); Berman & Massler (1958); Berman (1958a,b); Seltzer & Bender (1958); Swerdlow & Stanley (1958); Nyborg & Tullin (1965); Engstrom & Spangberg (1967) and Rowe (1967).

The consensus opinion among these workers was that the primary calcific bridge is probably related to the degenerating cells whereas the permanent (tubular) dentine bridge is a result of a vital cellular process. New odontoblasts differentiate in about 14 days from the time of wounding; tubular dentine follows and is seen between 21-28 days post-operatively.

Clarke (1970) undertook a study to further investigate the processes of pulp repair following exposure. He used dog molars as

suggested in the papers of Schroff (1952) and Nyborg (1955). Though he attempted to produce standardized wounds, he found this technically difficult. All wounds were dressed with a calcium hydroxide/ distilled water paste. Amalgam restorations were inserted and the teeth obtained by surgical extraction at 24 hours and 1, 2, 4 and 8 weeks post-operatively. Two planes of wound healing were studied by sectioning teeth buccolingually and also mesiodistally. Routine histological stains were used.

Specimens in the buccolingual plane correlated closely with the descriptions of Nyborg (1955). The mesiodistal sections gave "manifestly different results". The zones described by Nyborg now presented as concentric rings. Clarke (1970) concluded: "The present findings suggest that the teleologic concept of the dental pulp repairing breached dentine by bridge formation is inaccurate. Rather, the reparative dentine is a segment of an overall encapsulation of the pulp wound formed in a zone of stimulation around a central irritant of insufficient severity to cause pulp death. This response would seem to correspond to the localization of an area of damaged connective tissue elsewhere in the body."

Papers by Stanley & Lundy (1972) and Tronstad (1974) suggest that significant differences in the pattern of healing may occur under different calcium hydroxide preparations. Stanley & Lundy (1972) capped exposed pulps with Dycal. The results were evaluated histo-logically. Macrophages were reported to phagocytose necrotic tissue. Bridge formation occurred directly against the layer of Dycal. Stanley suggested that a revaluation of the criteria for pulp capping procedures was in order with regard to the potential depth of the

chemical cautery of pulp tissue, whether pulp capping or pulpotomy
is the treatment of choice and whether embolism of calcium hydroxide
is a possibility. Tronstad (1974) treated exposed monkeys' teeth
with Dycal and controls with calcium hydroxide paste. He observed
the typical zones as described by Glass & Zander (1949) beneath the
paste of calcium hydroxide. The necrotic zone was seen adjacent to
the vital tissue and interposed between the vital tissue and the
dressing. Dycal treated teeth did not have this necrotic zone.
Tronstad actually stated that the tissue did not become necrotic
(cf Stanley & Lundy, 1972). The formation of a dentine bridge
occurred directly at the Dycal-pulp tissue interface. The tissue
was infiltrated with macrophages and giant cells.

(iii) Following pulpotomy. Davis (1921) was the first to
perform vital pulp amputations and to examine the process histo-
logically. He considered that the nature of the applied dressing
was unimportant. In his opinion, if gross infection was avoided,
the remaining pulp tissue repaired by processes similar in nature to
the general processes in other connective tissues. The obliteration
of the pulp canal by a layer of osteoid tissue was frequently
observed. Seltzer & Bender (1965) and Massler (1972) were concerned
that the use of calcium hydroxide would cause a persistent stimula-
tion of vital pulp tissue and the eventual obliteration of canals by
hard tissue as described by Davis (1921).

Dausch & Sauerwien (1952) used Calxyl as a pulpotomy dressing.
They considered that the necrotising effect of this preparation
lasted for 2-3 days. After 1-2 days calcification occurs between

the necrotised tissue and the vital pulp.   They outlined the
following series of events.   Twelve to twenty-four hours post-
operatively there is a serious infiltration with concomitant
hyperaemia and dilated vessels in the tissue beneath the necrotic
zone.   A moderate number of leucocytes were found perivascularly.
This cellular exudate disappeared after two days.   Collagen fibres
were laid down at right angles to the demarcation zone.   Calcifica-
tion occurred and was related to these fibres.   A definite odonto-
blast palisade with dentine deposition was reported six to eight
months post-operatively.

Berk & Cohen (1954) performed pulpotomies on seven non-infected
primary and permanent teeth.   The results were evaluated histo-
logically after five weeks.   One case was evaluated after 52 weeks.
The dressings used were calcium hydroxide and a calcium hydroxide
methyl cellulose paste.   Tubular bridges in association with a
single layer of odontoblast-like cells were noted at five weeks.   The
authors reported pulp tissue of normal appearance but with an
increased number of fibroblasts.

Berman & Massler (1958) used rat molar teeth.   Pulpotomy
wounds were dressed with calcium hydroxide.   Progress was followed
over a period of 7-28 days.   They concluded that a distinctive
pattern of healing occurred under large and small exposures.   The
following zones were described at 21-28 days: (1) a zone of pulpal
injury degeneration; (2) a primary calcific bridge; (3) a permanent
bridge of reparative dentine and (4) a reformed odontoblast layer.
New odontoblasts took approximately 14 days to differentiate; a
regular tubular dentine barrier took at least 21-28 days to form.

Masterton (1963, 1964) reviewed the various schools of thought regarding the healing process in the pulp. He concluded that the weight of evidence supported the contention that an odontoblast palisade reforms and partakes in the laying down of a reparative dentine bridge. Masterton (1966b) reported on healing patterns following pulpotomy in monkey teeth. All wounds were dressed with Calxyl. The results were assessed histologically. Masterton found that healing in monkeys and humans followed the same pattern, the sequence of events resembling those in other healing connective tissues. With Calxyl, the inflammatory response was not marked. When the vessels were cleanly incised, there was minimal haemorrhage and a tubular type of dentine was formed. When haemorrhage was severe, healing occurred by organisation of clot with callus formation. This is contrary to the findings of Seltzer & Bender (1958) who investigated the role of the blood clot in pulpal healing and found it had no effect, but in agreement with Schroder (1973) who stated that the extra-pulpal blood clot impairs healing under otherwise optimal conditions.

Masterton stated that the sequence of events in the course of healing following pulp wounds was similar to those that take place during tooth formation. In the first stage of dentinogenesis a basement membrane is formed. This consists of argyrophilic fibres derived from pulp cells. These fibres are arranged parallel to the eventual amelo-dentinal junction. Basement membrane formation occurs before, and entirely independently of, odontoblast differentiation. In the wounded pulp, a similar basement membrane formation occurs in the presence of a necrotic layer. No epithelial

cells exist in the pulp.   Masterton, however, considered that this
basement membrane assumes the inductive functions of ameloblasts.
"It would seem logical to assume that the ameloblasts and the
necrotic layer associated with a calcium hydroxide dressing possess
the essential factors for the induction of dentinogenesis."

Seltzer & Bender (1958) considered that calcium hydroxide was
the only calcium salt capable of stimulating reparative dentine.
Masterton (1966a) stated that the success of pulpotomy operations
was partly due to the surgical procedure and partly to the dressing
of calcium hydroxide.   He admitted that the role of the dressing was
not completely understood.

Schroder & Granath (1971b) and Schroder (1972) studied tissue
changes following experimental pulpotomies by light microscopy and
routine histological staining.   A barrier of collagen formed within
seven days beneath a calcium hydroxide induced zone of coagulation
necrosis.   After one month, a layer of bone-like tissue was
reported to have formed.   By three months, a second layer, more like
dentine, had been produced pulpal to this osteoid tissue.   Schroder
& Sundstrom (1974) studied pattern changes following experimental
pulpotomies in three teeth dressed with calcium hydroxide.
Electron microscopic techniques were used.   Intervals of seven days,
one and three months were chosen to investigate events.   These post-
operative times were selected on the basis of previous light
microscopic investigations.

The results indicated that a layer of matrix-producing cells
lined the vital pulp surface by one month.   These cells resembled
odontoblasts in appearance and function.   Adjacent to these cells

was a predentine-like tissue. Calcification commenced as spherical
bodies within the necrotic zone. Similar calcified spheres were
found within the vital pulp in association with degenerate cells.
Mineralization of new-formed collagen was considered to start from
these centres of calcification. The initial mineralization of the
barrier resembled that of normal mantle dentine.

Harrop & Mackay (1968) had reported similar phenomena using
electron microscopy. Calcium hydroxide had been used as a dressing
following the exposure of 150 rat incisor pulps.

Mitotic proliferation of pulp cells occurred by the second day.
The cells then underwent morphological differentiation. By the
third day collagen fibres were laid down. In undecalcified sections,
clusters of crystals were observed at this time in intimate relation-
ship to these fibres. A progressive deposition of crystals resulted
in large expanses of calcified material of a uniform nature. Harrop
& Mackay (1968) considered that the mitotic figures seen in both
light microscopic and electron microscopic investigations indicate
that the process of healing (reparative dentinogenesis) is analogous
to the development of odontoblasts from the cells of the dentinal
papilla.

Only one other ultrastructural report is to be found in the
literature. Svejda & Melkova (1968) reported on the effects of
calcium hydroxide on calf incisors. The bridge formed in inflamed
pulp tissue. Initially osteodine was laid down and subsequently a
more tubular type of dentine was formed.

Schroder & Sundstrom (1974) were in general agreement with
Harrop & Mackay (1968) as regards the sequence of events, i.e. the

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laying down of newly formed collagen fibres at about three days post-operatively followed by calcification at four to seven days. Harrop & Mackay considered that the calcification centres were nucleated by the collagen. Schroder & Sundstrom disagreed with this view. They stated that mineralization of the barrier starts with calcification of spherical bodies found in the coagulation zone as well as in relationship to degenerate cells in the adjacent tissue. From these initial sites of deposition, calcium salts were considered by the authors to spread to the collagen-rich vital tissue. Schroder & Sundstrom had conducted their investigations at seven days, one and three months post-operatively, on the basis of previous light micro-scopic studies. At three months, the barrier was of a distinct two layers, the initial portion being bone-like, the subsequent pulpal layer being of a tubular-dentine nature. This description is identical to that given by Svejda & Melkova (1968) and confirmed the light microscopic observations of Schroder & Granath (1971a,b) and Schroder (1972) and the scanning electron microscopic study by Schroder & Granath (1972).

From the review of the literature so far presented, it can be concluded that, at the descriptive level, wound healing within the traumatized pulp follows the general pattern of wound healing in other connective tissues. Schroff (1959) stated that the three phases of inflammation, regeneration of lost tissue and repair of exposed surfaces, seen in all wounds, were observable in the pulp. He stated the clinical problems associated with pulp healing to be

(1) the removal of existing irritants, mainly bacteria and bacterial products, and the associated caries; (2) the provision of a seal protecting the pulp from the oral environment and (3) the incorporation, either in or beneath such a seal, of a biologic wound dressing which will encourage natural growth and healing. It is of interest to note that calcium hydroxide was not, in Schroff's view, an ideal biologic dressing. He condemned its necrotizing effect on pulp tissue.

Healing depends on the existence of an optimal relationship between the inflammatory phase and the proliferative phase. This seemingly holds true for pulp tissue as well as for other connective tissue. An ideal biologic dressing may be defined as a material which promotes such an optimal relationship. Glass & Zander (1949) clearly showed that an irritant necrotizing agent such as calcium hydroxide promoted healing in the exposed pulp. Zinc oxide eugenol, a renowned non-irritant lining material when used in conventional cavities, did not promote healing or 'bridge formation' when placed over the exposed pulp. It would appear that the necessary optimum relationship between the two phases is not promoted by the zinc oxide and eugenol. This is reminiscent of non-healing obtained under non-irritant wound dressings by Carrell (1921).

It is most important to appreciate that many variables exist, both in the clinical and experimental situation, which influence the pulp's response to injury. The difficulties involved in isolating these variables have been stressed by Langeland, Tobon & Langeland (1968).

Biological status of odontoblast precursor cells

Several authors have alluded to the biological status of the odontoblast precursor cells. Discussion has been limited by the difficulties in investigating this aspect of pulp healing. The subject is nonetheless more than of purely academic interest.

Sveen & Hawes (1968) found that labelled cells lay closely adjacent to blood vessels. In their view, perivascular cells were capable of differentiation into cells other than odontoblasts. The precursor cells were considered to be "undetermined". Sveen & Hawes did, however, accept that the possibility existed that precursor cells were specifically pre-odontoblastic. In such a case, the cells are determined by some previous event or events. The possibility that two kinds of precursor cell population exist need not be excluded. Friedenstein et al. (1968) and Friedenstein (1968) assumed the existence of two kinds of osteoblast precursor cell. The first is genetically determined; the second exhibits osteogenic potency only under the influence of an inductor.

Zach et al. (1969) regarded all pulpal cells as being potenti-ally dentinogenic, constituting a multipotential pool requiring a suitable enzymatic stimulus for activation. Both Zach et al. and Sveen & Hawes regarded the initial response of the stimulated cells to be towards cellular differentiation and not towards replication.

Variables affecting the pulp response

(a) The carious lesion. Caries is the most common process affecting the pulp. Caries does not develop in germ free animals (Orland et al., 1955). It can be classified as microbial in nature. Miller &

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Massler (1962) classified caries according to whether it was clinically active or arrested; the extent of bacterial penetration as judged from serial section; the age of the patient; the epidemiological group susceptibility. Baume (1970b) and Massler (1972) distinguished between slow, intermittent or arrested caries and rapidly penetrating caries in caries susceptible persons. Massler (1972) cautioned against considering the carious process as being a single entity. He stated that lesions differed with respect to bacteriology, pulpo-dentinal reactions and rates of progression.

The carious process and the associated dentinal and pulpal changes have been extensively studied in order to establish reliable clinical guidelines.

(i) Bacteriology. The following authors have reported on the bacteriology of the carious dentinal lesion: Seltzer (1940); Dorfman et al. (1943); Besic (1943); Orland et al. (1955); MacGregor et al. (1956); Bradford (1960); Whitehead et al. (1960); MacGregor (1962); Tukuma & Kurahashi (1962); Parikh et al. (1963); Kakehashi et al. (1965, 1969); Sarnat & Massler (1965); Shovelton (1965, 1968); Fusayama et al. (1966); Massler (1967, 1972); Crone (1968); McKay (1969); Baume (1970a); Paterson (1972, 1974); Brannstrom & Johnson (1974); Johnson & Brannstrom (1975); Mjor (1977); Forsten & Karjalainen (1977).

Wound infection is due to bacterial invasion. The importance of the elimination of bacterial contamination in wound healing is well understood. Kakehashi et al. (1965, 1969) and Paterson (1972, 1974) have clearly demonstrated the importance of bacterial contamination as a factor influencing pulp responses.

(ii)   The relationship between bacterial colonies, softening of dentine and discoloured dentine.   In order to establish reliable clinical criteria for the proper excavation of a carious lesion, it is a necessary prerequisite to know the relationship between the recognisable clinical signs of caries (softening and discolouration) and the frontmost bacterial colonies.   Five more or less regularly superimposed zones in carious dentine have been described by the following authors: Furrer (1922);   Scott & Albright (1954);   Tukuma & Kurahashi (1962);   Frank et al. (1964);   Sarnat & Massler (1965). These zones are: (1) the zone of vital reaction;   (2) the zone of transparency or sclerosis;   (3) the zone of opacity or of incipient decalcification;   (4) the zone of bacterial colonies (advanced); (5) the zone of dentine decomposition.

Seltzer (1940) found that, in 93% of cases in vivo, micro-organisms could be detected in the base of deep cavities after the removal of all gross caries.   Dorfman et al. (1943) found that the decalcified but intact dentine under the base of a cavity was almost always sterile.   Parikh et al. (1963) agreed with the findings of Dorfman.   MacGregor et al. (1956) used histological and bacterio-logical methods to investigate the problem.   Complete correlation was not achieved but it was concluded that softening of dentine occurred in front of the colonies of organisms.   Whitehead et al. (1960) extended the latter investigation.   Two hundred deciduous and two hundred permanent teeth were studied.   After all softened dentine was removed from the cavity floor, 51.5% of permanent teeth were free from

signs of bacteria; a further 37% had 1-20 infected dentinal tubules per section. Deciduous teeth showed a higher incidence of infected tubules. Shovelton (1965, 1968) investigated the problem clinically and histologically. A series of 102 permanent teeth had cavities prepared (Shovelton, 1968). In some, all traces of soft or discoloured dentine was removed; in others, a soft or discoloured layer was left. Each tooth was classified according to colour and texture. The teeth were fixed, decalcified and stained with a modified Gram's stain. The histological findings were correlated with the clinical findings. In 64% of cases, the clinical impression of a clean cavity floor was confirmed histologically. On the other hand, 28% of cavities which were definitely suspect clinically were free from organisms. This finding was supported by those of McKay (1969) who dissected into the lesion from the pulp. In many instances he found that very soft dentine so obtained gave negative results when cultured. Shovelton (1968) and Crone (1968) found that bacteria could be isolated from definitely hard cavity floors.

Fusayama et al. (1966) sectioned carious teeth vertically; one half was observed for colour and rated for hardness, using the Knoop indentation test. The other half was decalcified and stained with Gram's stain. Organisms did not penetrate as far as the discolouration front which, in turn, did not reach as far as the zone of softening. This pattern differed in acute and chronic caries. In the former, the discolouration was far in advance of the organisms and there were layers of dentine, up to 2mm thick, which were soft or discoloured but non-infected. In chronic lesions, the bacterial

colones were very close to the discolouration front.

The structure of the carious dentinal lesion was studied using light and electron microscopy by Sarnat & Massler (1965); Fusayama & Kurosaki (1972); Fusayama & Terashima (1972); Ohgushi (1973); Kurosaki & Fusayama (1973); Oghushi & Fusayama (1975); Kuboki et al. (1977). The Japanese school described it as consisting of two layers, a superficial outer layer characterized by extensive decalcification, degenerated collagen fibres and devoid of odonto- blast processes, and an underlying second layer characterized by intermittent decalcification, sound collagen fibres and vital odonto- blast processes. The superficial layer is not recalcifiable; the deep layer is.

The electron microscopic studies of Sarnat & Massler and Fusayama confirmed that the deepest demineralized layers were bacteria free.

Shovelton (1972) reviewed the literature, including his own investigations, relating to bacterial penetration within the carious cavity. He concluded that not only was it unnecessary to remove all the softened dentine on the pulpal floor, since it was frequently sterile, but, paradoxically, seemingly hard dentine is often infected.

(iii) <u>The pulpal response to the carious lesion.</u> Shovelton (1972) stated that the correct lines of treatment should be based on an assessment of the carious lesion and the pulpal response to this lesion. This necessarily involves consideration of any dentinal defence reaction to the carious lesion and an appreciation of the extent to which this will modify pulp inflammatory processes. MacGregor et al. (1956) noted that there was an absence of inflammation

and bacterial contamination even when the carious lesion was well advanced. Seltzer & Bender (1965) stated that inflammatory changes only occur immediately prior to a carious exposure of the pulp. These observations substantiate the views of Fish (1932); Orban (1941); Bradford (1960) and Massler (1967) who maintain that, in the first instance, the pulpal reaction to caries is the production of a vital protective barrier. This is expressed by an accelerated sclerosis of existing dentine and the laying down of tertiary (reparative) dentine. Pulpal pathology is delayed until a late stage in the progress of the carious lesion.

The following authors have reported inflammatory reactions within the pulp in relation to incipient enamel caries: Nyborg (1955); Langeland (1957); Stanley (1962); Brannstrom & Lind (1965); Stanley et al. (1966) and Langeland & Langeland (1968). They contend that an acute pulpal reaction precedes any reparative response. In Langeland's opinion (personal communication) "reparative" dentine is a scar tissue replacing atrophied pulp tissue.

Bevelander & Benzer (1943); Whitehead et al. (1960) and Corbett (1963) dealt with the incidence of reparative dentine beneath carious lesions. Corbett (1963) found that reparative dentine was present in under half of the permanent teeth and in nearly three quarters of the deciduous teeth.

Corbett (1963) and Shovelton (1968) found that pulpal distur-bance was sometimes seen before there was bacterial infection of reparative dentine. Reeves & Stanley (1966) found that "once the carious process was within 0.55mm of the pulp, more pathological changes were seen but it was not until the secondary dentine itself

was involved that pathosis of real consequence was seen". Baume

(1970a) observed histologically some 400 teeth affected by caries.

He found a wide variation in pulpal response and considered this to

be a consequence of extrinsic carious factors and intrinsic host

factors. Deep caries was characterized by bacterial invasion of

tertiary dentine. When tertiary dentine was infected, this was

usually accompanied by the formation of an acute (polymorphonuclear)

micro-abscess. Baume was unable to establish any quantitative

correlation between depth of bacterial penetration and severity of

inflammation due to, in his opinion, method error inherent in histo-

logical serial observations. His observations were in general

agreement with Reeves & Stanley (1966) and Shovelton (1968) who had

presented their findings in quantitative terms.

Reeves & Stanley (1966) studied 46 teeth. They found no

significant pulpal involvement until the carious lesion was 1.1mm

from the pulp. When the remaining dentine was 0.5mm or less, the

amount of inflammation increased.

Shovelton (1968, 1970) found no signs of inflammation when there

was more than 0.8mm of dentine interposed between the bacterial

colonies and the pulp tissue. Severe inflammation ensued when the

remaining dentine was less than 0.3mm thick. Bacterial contamina-

tion of the pulp was found when the remaining dentine thickness was

less than 0.2mm. Rayner & Southam (1979) recorded comparable

results in deciduous teeth.

Thoma & Goldmann (1960); Kuwabara & Massler (1966); Massler

(1967) and Baume (1970b) have stressed the intermittency of the

progress of the carious lesion. In the view of Massler (1967)

variations in the pulpo-dentinal reaction are related to the character of the overlying lesion, i.e. whether the lesion was active or arrested. In addition to these extrinsic carious factors, Baume (1970a) considered that intrinsic host factors, such as caries susceptibility, modified the dentinal response and the associated pulp pathology.

Zerosi (1970) concluded that the normal pulp reaction to dentinal caries is to form tertiary dentine. In agreement with Yoshida & Massler (1964), Sayegh (1967) and Baume (1970a) stated that the width and structure of this formed dentine is related to the intensity, nature and duration of the stimuli. Under superficial caries, the stimuli would normally be of a mild nature leading to the formation of regular tubular tertiary dentine. More amorphous dentine is formed when the cavity is active and rapidly penetrating.

Anderson et al. (1968) concluded that the dentinal response reflected existing pulp morphology and vice versa. A range of types of reparative dentine was noted in experimental tooth replants. In the apical portions, where the blood supply was good, healing was quicker and the dentine more highly organised than in coronal areas where the pulp was degenerate and the blood supply poor.

It should not be concluded from this section that pulp pathology is always due to bacterial infection. Nor should it be thought that a non-infected pulp is free from pathological changes.

Massler (1972) differentiated between an "infected pulp" and an "affected pulp". Shovelton (1962) and Massler (1972) agreed that bacterial toxins, as well as bacterial contamination, can cause pulp reactions. Baume (1970a) reported acute polymorphonuclear micro-abscesses

in relationship to infected dentinal tubules. Serial sections, however, showed that a zone of non-infected tertiary dentine was interposed between the infected dentine and the non-infected pulp tissues. Baume concluded that, in caries susceptible teeth, where the carious lesion was of a rapidly penetrating type, and had progressed to a considerable depth, pulp abscesses were prevalent. He stressed that this type of pulp pathology occurred prior to pulp exposure and bacterial infection of the pulp tissues.

Mjor (1972) sealed carious, infected dentine in cavities prepared in non-carious monkey teeth in vivo. Severe pulp reactions were observed. Mjor suggested that the cause was likely to be toxic substances originating from the infected dentine. He further suggested that immunological mechanisms may have been involved. There is an increasing awareness that immunological mechanisms may contribute to pulpal inflammation. This notion has been expressed by various authors including Langeland (1972); Seltzer & Bender (1975); Pulver et al. (1977); Seltzer et al. (1977); Bergenholtz et al. (1977) and Torneck (1978).

It is relevant to consider at this point the evidence which indicates that pulp tissue injury may be initiated or maintained by immunological mechanisms.

Torneck (1974a,b) conducted investigations with light and electron microscopy on human teeth exhibiting a carious pulpitis. The electron-microscopic observations indicated degenerative changes in blood vessels ranging from minor alterations in endothelial cell morphology to complete necrosis and lysis. Advanced changes in the blood vessels appeared to result in an increased vascular permeability

causing oedema.   It was suggested that some of the degenerative

changes might be related to the humoral phase of the inflammatory

response.

The basic inflammatory infiltrate was shown to be predominantly

mononuclear, with plasma cells predominating.   From their morpho-

logical appearance, it was considered that these cells were engaged

in protein (antibody) synthesis.   The stimulus for antibody produc-

tion could arise from substances emanating in the carious lesion or

from locally sensitized dental pulp tissue.

Kearney (1974) suggested that chemotactic substances produced by

lymphocytes present in inflamed tissue may produce alterations in the

inflammatory cell population as seen in caries induced pulpitis.

Torneck (1977) noted that a common finding was the change in cell

population of the inflammatory infiltrate.   He noted that lymphocytes

and plasma cells were associated with inflammatory changes related to

early caries.   As the carious lesion advanced, there was an observed

increase in macrophages and polymorphonuclear cells.   Torneck

suggested that these changes in cell populations may be due to an

increase in the severity of the stimulus or an increase in diffusi-

bility of antigenic substances present in the pulp.   He also was

aware that the number of phagocytic-type cells in advanced

inflammatory states may be responsible for cellular pulp injury.   The

presence of extracellular lysosomes from degranulating or ruptured

phagocytic cells was a frequent finding.   This was in contrast to

early carious pulpitis when the presence of extracellular lysosomes

was markedly less.

Bergenholtz & Lindhe (1975) and Bergenholtz (1977) sealed crude extracts from human dental plaque, and also material produced from micro-organisms cultivated from human dental plaque, into cavities cut in non-carious teeth. Both substances had the capacity to induce or mediate acute inflammatory reactions in the dental pulp. Bergenholtz concluded that freshly cut coronal dentine has a poor capacity to protect the pulp from noxious agents. From his observations he was not surprised that under 'normal' conditions pathological reactions are detectable when the carious lesion reaches the dentine.

Bergenholtz et al. (1977) applied bovine serum albumin to exposed dentine to assess whether inflammatory reactions can be induced in the pulps of monkeys immunized against bovine serum albumin. Controls were used. Severe inflammatory lesions were observed in the experimental teeth, characterized by haemorrhage and a large leucocyte infiltration. Extensive tissue damage ensued. The results suggest that antigen-antibody interactions can occur within the pulp and mediate pulp tissue injury.

Torneck (1974a, 1977) showed that the inflammatory infiltrate associated with the deep carious lesion is primarily mononuclear. This suggests that a delayed type of hypersensitivity reaction may be involved. Modification of an Arthus type or an immune complex type reaction may lead to tissue injury. Baume (1970a) described micro-abscesses associated with a polymorphonuclear infiltrate. These abscesses were prevalent beneath deep carious lesions and occurred before pulp exposure.

It appears that these pathological changes are possibly of an immunological nature and that they occur before actual bacterial

invasion of the pulp.   Antigenic material arising in the carious

lesion must gain access to the tissues by penetrating the remaining

dentine.   A tubular pathway is frequently suggested.   Inflammatory

changes are seen in relationship to tubules associated with the

cavity base.   Moreover, as shown by Seltzer et al. (1961a,b)

bacteria placed on the base of freshly cut deep cavities in dogs'

teeth can reach the pulp, particularly if pressure is applied or if

the dental tubules are opened by the application of phenol.   The

many variables contributing to bacterial ingress to pulp tissue will

be discussed later.   It remains to note the type of antigenic

material available as possible immunological tissue injury

initiating substances.   Metabolites or exotoxins produced by

micro-organisms in the carious lesion or by micro-organisms which

gain access to the restoration by micro-leakage are antigenic.

Metabolites and exotoxins are the products of viable organisms.

Seltzer & Bender (1965) state that the use of cavity-sterilizing

agents to kill micro-organisms in the dentinal tubules is to be

discouraged since the medicaments are often more damaging to the

pulp than are the few micro-organisms within the dentinal tubules.

Antigenicity is not a property exclusive to the viable organism.

Immunological reactions may be initiated by substances that arise

from micro-organisms subsequent to their death, i.e. portions of

their cell wall and endotoxins.

It is beyond the scope of the present text to discuss the

immunogenicity of these substances in further detail.   It is, however,

important to appreciate that such mechanisms may be involved.

Massler (1972) distinguished between the infected pulp and the

affected pulp.   He made this distinction for therapeutic purposes.

He stated that, if the tissue is infected, it should be removed.   If
the tissue is affected by microbial products, but not invaded by large
numbers of pathogens, therapy should, in his opinion, be directed
towards removing the source of the irritants.

(iv) The fate of organisms remaining in the base of prepared
cavities.   Besic (1943); King et al. (1965); Aponte et al. (1966)
and Fisher (1966, 1972) sealed carious cavities and followed the
progress of the lesion and the bacterial populations over a period of
time.   Besic found that the progress of the lesion gradually
diminished over a 12 month period once it had been sealed from the
environment.   Lactobacilli, staphylococci and streptococci appeared
to have a variable resistance to these conditions.   Lactobacilli
disappeared between 2 and 10 months; streptococci were cultured up
to 12 months and were obtained in one third of the cases at the end
of the investigation.   King et al. (1965) found that a higher
percentage of negative cultures were obtained when zinc oxide and
eugenol were used to line the cavities than when calcium hydroxide
was used.   King et al. (1965) left the deepest layer of caries
beneath fillings and cultivated this at various post-operative times.
Organisms were obtained routinely under unlined amalgam but in 82%
of cases where zinc oxide was used as a lining, and in 61% of cases
where calcium hydroxide was used, no viable organisms were cultured.
These results suggest that the sealing properties of the zinc oxide
and eugenol are a significant factor in the elimination and prevention
of bacterial contamination beneath restorations.   The sealing
property of any lining may be as important a long term factor in the
maintenance of pulp vitality as is its non-irritant or bactericidal

effect. Fisher (1966) found lactobacilli persisted for long periods under non-antiseptic amalgam restorations. However, Fisher (1972) found no viable bacteria could be cultivated from previously infected carious dentine after six months when this had been lined with calcium hydroxide.

Aponte et al. (1966) studied teeth over periods ranging from six months to four years. Calcium hydroxide had been used to line residual infected caries. On re-opening the teeth, 93% of all cases were reported as giving negative cultures.

The consensus opinion, as expressed in various papers already cited, is that living micro-organisms do not normally occur in the non-demineralized dentine beneath a carious lesion. This may not be the case subsequent to cavity preparation. Lundy & Stanley (1969) left prepared cavities in human teeth exposed to saliva and to plaque formation. Bacterial penetration into dentinal tubules was observed after six days. Maximum penetration occurred by 210 days and the average depth of penetration was 0.52mm after an average period of 84.2 days. They concluded that "the visualized bacteria moved through the patent dentinal tubules at no great speed."

Tronstad & Langeland (1971) observed that bacterial invasion occurred in dentinal tubules affected by abrasion. The areas of penetration were confined to the incisal half of the dentine exposed by attrition.

Vojinovic et al. (1973) and Olgart et al. (1974) agreed that conditions at the outer apertures of tubules seem to affect the ingrowth of micro-organisms. Acid treatment of dentine facilitates penetration of bacteria into dentinal tubules beneath cavities filled

with composite material (Vojinovic et al., 1973).   In in vivo and in vitro experiments, Olgart et al. found that the outward flow of fluid in the dentinal tubules due to intrapulpal pressure may mechanically hinder bacterial growth.   Of greater importance as an obstruction was the blocking of the outer apertures by grinding debris.

Mjor (1977) also demonstrated, histologically, bacteria subjacent to dental restorations.   He considered that the disinfection of cavities as an additional step to conventional preparation techniques, was unnecessary.

Forsten & Karjalainen (1977) compared the effectiveness of cavity cleaning agents on the microbial activity in carious dentine. Occlusal cavities were cut in human molars.   The teeth were removed by conventional means and preserved in continuous humidity after extraction.   Bacteriological samples were obtained after the cavities had been rinsed with water only, after experimentally infecting the cavity and after treating the experimentally infected and the uninfected cavities either with saturated calcium hydroxide or with chlorhexidine.   Samples were cultured on blood agar plates aerobic- ally and anaerobically.   Cavities rinsed only with water showed sparse bacterial growth.   The authors concluded that this was an adequate clinical procedure.   Their conclusions are in general agreement with Shovelton (1969).   He, moreover, was voicing the generally accepted notion, as obtained from a review of the literature, that the remaining micro-organisms will lose their vitality once a restoration has been inserted.   This is especially so when a calcium hydroxide lining is used.   Though this would seem to be the consensus opinion, it is not universally accepted.   Brannstrom & Johnson (1974)

and Johnson & Brannstrom (1975) question this notion. They
emphasized that bacteria remaining in the floor of a cavity will have
an irritant effect on the pulp. Brannstrom & Nyborg (1971, 1974)
have reported bacterial growth in dentinal tubules, especially
beneath silicate cement. They assumed that the toxins from such
a bacterial colony, rather than the irritant qualities of the
silicate, were the cause of pulp inflammation. They suggested
that such bacteria may have existed on the cavity walls before
restoration or may have entered from the oral cavity through leakage.

Viable organisms, from whatever source, have the potential to
reactivate the carious lesion. In electron microscopic investiga-
tions, Takuma & Kurahashi (1962) showed that dentinal caries started
in dentinal tubules, progressed into the peritubular dentine and
thence to the intertubular substance. Demineralization occurs
first; this is followed by dissolution of the organic matrix. This
would suggest that it is important to eliminate bacteria from tubules
prior to cavity restoration. Some of the commercially available
cavity cleansers have a demineralizing action and are not microbicidal
(Brannstrom & Nyborg, 1973).

(v) Remineralization of carious dentine. Sobel et al. (1957);
Solomons & Neuman (1960); Sobel (1961); Eidelman et al. (1965);
Ehrenreich (1968) and Kato & Fusayama (1970) have demonstrated
that demineralized dentine is capable of undergoing remineralization.
A remineralization of demineralized carious dentine is considered to
retard further progress of the lesion. Eidelman et al. (1965)
measured the phosphorus content of dentine before and after the
application of calcium hydroxide. An increase in mineral content

was attributed to an exchange of minerals from the pulp into the
dentinal tissues.

Mjor et al. (1961) evaluated the effect of calcium hydroxide
and amalgam on non-carious vital dentine.   The effect of zinc oxide
and eugenol on dentine was evaluated by micro-hardness tests (Mjor,
1962).   Microradiographic techniques were employed to assess the
increase in radiopacity of human coronal dentine following the
insertion of various filling materials (Mjor, 1967).   An increase
in the mineral content of dentine covered by $Ca(OH)_2$ was attributed
to a precipitation of mineral salts from the oral environment, as
was the increase in the mineral content of the surface layer of
dentine exposed to the oral environment.   The increased mineral
content of corticosteroid covered dentine appeared to be a progressive
and active mineralization involving distinct alterations in the
organic matrix.   Kuboki et al. (1977) considered that the presence of
a vital odontoblast process may be a necessary factor for the recalci-
fication of carious dentine.   The presence of vital processes would
account for the physiological recalcification of the demineralized
zone deep to the carious lesion.   He also considered that, in this
zone, the character of the organic fraction and the nature of the
collagen fibres predisposed to the precipitation of calcium and other
metallic salts.   Kurosaki & Fusayama (1973) showed that metallic
elements penetrated the superficial layers of carious dentine and
accumulated in the second layer.   Electron microscopy indicated that
the second layer had dense regularly arranged collagen fibres with
characteristic crossbands similar to sound dentine (Ohgushi & Fusayama,
1975).   In a further study, Kuboki et al. (1977) compared the collagen

fibres of both layers chromatographically with sound dentine.   The

deep layer showed a change in cross-linkage but this was considered

reversible.   The superficial layer was considered to be irreversibly

denatured.   The importance of collagen fibres in the process of

remineralization has been reported by Johansen & Parks (1961) and

Johnson et al. (1969).   It is generally accepted that the remineral-

ized layer retards the progress of any future dentinal lesion

initiated by bacterial action.   Bradford (1960) and Young & Massler

(1963) suggested that the penetration of bacteria may be impeded by a

superficial layer of carious dentine.   This was based on the observa-

tion that carious dentine was more resistant to decomposition by acids

and proteolysis than was normal mineralized dentine.   Stated this

way, it may imply that a layer of carious dentine is more protective

to the pulp than a layer of remineralized dentine.   Electron micro-

scopy investigations by Takuma & Kurahashi (1962) have indicated that

the dentinal caries progresses by dissolution of the inorganic

constituents and then the breakdown of the organix matrix and that

the process commences within the dentinal tubules.   A remineralized

zone will retard the ingress of bacterial colonies into these tubules.

(b) Cavity preparation.   It has long been known and accepted that the

physical act of cavity preparation must have, in itself, an effect on

the pulp tissues.   Langeland (1967) and Stanley (1968a,b) outline

criteria for human pulp study and the evaluation of operative techniques

and restorative materials.   In an attempt to reduce variables, teeth

with intact, uninflamed pulps are most frequently used in such investi-

gations.   As a consequence of this approach, less is known about the

influence of clinical procedures on the inflamed pulp. Moreover, this

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widespread use of caries free teeth usually scheduled for ortho-
dontic extraction tends to preclude long term experimental periods
between the preparation of the cavities and extraction. Brannstrom
(1960a) considered a two month period as long.

Shovelton (1972) succinctly reviewed the literature. He
emphasized the need to prevent pulp damage during operative procedures
as well as outlining the ways of minimising pulp damage during
restorative procedures. Seltzer & Bender (1965) discussed the
various parameters, i.e. cavity depth, speed of instrument rotation,
heat and pressure, coolants, and their influence on the pulp tissue.
Marsland & Shovelton (1957) and Langeland (1961a) indicated that, at
very low speeds (below 500 rpm), odontoblast reactions (a sensitive
indicator of pulpal injury)were absent or minimal. With the advent
of high and ultra-high speed instruments, it was appreciated that
the chief hazard to the pulp would arise from frictional heat.
Coolants, especially water, are essential if severe pulpal damage is
to be avoided.

The following papers report on the immediate and deferred
responses of the pulp to operative techniques. Kramer (1959)
reviewed the effects of cavity preparation and concluded that the
main threat to the pulp was the frictional heat generated during the
procedures. This was in agreement with the observations of Marsland
& Shovelton. These authors conducted further experiments (Marsland
& Shovelton, 1970) and found that increasing cavity depth produced
increased damage at the same speed. Young teeth had a greater
resistance than older teeth to the iatrogenic damage of cavity

preparation.   Moreover, carious teeth showed less pulpal irritation following cavity preparation than did sound teeth.   They proposed that this was due to the existing defence responses in carious teeth.

Swerdlow & Stanley (1958) noted that speeds in excess of 20,000 rpm produced odontoblast damage, with or without coolant.   The damage was, however, more severe when coolant was not used.   With the proper use of coolant, pulp reactions were minimal and clinically acceptable even at speeds of 50,000 to 250,000 rpm.   Langeland (1959) found that odontoblast damage was eliminated when water spray was used at speeds of 50,000 rpm.   Morrant & Kramer (1963) and Marsland & Shovelton (1970) indicated that water coolants do not completely prevent pulpal damage.

Dehydration has been cited as another stimulus which is injurious to the pulp (Brannstrom, 1960b; Linden & Brannstrom, 1967).   Seltzer & Bender (1965) were of the opinion that the inevitable consequence of all operative procedures "is to age the pulp tissues, i.e. to bring about the quantitative reduction of cells and increase in fibrosis."   Marsland (1965) had a somewhat conflicting opinion. He stated that ultimately there is the same degree of repair irrespective of the type of coolant and this is especially so in young, healthy teeth.   There is no decrease in the number of pulp cells and no fibrosis correlated with an initial degree of damage. This is in agreement with Weinreb et al. (1967) who indicated the enormous recuperative capacity of the pulp, far beyond that normally described in short term histological investigations of clinical procedures.

Langeland (1959) evaluated pulp responses to Class I and V type cavity preparations cut at speeds of 6,000 rpm and 300,000 rpm and variable coolants. Water spray eliminated odontoblast displacement and hyperaemia produced when air alone was used to cool instrument tips revolving at 50,000 rpm. He was not completely convinced that lack of coolant alone was the decisive factor in producing adverse pulpal responses. This view is substantiated in a paper by Bhaskar & Lilly (1965). They monitored temperature changes in the pulp in association with the use of high speed instruments. It is of considerable interest to note that a temperature drop of 2.5°C was registered when coolants were not used. A temperature drop of 8.1°C was observed when coolants were used. The authors suggested that the obvious conclusion was that pulpal changes and damage associated with cavity preparations were not due to heat per se.

Bodecker (1939); Peyton (1958); Vale (1959); Schuchard & Watkins (1961, 1965); Zach & Cohen (1963) and Prensky (1971) monitored pulpal changes in association with the use of high speed instrumentation. All reported pulpal temperature increases with operative procedures. Zach & Cohen (1963) found that drilling at high speeds without water spray produced a linear progressive intra-pulpal temperature increase. Coolants were advocated at all speeds as a necessary precaution against pulpal damage.

Zach & Cohen reported on the pulp responses to injury. An increase in alkaline phosphatase concentration was observed in the reactive area. This was heaviest in the layer of Weil. Changes in the ground substances and a shift towards acidity of the mucopoly-saccharide matrix in the tissue undergoing repair was reported. This

is consistent with histochemical reports on alkaline phosphatase
activity in developing teeth as reported by Nuki & Bonting (1961);
Ten Cate (1962); Baratieri (1964) and Goggins & Fullmer (1967).
In the developing tooth the greatest alkaline phosphatase concentra-
tion is found in the narrow zone of pulp tissue immediately deep to
the odontoblast layer and along the course of the von Korff fibres.
The odontoblasts and general pulp tissue show a lower level of
enzyme activity.   This distribution of alkaline phosphatase activity
is in keeping with the view that the enzyme is implicated in fibrous
protein synthesis.   Evidence to substantiate this is presented by
Zach et al. (1969).   Small deep cavity preparations were cut in rat
molar teeth, subsequent to the animals being injected with tritiated
thymidine.   Mitotic activity was noted in the pulp stromal cells
two days post-operatively and labelling reached a peak at eight days.
The authors concluded that the early response of pulpal fibroblasts is
to organise collagen as dentine matrix following a sublethal injury.

The consensus opinion is that teeth can be drilled or ground
without fear of lasting pulp damage provided adequate coolants are
used (Marsland & Shovelton, 1970;   Langeland et al., 1971;
Shovelton, 1972).

(c)   Remaining dentine thickness.   Schroff (1946), Swerdlow & Stanley
(1959) and Stanwich & Stanley (1967) reported on the effect of the
remaining dentine thickness and its effect on pulp responses to
thermogenic procedures.   It was statistically substantiated that
the remaining dentine thickness is the most important single parameter

in deciding the incidence of pulpal response. A remaining dentine
thickness of 2mm or more will provide an adequate insulating
barrier against even the most traumatic operative procedures.
With a decrease in dentine thickness, there is an increase in pulpal
damage even if the frictional heat is controlled. Stanwich & Stanley
(1967) stress that a response to cutting only occurs in areas
beneath freshly cut virgin dentinal tubules not lined with repara-
tive or irregular dentine. Minimal responses are seen when repara-
tive dentine is involved.

The remaining dentine thickness qualitatively and quantitatively
is now generally accepted as the most important parameter in the
understanding and interpretation of the human dental pulp to known
traumatic stimuli (Schroff, 1946; Swerdlow & Stanley, 1958, 1959;
Stanley, 1962). It is more important than the clinical depth of
the cavity. A cavity is defined as being 'deep' if it is thought to
have a remaining dentine thickness of 2mm or less. Clinically,
this may not be quite so easy to assess. Reparative dentine, if
present, being less highly mineralized than normal dentine, is often
undetectable on radiographs.

(d) Dental materials. Pulpal response to caries and restorative
procedures may be modified by the presence of dental materials. The
biological principles relating to restorative techniques, now more
or less universally accepted, stemmed from the pioneering work of
Fish (1932); Van Huysen (1933) and Manley (1936, 1941). A direct
consequence of this early work was the emergence of bland cavity
liners to reduce the adverse effects of non-biological irritant

restorative materials. A 2mm dentine barrier between the floor of the cavity preparation and the pulp provides an adequate insulating barrier against the more traumatic thermogenic operative procedures and most restorative materials (Stanley, 1968a). This remaining dentine thickness represents an extremely critical factor in determining the severity and incidence of pulp lesions to various experimental procedures. The nature of the dentine covering the pulp also determines the severity of the response (Stanwich & Stanley, 1967). In a deep cavity, restorative materials and liners can be expected to have some effect on the pulpal response. The effect of the same material on the exposed pulp may not be the same as that mediated via intact dentine.

The action of the materials employed in the clinical study will be considered in relation to their effect when placed within a deep cavity and also when placed in contact with exposed pulp tissue.

(i) Zinc oxide and eugenol

(1) The effect in the deep cavity. The following investigators conducted histologically assessed trials. All agree that zinc oxide eugenol is not irritant when applied to intact dentine.

Human studies:

Kramer & McLean (1952); Stewart & Kramer (1958); Langeland (1959, 1961a); Dubner & Stanley (1962).

Animal studies:

Manley (1936, 1941); James & Schour (1955); Schour & Mohammed (1955); Silberkweit et al. (1955); Mohammed et al. (1961); Bhaskar et al. (1969).

Bhaskar et al. (1969) compared four zinc oxide based materials. Their effects were assessed according to the degree of inflammation and odontoblast disruption. All materials were found to be biologically acceptable.

Stanley (1968b) advocated the continuing use of zinc oxide and eugenol as a control restorative material.

Kerkhove et al. (1967); Mjor (1962, 1967) and Ehrenreich (1968) presented evidence which indicated that zinc oxide and eugenol will cause a remineralization of carious dentine.

Turkheim (1953) showed that in vitro micro-organisms were inhibited by zinc oxide and eugenol paste. The carious dentine was found to be sterile after 24 hours. King et al. (1965) showed that in vivo the bacterial count within deep carious lesions was reduced or eliminated by zinc oxide and eugenol linings.

(2) The effect on the exposed pulp. Glass & Zander (1949) categorically stated that healing does not take place when zinc oxide and eugenol dressings are placed directly in contact with the exposed pulp. This opinion has become generally accepted and zinc oxide and eugenol is considered an unsatisfactory dressing material whenever there is an expected exposure or a frank exposure. A brief review of the papers shows, however, that this is not the unequivocal view.

Rzeszotarski (1939) found that an extensive inflammatory reaction occurred and he considered the treatment a failure.

Luks (1954) on similar grounds condemned the use of the material in connection with the exposed pulp. These authors are in general agreement with the opinions of Glass & Zander (1949). In human premolars capped with zinc oxide eugenol, a polymorpho-nuclear infiltration was observed at the exposure site after 24 hours. There was no evidence of repair at eight weeks. The exposure was walled off by chronic inflammatory cells and there was no evidence of calcific repair except in relationship to dentine fragments. Sayegh & Reed (1967a,b) reported diffuse inflammation at the site of exposure in 12 human teeth. No barrier formation was reported.

In contrast to these findings, Gardner (1950) and Berman (1958b) reported some success with zinc oxide eugenol. The latter reported the formation of a tubular dentine barrier in some cases. Nakamura et al. (1966) also reported bridge formation and stated that the material had "no dangerous effect on pulp tissue".

Shovelton et al. (1971) conducted a comparative trial on the efficacy of several pulp capping materials, including zinc oxide eugenol. It was found that the differences in success rate were not statistically different at the 5% level (χ^2 test) though zinc oxide eugenol came last with 76% success rate at 12 months, dropping to 69% after two years.

Langer et al. (1970), using contra-lateral human premolars, compared Calxyl along with Calxyl and zinc oxide eugenol in the treatment of exposed pulps. They considered that a

chemical reaction occurs between Calxyl and zinc oxide eugenol which affects the properties of the combined medicament. Dentine bridge formation was found to be better in the zinc oxide eugenol plus Calxyl group.

In the following papers the effect of zinc oxide eugenol is reported when used as a dressing in exposed pulps in various non-human studies: Jensen (1957); Berman (1958); Berman & Massler (1958); Mohammed et al. (1961); Sela & Ulmansky (1970); Weiss & Bjorvatn (1970).

The consensus opinion was that the material evoked a persistent type of chronic inflammation. Bridge formation was seldom observed and, where evidenced, was often in relationship to dentine chips.

(3) <u>The effect of implantation in subcutaneous tissues</u>. Beagrie et al. (1972) compared the reaction of subcutaneous rat tissue to the implantation of zinc oxide eugenol cements and zinc polyacrylates. At all intervals from 2 to 32 days, the tissue reaction to zinc polyacrylate was less than to zinc oxide eugenol. The response to zinc oxide eugenol was severe during the first week, though, by 32 days, both materials produced a mild reaction. The general trend of these implantation results was found by the authors to be similar to a number of recent studies of pulp reaction to the same materials.

(ii) <u>Calcium hydroxide</u>

(1) <u>The effect in the deep cavity</u>. The use of calcium hydroxide as a cavity liner was first employed to reduce the sensitivity of cut

dentine and to protect the pulp from the deleterious effects of silicates and zinc phosphate cements (Berk, 1957).

The following are among the papers which assessed the effect of calcium hydroxide on the pulp by routine histological methods.   In all cases the calcium hydroxide was used to line experimental deep cavities and, in most cases, controls lined with zinc oxide eugenol were used.

Stewart & Kramer (1958), using human premolars, found no difference in pulpal response to calcium hydroxide and zinc oxide eugenol after 21-24 days.

James & Schour (1955) and Mohammed et al. (1961) used dogs' teeth to investigate the pulpal response to calcium hydroxide. The former authors reported a mild inflammatory reaction which decreased over a two week period.   Normal reparative dentine was formed.   The latter authors compared the effects of calcium hydroxide with zinc oxide eugenol.   The remaining dentine thickness ranged from 25 to 275 μm.   There was little evidence of pulpal injury whether calcium hydroxide or zinc oxide eugenol was used.

Sayegh & Reed (1967a,b) used Hydrex to indirectly cap human pulps.   The response was assessed histologically.   They reported that Hydrex caused a more intense repair response than zinc oxide eugenol.   There was less chronic inflammation however with the calcium hydroxide based material.   The remaining dentine thickness was not reported.

Tronstad & Mjor (1972), using intact human bicuspids, compared the four calcium hydroxide based products. Cavities, without exposures, were prepared and lined with one of the products. The pulpal response was assessed histologically after eight days. All products elicited reactions similar in nature and characterised by slight to moderate inflammatory changes in the underlying pulp.

Heys et al. (1976), using monkey teeth, compared Dycal, Pulpdent, MPC10 and MPC12. The products were applied to cavities without exposures. All products elicited similar responses at all time intervals. The authors did, however, consider Pulpdent to have a more severe action and attributed this to its water based nature and greater penetrability via the dentinal tubules.

Mjor et al. (1961); Mjor (1967) and Mjor & Furseth (1968) have studied the remineralization of dentine using microhardness tests, densitometric evaluation of microradiographs and electromicroscopic methods. Mjor concluded that the increase in mineral content of calcium hydroxide covered dentine is due to a precipitation of mineral salts. He compared this with the increased mineral content of corticosteroid covered dentine which was attributed to a progressive and active mineralization involving distinct alterations in the organic matrix.

Eidelman et al. (1965) estimated the phosphorus content of dentine before and after the application of calcium hydroxide. The increase in mineral content was suggested to be due to an

exchange of minerals from the pulp to the dental tissues.

Kato & Fusayama (1970) set up an experimental model to investigate remineralization. Dentine at the base of Class V cavities was softened by acid to simulate caries. The cavities were dressed with calcium hydroxide, zinc oxide eugenol, or left open.

Calcium hydroxide has been shown to have bactericidal action when placed as a lining in prepared cavities (King et al., 1965; Aponte et al., 1966; Fisher, 1972; Forsten et al., 1977 and Fisher & McCabe, 1978). Eidelman et al. (1965) considered that the bacteriostatic and bactericidal action of calcium hydroxide, together with the high alkalinity of the lining, aided in the arrest of the carious process.

(2) The effect on the exposed pulp. The effect of calcium hydroxide on the exposed pulp has been dealt with in a previous section.

(3) Variations in reactions to different calcium hydroxide based materials. Masterton (1964) considered that calcium hydroxide and water paste and Pulpdent had similar actions. Calxyl has a lower pH ($\bar{c}$ 11) due to the addition of serum salts. According to Masterton, the action of Calxyl was more gentle and physiological and this dressing was therefore superior to the other two.

A different healing pattern was reported following the use of Dycal (Stanley & Lundy, 1972; Tronstad, 1974). Bridge

formation occurs directly against the dressing following phagocytosis of the chemically cauterized tissue by macrophages.

Hydrex has an unfavourable effect when placed on the exposed pulp. ·Delaney & Seyler (1966); Sayegh & Reed (1967b); Phaneuf et al. (1968) and Hirchfeld et al. (1972) have assessed the effects of Hydrex histologically. The latter capped pulp exposures in rat molars with Hydrex or amalgam. Normal healing patterns with bridging were seen beneath amalgam but necrosis occurred when Hydrex was used. It was concluded that some component of Hydrex was toxic to pulp tissue.

Sela, Hirschfeld & Ulmansky (1973) investigated the toxic effect of Hydrex. In a study using rat molars, exposures were dressed with either the Hydrex base (the calcium hydroxide containing paste) or with the catalyst paste. All teeth treated with the base were reported to have healed as assessed histologically. Teeth dressed with the catalyst were found to be necrosed.

Barker & Lockett (1972) acknowledged that the reaction of the pulp tissue to calcium hydroxide had been extensively studied. They did not accept that variations in reactions had been fully documented. An unusual reaction of exposed, uninfected dog pulp tissue to calcium hydroxide was reported. There was a rapid appearance of a very dense radiopaque area beneath the calcium hydroxide in three teeth. Histologically, this was not shown to be a hard tissue barrier but a

basophilic zone of cellular degeneration. Further long term investigations were proposed to clarify the outcome of this healing pattern.

(4) <u>The effect of implantation in subcutaneous tissues.</u>
Bhaskar et al. (1969) implanted calcium hydroxide pellets with and without the addition of corticosteroid compounds in the subcutaneous tissues of the rat abdomen. The steroid-enriched preparation reduced necrosis, oedema and infiltrate. Dystrophic calcification was reduced or eliminated.

Mitchell & Shankwalker (1958), Yoshiki & Mori (1961) and Binnie & Mitchell (1973) have demonstrated the osteogenic potential of calcium hydroxide when implanted subcutaneously in the rat. Rasmussen & Mjor (1971) could not demonstrate any osteogenic effect when calcium hydroxide was implanted subcutaneously under similar conditions.

(iii) <u>The effect of dentine particles on the exposed pulp.</u>
Dentine chips are considered to play an important role in dentine bridge formation (Seltzer & Bender, 1965). In a series of investigations into reparative dentinogenesis (Seltzer & Bender, 1958; Seltzer et al., 1962), matrix was found in apposition to dentine; in the pulp it was only found around dentine chips which had been forced into the tissue accidentally.

Rzesztorski (1939) and Manley et al. (1946) stated that homogenous dentine particles can stimulate callus formation. Manley et al. postulated that dentine particles may assume an osteogenic

function within the pulp tissue.   Fish (1948);   James & Englander (1956);   Kainins & Frisbie (1956, 1960) and Seeling (1956) found that degeneration and tissue necrosis occurred in spite of attempts at calcification when dentine was used as a pulp dressing.   Seeling stated that the pulps of exposed teeth are always infected and that homogenous carious dentine chips increase this infection.

Massler (1963) reported on histological investigations.   The presence of dentine spicules pushed into the pulp during operative procedures appeared to accelerate the formation of secondary dentine.

Luostarinen (1971) conducted a study into the part played by dentine fragments in bridge formation.   It was observed that the dentine was either completely passive or that collagen formation occurred near these fragments at an early stage.   No resorption of dentine fragments was observed.   Dentine fragments which had been forced into the pulp tissue were surrounded by osteoid tissue during the first few days;   a layer of odontoblast-like cells then developed and became associated with predentine and dentine formation.   The dentine fragments were considered by the author to accelerate the development of the organic matrix.

Obersztyn (1966, 1968) applied lyophilized dentine and enamel chips to the exposed pulps of dogs and rats.   Dentine formation occurred around these chips in both species.   Positive results were also obtained in humans.

The induction of heterotropic osteogenesis has been described in several experimental systems.   This phenomenon may be looked upon not only as an example of experimental metaplasia but also as a system similar, if not identical with, the induction of specific differ-entiation.

The osteogenic properties of dental tissues have been well documented.   Huggins et al. (1936) found that subcutaneous grafting of teeth into six to eight week old puppies induced bone formation.   This inductive ability was abolished by boiling the grafts.

Bang & Urist (1967) found that mature allogeneic dentine, decalcified and lyophilized, induced bone formation within four weeks when implanted in the anterior abdominal wall of rats and rabbits. The end product was an ossicle of bone filled with marrow.   This was identical to bone induced by bone matrix.   Undecalcified dentine had a slower induction time.   The presence of a mineralized phase apparently impeded the process.

The following papers report that heterotropic bone formation occurred when non-treated whole dentine from rat incisors was implanted into allogeneic sites: Bang & Urist (1967);   Bang (1973) and Ronning & Luostarinen (1973).

Osteogenic induction was reported when decalcified lyophilized dentine was implanted (Huggins & Urist, 1970;   Bang, 1973;   Linden, 1975).

The properties of implants were defined by Urist (1971) in terms of histogenetic induction effects and of bone morphogenetic effects.   Morris (1972) and Huggins & Urist (1972) both reported bone marrow formation following the allogeneic implantation of demineralized dentine.   This extent of morphodifferentiation was not observed by Luostarinen & Ronning (1977) in their experiments using decalcified dentine.

In a series of experiments, Ronning & Luostarinen investigated

the possible variations in osteo-inductive potentials possessed by

different parts of the donor tooth.   They noted that bone is not

always associated with dentine transplants (Ronning & Luostarinen,

1973; Luostarinen & Ronning, 1975).   They continued these investi-

gations using the incisal and basal dentine from the mandibular

incisors of rats.   Enamel and pulp tissue were used as controls.

The dental tissues procured from one litter of rats was implanted

subcutaneously or intra-cerebrally in sex matched, five day old

animals of the subsequent litter of the same parent.   As further

controls, the implantation instrument was inserted into corres-

ponding sites without accompanying tissue.   The animals were

sacrificed at 4, 32, 128, 138 and 210 days post-operatively.

Osseous tissue was observed at 128 days post-operatively in

association with pulp tissue.   Bone was found in association with

basal dentine at this time period.   No bone tissue was, however,

observed in relationship to implanted enamel or incisal dentine.

The osteo-inductive activity of transplanted tissue seemed

reduced by the mineral phase.   Moreover, non-demineralized tissue

did not show the same bone morphogenetic properties exhibited by

demineralized hard tissue.   The authors suggested that the difference

in osteogenic potential was related to the "vitality" of the dentine.

Dentine from the incisal portion of rat incisors was heavily mineral-

ized.   The basal portion still contained dentinal tubules.   They

cited the work of Slavkin et al. (1969) in support of this hypothesis.

The latter observed that the extracellular matrix of embryonic teeth

is capable of inducing mesenchymal cells to differentiate in cells

suggestive of preodontoblasts.

It is difficult to assess the relative merits of dentine chips vis-à-vis other pulp capping materials because of the many variables which exist among the reported investigations. The effect of any particular medicament is almost certainly modified by the presence of some amount of dentine dust following operative procedures.

It can be concluded that surface decalcification enhances the osteogenic potency of dentine. Clinically better results, in terms of calcified bridge formation, would be expected if dentine from the more incisal areas was prevented from entering the pulp vis-à-vis the partly decalcified dentine from the second layer within the carious cavity (Ohgushi & Fusayama, 1975). This layer is also more likely to be non-infective (Shovelton, 1972), though not necessarily so.

The foreign body reaction noted by James & Englander (1956) and Kainins & Frisbie (1956) may have been due to the extent of mineralization of the dentine chips. Lyophilized dentine is known to reduce the foreign body reaction following implantation. Autologous dentine is unlikely to cause any deleterious immunological response.

(iv) Corticosteroids

(1) The effect in the deep cavity. Mosteller (1962); Dachi et al. (1964); Schroeder (1965) and Massler (1967) suggested the use of corticosteroids in reducing post-operative pain following operative procedures. They based their suggestions on clinical evaluation.

The effects of corticosteroids, placed in deep cavities, have been assessed histologically by the following: Baume & Fiore-Donno

(1963, 1970); Mosteller (1963); Baume (1966); Mjor &
Ostby (1966); Mjor (1967); Langeland & Langeland (1968);
Langeland et al. (1968, 1971); Hasselgren & Tronstad (1977).

Mosteller (1963) prepared deep cavities in sound teeth and
dressed these with prednisolone.   Controls were left untreated
by steroids.   All cavities were sealed using zinc oxide
eugenol.   Marked inflammatory changes were seen in all control
teeth.   Prednisolone reduced but did not eliminate the
inflammatory response.   Langeland & Langeland (1968) and
Langeland et al. (1968, 1971) stated that pulpal inflammation,
caused by dry cavity preparation, was not prevented or
eliminated by the use of corticosteroids.   They suggested that
the assessment of the reduction in inflammation depended on the
amount of inflammation present prior to the application of the
steroids.

The use of H^3 labelled prednisolone (Langeland et al., 1968,
1971) showed that the drug did penetrate into the pulpal
tissues where it was phagocytosed by macrophages.

Dentine formation is inhibited by the application of
corticosteroids according to Fiore-Donno (1963, 1970);   Baume
(1966);   Mjor & Ostby (1966) and Mjor (1967).

Mjor & Ostby (1966) used Ledermix in the treatment of deep
carious lesions.   Changes in the odontoblast zone and a
decrease in the number of odontoblast cells were observed.
Predentine formation was reduced.   The reduction in dentine
matrix formation was confirmed by Mjor (1967).

Baume & Fiore-Donno (1970) lined cavities in 15 teeth with a corticosteroid. They reported pulp fibrosis and odontoblast atrophy. They advocated the limitation of the use of corticosteroids to the temporary dressing of painful teeth without exposures.

Schroeder (1968) did not confirm the inhibition of dentine formation following the application of corticosteroids. In a histological trial, using monkey teeth, Hasselgran & Tronstad (1977) showed that there was no apparent effect on enzyme activity following the application of corticosteroids.

It must be concluded that the reports on the effect of corticosteroids placed in the deep cavity are conflicting and require further investigation.

Mjor & Furseth (1968) found that many dentinal tubules were obliterated by an electron dense material when corticosteroids were used to line cavities. Mjor (1968) suggested that the increase in mineral content of corticosteroid-covered dentine was due to "a progressive and active mineralization involving fairly distinct alterations in the organic matrix".

(2) The effect on the exposed pulp. The treatment of exposed vital pulps with a corticosteroid/antibiotic mixture was first reported by Kiryati (1958) and Rapoport & Abramson (1958). Schroeder & Triadan (1962) formulated the preparation marketed as Ledermix. The corticosteroid/antibiotic preparation was intended to suppress the undesirable sequelae of pulpal inflammation and inhibit bacterial growth.

Several clinical studies, assessed mainly by vitality tests, have reported the "successful" use of corticosteroids in the treatment of the inflamed, exposed pulp. These include papers by Rowe (1963); Schroeder (1963); Ehrmann (1965); Allwright & Wong (1966); Cowan (1966); Olsen (1966); Leibur (1969, 1970) and Shovelton et al. (1971).

Histologically controlled trials have been conducted by several authors. The following reported that no calcific repair accompanied the use of corticosteroids when applied to the exposed pulp: Kiryati (1958); Fiore-Donno & Baume (1963, 1966); Ehrmann (1965); Cowan (1966); Harris & Bull (1966); Laws (1967); Sykaras (1970, 1972). However, maintenance of pulp vitality was reported without accompanying calcific repair by Baume (1964); Ehrmann (1965); Cowan (1966); Clarke (1968); Barker & Ehrmann (1969); Hansen (1970); Sykaras (1972) and Ivanov & Leibur (1974).

Cowan commented that the maintenance of a vital pulp was of first importance. The formation of a reparative dental bridge was not considered to be necessary for healing. This is an unacceptable point of view. True healing requires the reformation and function of an intact pulpo-dentinal organ. This is manifest by the formation of a calcified (tubular) dentine bridge at the site of exposure. Ivanov & Leibur (1974) stated that corticosteroids on their own do not stimulate secondary dentine. Calcification may occur with the addition of additives which eliminate infection, e.g. antibiotics, or stimulate pulp tissue, e.g. calcium hydroxide. Sinkford

& Harris (1964) condemned the use of corticosteroids for two
reasons: they suppressed the inflammatory reaction which is
a primary defence mechanism and there is a very limited
knowledge of the mechanisms involved.

Klotz et al. (1965) demonstrated that the application of
steroids to the exposed pulp facilitated the systemic spread
of bacteria from the pulp.

Kozlov & Massler (1960) reported on a histologically controlled
trial and stated that, following the use of Ledermix, calcific
repair did occur.   Clarke (1968) applied Ledermix to the
exposed pulp of sound human teeth.   The teeth were extracted
and examined histologically at periods ranging from 2 days to
21 weeks.   The effect of cement on the mechanically trauma-
tized pulp was minimal.   The pulp cells remained unaffected
with little or no signs of inflammation.   Secondary dentine
formation was observed on the wall of the exposed cornu, and
in association with dentine fragments in the 21 week specimen.
Clarke stated that the findings were not in agreement with those
of papers reporting on similar investigations (Schroeder,
1962 and Rowe, 1967).   It is perhaps a significant comment
that new dentine formation occurred in relation to (1)
undamaged odontoblasts on the wall of the cornu and (2)
where stimulated by dentine chips.   Clarke's findings are
more in step with Langeland & Langeland (1968) who found
that corticosteroids do not affect the pulp physiology.

De Souza & Holland (1974) histologically examined the pulps
of dogs' teeth which had been dressed with either an anti-
biotic, corticosteroid or calcium hydroxide, either alone or
in combination. The pulps had previously been exposed
to the oral fluids for 48 hours. There was an observed
relationship between the state of the coronal tissue, the
type of capping material used and the success or failure of
the treatment. Success was highest when inflammation was
first controlled by corticosteroids and then dressed with
calcium hydroxide. A similar two step regime was carried out
by Ivanov & Leibur (1974). They histologically examined 29
of a series of 784 teeth which had been treated in the first
instance with an antibiotic/glucosteroid supplied directly to
the exposed pulp, and then by either Dycal, Hydrex or Pulpdent.
They concluded that the two step regime allowed the inflamma-
tory processes to resolve and found that the subsequent
application of calcium hydroxide stimulated the formation of
secondary dentine.

Sykaras (1972) carried out a clinical and histopathological
study into the effect of corticosteroid/antibiotic compounds
on the exposed pulp. Teeth with clinical signs of
inflammation were treated with Ledermix. A clinical assess-
ment of the results was made at post-operative intervals
ranging from 3 to 30 months using vitality tests and radiographs
were taken to assess apical changes. On the basis of this
clinical assessment, 62 out of 90 treated teeth were considered
successful.

A histological assessment was then undertaken. No bridge formation was observed at the exposure site at post-operative intervals ranging from 3 to 40 days. The unaffected pulp was, however, seen to be walled off by a transverse arrangement of fibroblasts and associated collagen fibres. The authors concluded that the corticosteroid did not inhibit the action or differentiation of mesenchymal cells, although it was considered that this differentiation was modified.

Paterson (1976) reviewed the literature on pulp capping with anti-inflammatory agents. Three commercially available corticosteroid/antibiotic preparations gave poor results in the treatment of the exposed rat pulp molar. In the case of Ledermix paste, the carrier itself was irritant to the pulp. Further, there was no significant difference between the pulp response to the carrier alone and the complete formula. Paterson concluded by stating that, on the basis of current evidence, routine use of corticosteroid/antibiotic preparations as pulp capping agents cannot be supported.

A bacteraemia arising from infected pulp tissue was considered to arise only when the periapical tissues were traumatized (Beechen et al., 1956; Kennedy et al., 1957; Bender et al., 1960). Klotz et al. (1965) found that the local application of pred- nisolone to an infected pulp could produce a bacteraemia even when the apical tissues were not traumatized.

Assessment of pulpal response to trauma

The pulp response to trauma can be assessed histologically or clinically.

(a) Histological assessment. Most histological assessment methods adopt an inflammatory index and a calcific index.

Kramer & McLean (1952) described the presence, location and severity of pulp reactions in human teeth using the following criteria.

1. Polymorphs in the region of the odontoblast layer (+ → ++++).

2. Cell infiltration of pulp tissue other than the odontoblast layer (+ → ++++).

3. The presence of secondary dentine thickness (+ → +++).

4. The presence of aspirated odontoblasts.

James & Schour (1955) adopted a similar system with an inflammatory index: mild, moderate or severe. They assessed the odontoblast response according to the type of reparative dentine. Cavity depth was considered an important factor; they assessed the degree of inflammation according to cavity depth and always evaluated the severest response.

Schroff (1946) and Swerdlow & Stanley (1959) have shown that the remaining dentine thickness is a much more important parameter than the clinical depth of the cavity.

Silberweit et al. (1955) measured both the amount and character of the post-operatively formed dentine. Odontoblast disturbance

and reduction in cell numbers were recorded.   Langeland (1957)
listed definite criteria for assessing pulp responses to injury
without quantifying this response.   The criteria were based on
odontoblast aspiration and inflammatory and degenerative reactions
within the pulp.   Mjor (1972) used similar criteria and graded
the reactions as slight, moderate or severe.

In two papers on the design for human pulp studies, Stanley
(1968a,b) proposed the following histopatholgical criteria:

1.   Cellular displacement (odontoblast and leucocyte aspiration into
      dentinal tubules).

      This characteristic was said by Stanley to occur within the first
      few days and persist for over 30 days.   It therefore provides
      excellent evidence of the acuteness of the initial response long
      after other inflammatory characteristics have resolved.

2.   Inflammatory cell infiltrate.

      This was graded (A) in the superficial tissues, i.e. the odonto-
      blast zone, zone of Weil and cell rich zone, and (B) in the deeper
      tissues.   The predominating inflammatory cell type was recorded
      at the same time as the intensity of the response.   Emphasis
      was placed on the remaining dentine thickness; as pulp
      haemorrhage and cytoplasmic vacuolization have not been shown
      to vary in relationship to dentine thickness, these latter
      features were not emphasized as significant criteria.
      Estimation of degrees of response was recognised as a purely
      subjective exercise which would be reflected in different

examiners arriving at different quantitative scores.

The following papers deal with the assessment of pulpal response using variations of the inflammatory and calcific indices mentioned above. Salient comments by the authors are included.

Nyborg (1955, 1958) used an inflammatory index and stated that the pulp should exhibit a normal structure, staining properties and an apparently functional vascular system when healing has occurred. No special requirements were detailed with reference to the structure of the barrier at an exposure site. Hyperaemia, vasodilation, reticulation, extravasated lymphocytes, blood pigment and fibrin were not regarded as excluding pulp recovery.

Berk & Stanley (1958) assessed the structure of the reparative bridge as tubular or non-tubular and related this to the quality of the healing process.

Seltzer & Bender (1958) considered the formation of a dentine bridge as a questionable criterion of successful repair. They stated that the longer the post-operative period, the greater the likelihood of its covering permanent pulp damage.

Paterson (1976), on the other hand, considered that the formation of a dentine bridge was a good sign of restoration of odontoblast function and agreed with Masterton (1966a) that the formation of a dentine bridge suggested a favourable prognosis and maintenance of pulp vitality. Cowan (1966) considered that the formation of a calcific bridge was unimportant. Maintenance of pulp vitality

was his only criterion of success.

Nakomora et al. (1966); Massler (1968); Sayegh (1968) and Schroder (1972) all indicated that the formation of a dentinal bridge was the most significant histological indication of a healed pulp.   Schroder noted that the term "dentine bridge" was often used without clarification of the type of new-formed tissue.   Barrier formation must be assessed for completeness and for the type of tissue formed.   Moreover, in the evaluation of the post-operative events, a clear distinction must be made between wound healing per se and the disappearance of inflammatory changes.   Massler (1972) reiterated this view.   He acknowledged that the histological criteria commonly used to assess pulp healing was the formation of a mineralized bridge and the observance of absence of inflammation in the pulp.   He stated that a clear definition of what was meant by healing was necessary.   He listed the pulpo-dentinal damage that occurred with increasing severity of trauma and the histological criteria considered necessary for healing.

Recent publications by Stanley (1970) and Baume et al. (1971) have suggested uniform systems for the assessment of pulp responses.   These are based on a recognition of variables inherently present in any pulp study and the minimising of these variables. Suggestions are given for indexing the histopathological responses.

Cabrini et al. (1960) reported on the alkaline phosphatase, mucopolysaccharide and glycogen content of healing pulps. Alkaline

phosphatase activity increased slightly in wounded pulps.   In teeth treated with calcium hydroxide, large amounts of P.A.S. positive, ptyalin removable granules (glycogen) were observed within odontoblasts.   It was concluded that the histochemical demonstration of glycogen evidenced pulp healing.   This implied that the normal or healed pulp engaged in aerobic metabolism whereas in the traumatized pulp, anaerobic metabolism, due to functional disruption of pulp physiology, depleted glycogen stores.

Weinreb et al. (1967) confirmed the opinion of Cabrini et al., and showed that fully functional odontoblasts contain large amounts of P.A.S. positive material.   They found that odontoblasts before, and immediately after, transplantation stained P.A.S. positive. During the post-implantation degenerative stage (3-20 days), this staining was lost.   A positive staining was obtained after 14 days. Contrary to the findings of Cabrini et al. (1960), the P.A.S. stain was not removed by salivary amalase.   Weinreb et al. concluded that this represented neutral polysaccharides rather than glycogen.

Zerlotti (1964) did not succeed in demonstrating P.A.S. material in the odontoblasts, though it must be stated that, due to the experimental methods employed, the odontoblasts were not easily studied.

Del Balso et al. (1976) undertook investigations to determine the possible quantitative changes in porcine pulpal histamine levels following trauma using quantitative spectro-photo fluometric assays. The findings revealed a four fold increase in pulpal histamine levels following injury.   Pulp histamine levels were considered to be a method of assessing pulp response to trauma.

Spanberg & Langeland (1973) recommended that endodontic materials be tested in vitro and by standardized implantation tests prior to being released for general clinical use.   They did not detail indices for use with implantation studies.   Beagrie et al. (1972) reported on the inflammatory reactions invoked by zinc polyacrylate and zinc eugenate cement when implanted subcutaneously in the rat. The degree of inflammation was assessed microscopically using a variation of the criteria recommended by the American Dental Association and the Federation Dentaire International for the biological testing of dental materials.

(b) Clinical assessment.   The most commonly employed methods for the clinical assessment of pulp healing are (i) assessment of symptoms and signs, (ii) vitality tests (thermal and electrical) and (iii) radiographs.

Seltzer & Bender (1965) warned that the practitioner should be fully aware of the limitations of all diagnostic techniques including radiographs and vitality tests.

(i) Assessment of symptoms and signs.   It is a well documented fact that the histopathology of the pulp cannot be accurately assessed from clinical signs and symptoms.   Many investigators (Thoma, 1929; Herbert, 1945; McDonald, 1956; Weiss, 1959; Seltzer et al., 1963; Pheulpin et al., 1967; Lundy & Stanley, 1969; Baume, 1970a; Tyldesley & Mumford, 1970; Garfunkel, Sela & Ulmansky, 1973) have found no significant correlation to exist between a presumptive clinical diagnosis and histologically evidenced pathology. Nicholls (1967) stated that it is as difficult to assess the state

of the pulp following treatment as it is to assess the pathology prior to treatment when only clinical methods are employed.

(ii) Vitality tests

(1) Thermal tests. The normal response of the intact, uninflamed tooth to heat or cold is pain. The pain subsides as soon as the stimulus is removed. In pathologically affected teeth, the pain persists after the stimulus has been removed.

Isolation and drying of the teeth give better results. Controls should be used.

Macdonald (1956) and Seltzer et al. (1965) found that neither heat nor cold was a reliable indicator of pulp damage.

(2) Electric pulp testers. Mumford (1963) found electric pulp testers to be far from reliable. He stated they would be more dependable if pain threshold values could be established for normal teeth.

When no response is obtained, a necrotic pulp is indicated. The converse need not be true.

Both thermal and electrical pulp tests are, at best, tests of pulp sensitivity and not of pulp vitality. They should be employed as part of a total diagnostic procedure and as methods of substantiating the accuracy of other clinical diagnostic methods.

(iii) Radiographs. An important guideline to treatment of the deep carious lesion is the fact that pulp involvement of a significant extent does not occur until a late stage in the carious process (Reeves & Stanley, 1966; Shovelton et al., 1971).

The depth of the carious lesion can be assessed from radiographs. The amount of dentine remaining beneath the cavity plays a most important role in the incidence of a pulpal response (Swerdlow & Stanley, 1962). This remaining dentine thickness (Schroff's effective depth) is much more difficult to assess. The following points should always be borne in mind. Reparative dentine is of a lower degree of mineralization than physiological secondary dentine and may not always be visualized on radiographs. Moreover, once involved by the carious process, reparative dentine is destroyed faster than physiological secondary dentine.

Radiographic assessment of the carious lesion should be made following a presumptive rating of the type of carious lesion that is being treated.

Radiolucent periapical areas and widening of the periodontal ligament are taken as indicative of pulp pathosis. In vital pulp therapeutic procedures, their usefulness is in the elimination of teeth or indicating failures following treatment. They are unreliable indicators of early pulp changes.

Jordon et al. (1978) reported on the treatment of 24 teeth. These teeth had deep carious lesions and radiographs demonstrated periapical involvement. The teeth were treated by a standard, indirect pulp-capping procedure. Patients were recalled after 48 hours and thereafter at six monthly intervals. Comparison periapical radiographs and vitality tests were carried out. Eleven teeth showed apparent resolution of periapical pathology, absence of pain and continued vitality for up to seven years post-operatively.

where resolution occurred, the periapical radiolucent area generally disappeared within six months of treatment, leaving a zone of condensing osteitis. This resolved from 12 to 18 months post-operatively. The authors concluded that periapical lesions are not always irreversible. They suggested a histological investigation to research the process of periapical inflammation and resolution.

The clinical assessment of bridging

Following exposure of the pulp, healing is generally accepted to have occurred with reparative dentinogenesis. Histological studies indicate that the formation of a calcified 'bridge' at the exposure site correlates well with the resolution of inflammation in the pulp tissues.

Bridging can be assessed by two clinical methods: (a) radiographic and (b) direct inspection.

(a) Radiographic. Bridge formation is usually described as a dense radio-opaque line beneath, and separate from, the lining/restoration. This description is in keeping with the classical account of wound healing patterns associated with a calcium hydroxide dressing (Glass & Zander, 1949).

Variation in healing patterns has been reported following Dycal (Stanley & Lundy, 1972; Tronstad, 1974). Bridging is said to occur in direct contiguity with the lining. Radiographic visualization and differentiation between lining and 'bridge' is more difficult in such cases.

The low degree of mineralization of reparative dentine makes the early detection of bridge formation difficult. The long term

assessment, using standard radiographic techniques for comparison, is a useful clinical criterion of successful wound healing (Shovelton,1972).

Radiographs do not show the completeness of bridging. Histological examination of serial sections showed defects in the bridge in every case (Paterson, 1976; Langeland, 1979).

(b) Direct inspection. Following the assessment of wound healing by both histological and clinical methods, Masterton (1966a) and Schroeder (1972) suggested that a favourable prognosis is given by the clinical detection of bridge formation. To overcome the short-comings of radiographs, they both suggested that completeness of bridging be assessed by removing the restoration and confirmation made by direct clinical inspection.

Shankel & Brauer (1962) re-opened teeth which had been treated by direct capping procedures. They reported a 70% success rate as assessed by the observation of bridging at the site of exposure.

It is the present author's experience that the detection of a healed exposure following direct pulp capping is an extremely difficult exercise. Following pulpotomy, the formation of a bridge is a much more quantifiable entity.

Brinsden (1955), in a study of the reparative powers of the mature dental pulp following pulpotomy, found that there was only a 66% agreement between radiographic and clinical evidence of bridging. This tends to support Masterton's method for evaluating the post-operative status of the pulp by direct inspection for bridge formation.

Within the last decade, several clinically assessed trials have been conducted on human material to assess the efficacy of operative procedures such as direct pulp capping with calcium hydroxide (Weiss, 1970; Shovelton et al., 1971; Haskell et al., 1978). The assessment in each case was by serial radiographs and vitality tests.

The most comprehensive study was undertaken by Shovelton et al. (1971). The efficacy of materials for direct capping of the exposed pulp was investigated.

The authors listed the problems associated with a clinical trial of this nature as (1) the difficulty in obtaining sufficient patient numbers presenting with the appropriate clinical condition; (2) the difficulty of following up the patients for a reasonable period of time when histological evaluation is ruled out and (3) the difficulty in assessment of results.

To overcome the first problem, eight dental schools co-operated and this allowed the accumulation of 412 treated teeth. A code of standardized procedures was drawn up to reduce the variables introduced by different operators/assessors. Materials were also obtained from a common source.

Teeth were classified as being exposed, vital and symptomless or exposed, vital and with a history of pain. A detailed history was taken and electrical vitality tests and radiographs were obtained.

The criteria used in the assessment of success were acknowledged by the authors to be open to some criticism. This expresses the

awareness that no clinically conducted trial conforms fully with
ideal scientific investigatory procedures.   Shovelton et al. did
consider that, over the period of time (one to two years) the study
was conducted, the criteria chosen were reasonable.

Pulpotomy procedures have been assessed in humans using
clinical assessment methods by Adams et al. (1962);   Masterton (1966a);
Frankl (1974) and Cvek (1978).

Adams et al. (1962) evaluated the treatment at one year post-
operatively by clinical symptoms, and radiographically for signs of
pathology.   Frankl (1974) used vitality tests and emphasised the
importance of radiographic evaluation with special reference to the
observation of an intact periodontal membrane.

Masterton (1966a) and Cvek (1978), in addition to the usual
vitality tests and radiographic tests, clinically verified the
presence of a hard tissue barrier by direct inspection.

The direct inspection for the presence of an intact hard tissue
barrier is the only non-subjective clinical test detailed in the
literature for the assessment of healing following exposure.
Subsequent to pulpotomy, it allows a definite yes/no evaluation to
be made and so facilitates statistical analysis of results.

Synopsis of section

A review of the literature shows that wound healing in the pulp
follows the general pattern of wound healing in other connective
tissues.   There are many aspects of pulp biology on which little
information is available.   As with wound healing in general,

knowledge does not extend far beyond the descriptive phase. The literature relating to the main factors affecting the wounded pulp is summarised. These factors include the carious process, operative techniques, and the effect of pulp capping materials. Included in this section, for convenience, is a review of the dentinogenic effect of dentine chips. The difficulty in isolating individual components for assessment is noted. The section is concluded by a review of articles dealing with methods of assessing pulp response to injury.

C. DENTINE PERMEABILITY

Numerous reports are available concerning the permeability of dentine to dyes. These studies are of a qualitative nature (Fish, 1933; Bodecker & Lefkowitz, 1937; Blake, 1958).

In vivo experiments have shown that dentine is readily permeable from both the pulp and amelo-dentinal junction. Lefkowitz (1943) injected argyrol into the pulp through small holes bored in the neck of dogs' teeth in vivo. The teeth were extracted at varying time intervals and the distance of stain permeation ascertained. Transverse sectioning showed stain within the odontoblast tubules. This was interpreted as indicating that at least one of the routes of diffusion, for substances originating from the pulp, was via the odontoblast process.

The highly permeable nature of dentine was confirmed by Wainwright & Lemoine (1950) and Wainwright (1954). To exclude the possibility of damage to the pulpo-dentinal anatomy, trypan blue was injected into the blood stream of cats and dogs. The dentine formed prior to the injection was stained. This type of experimental procedure suggests that stain enters the dentine through the intact pulp and across the undamaged odontoblast palisade.

Wainwright (1954) placed solutions of radioactive substances inside pulp chambers of extracted teeth kept at 37^0C in a humid chamber. The teeth were sectioned and studied autoradiographically at varying time intervals. Small molecules of nicotinamide, urea and thiourea can permeate the whole thickness of dentine (3-4mm) in 20-30 minutes.

The times are so similar to those for dyes placed in teeth with intact blood supplies that it is indicated that diffusion alone and not tissue pressure is largely responsible for movement of substances through dentine.

Blake (1958) traced diffusion channels in dentine using sound teeth extracted for orthodontic reasons. Teeth were either fixed in 5% formol saline or fresh. Similar results were obtained for both types. The tooth was first immersed in mercuric chloride for 12 weeks; this was then precipitated by immersing the whole tooth in ammonium chloride for four weeks. The author considered the demonstrated paths to be anatomical channels available for the passage of solutions either by flow or diffusion.

Several investigators have studied the effect on diffusion brought about by changes in tooth structure following cavity preparation and caries. The establishment of barriers to diffusion of harmful substances is an important defence reaction. Fish (1933) prepared cavities in dogs' teeth. Four months were allowed for any protective reaction to develop. In response to severe attrition, odontoblasts within the affected tubules were killed and a 'dead tract' resulted. Stain was allowed to permeate from the pulp of extracted teeth. No stain entered the dead tract because reparative dentine sealed off this area. The initial barrier was a hyaline layer of calcified non-tubular dentine. Fish noted that different types of reparative dentine were formed pulpal to this zone of hyaline material at different times. Initially the dentine was atubular; later it was permeable to dyes and clearly tubular in nature.

Bodecker & Lefkowitz (1946) sealed stain into dentinal cavities. The stain moved towards the pulp; once within the pulp tissues it penetrated back into the dentine. The authors concluded that diffusion occurs in both directions and was not solely dictated by intra-pulpal pressure. Blake (1958) studied changes in permeability as a result of age and caries. He showed that translucent areas are completely impermeable to mercuric chloride (in agreement with Fish (1932) and Beust (1934)). Mercuric chloride applied solely to the enamel surface permeated as far as the hyaline plug at the pulpal end of 'dead tracts'. Lateral to the carious lesion were opaque zones which were impermeable either from the pulpal aspect or from the amelo-dentinal surface. These results confirmed the findings of Fish (1932). Barber & Massler (1964) examined the permeability of active and arrested carious lesions to four different tracer solutions. Attention was focused on the defensive reaction of dentine. Radio-calcium provided a double bonded cation; radio-sulphur provided a double bonded anion. Orange G. and toluidine blue provided a positive and negative charged dye respectively. The authors concluded that there is a natural defence reaction to the carious attack characterized by the formation of reparative dentine and the sclerosis of the affected tubules. These parameters contributed to the impermeability of the arrested lesion. Active lesions were highly permeable to dyes and radioisotopes.

Autoradiographic studies have confirmed the permeability of dentine to various isotopes (Wainwright & Lemoine, 1950; Bartelstone, 1951; Sognnaes & Shaw, 1952; Sognnaes et al., 1955). Fremlin & Mathieson (1961) cautioned against the misinterpretation of results which may occur due to contamination caused by isotope leakage.

Smith & De Vincenzo (1968) reported on a new in vitro technique for studying tooth permeability while attempting to maintain physiologic conditions. They reported on the difficulty in obtaining isotope-impermeable seals and the significant effect which leakage could have on the overall results.

Bulk fluid flow through dentine has been demonstrated by Brannstrom and his co-workers following the application of an air blast to exposed dentine (Brannstrom, 1960a,b); pressure (Brannstrom, 1961); chemical stimuli (Brannstrom, 1962b); heat and pressure (Brannstrom, 1962a ; Brannstrom et al., 1967); osmotic pressure (Linden & Brannstrom, 1967); hydrostatic pressure (Johnson et al., 1973).

The movement of the fluid contents of the dentinal tubules is attributed by Brannstrom to the "mobilization of capillary forces". The experiments used unerupted molar teeth or young permanent premolars but the papers did not state whether the latter teeth were unerupted or not. There are no reports investigating fluid flow through dentine altered by attrition or caries.

Horiuchi & Matthews (1973) recorded pressure changes in pulp chambers of recently extracted teeth and estimated fluid movement through human dentine produced by solutions of $CaCl_2$, NH_4Cl, $NaCl$, urea and Golden Syrup. The authors concluded that the fluid movement caused by different substances could not always be predicted on the basis of their osmotic pressure. They also concluded that dentine did not act as an ideal semipermeable (osmotic) membrane.

Reeder et al. (1977) stated that a common deficiency in the study of fluid movement in dentine is that flow is measured under poorly defined conditions. To resolve this problem they stated that it was

necessary to quantify the hydraulic conductance of dentine. They defined hydraulic conductance as the measure of ease with which fluid under hydrostatic or osmotic pressure can pass through a permeable barrier under defined conditions. Using a split chamber device (Outhwaite et al., 1974), they investigated the hydraulic conductance of ground and acid etched dentine discs including the effects of thickness and surface area. Their results showed that, for any given surface area, there is a progressive increase in filtration rate with reduced dentine thickness. At zero hydrostatic pressure, neither ground nor acid etched discs exhibited bulk fluid movement. Etched discs resulted in much greater filtration rates than ground, unetched discs when hydrostatic pressures were applied. They clearly illustrated the origin of the prepared dentine discs but omitted to comment on the relevance of this to their results.

Outhwaite et al. (1976), using discs of dentine cut from freshly extracted, unerupted third molars, measured steady-state permeability values using radio-active iodine. Their data supported the Fick equation's prediction that the rate of diffusion of substances through a membrane is related to membrane thickness and surface area. The results showed the necessity of defining exactly the level of section of coronal dentine as the slopes relating dentine permeability to surface area varied depending on the distance from the pulp chamber. The Fick equation assumes that membrane geometry is constant, i.e. the number and dimensions of pores per unit volume is constant. In dentine, the number of tubules per unit area increases as the pulp is approached. In addition, the tubular diameter decreases from the pulp to the enamel. For these reasons alone, the flux of a substance

will depend on the preparation of the dentinal discs.

Merchant et al. (1977) compared the rate of iodide permeation of dentine by diffusion and by filtration. Dentine discs were prepared approximately 1mm thick. Before acid etching, filtration doubled the rate of iodide permeation relative to diffusion. After acid etching, filtration produced a 32 fold increase in permeation. Acid etching, by removing occluding debris, facilitated filtration (bulk fluid flow) more than diffusion.

Chelating agents have been used to soften and widen root canals since the mid-1950s. E.D.T.A. was found to increase the permeation of dentine to medicaments sealed in the root canals (Hampson & Atkinson, 1964; Stewart et al., 1969; Cohen et al., 1970). Decreased dentine permeation was reported following E.D.T.A. by Marshall (1960) and Fraser & Law (1976) suggested that occlusion of tubules by Ca-E.D.T.A. sludge may contribute to the decreased permeation.

SECTION 2

CLINICAL TRIALS

A.   INTRODUCTION

An extensive review of the literature shows that the present
knowledge of the biology of wound healing does not extend far beyond
the descriptive.

Experimental studies in animals have compared the reaction of
the dental pulp to various materials. Though the results of such
studies cannot be related directly to man, they indicate the likely
effect of materials on the human pulp.   Histological studies in
animals and man have indicated that the three essential processes of
wound healing, i.e. active migration of cells, the greatly increased
mitotic rate by which cell loss is made good and the production of
new connective tissue take place in the wounded dental pulp.   A
fundamental problem, still unresolved, is whether materials such as
calcium hydroxide actively stimulate healing or merely allow the
inherent biological processes to take place within a suitable
environment.

There is still dispute about the efficacy of materials used to
preserve the vitality of the exposed human pulp. Calcium hydroxide
promotes reparative dentinogenesis at an exposure site. Undesirable
side effects such as internal resorption or complete calcification of
the root canal have been reported.   The advent of proprietary
corticosteroid antibiotic compounds aroused fresh debate on the
subject.   The consensus opinion is that these compounds are
unsuitable as biological pulp dressings in humans.   There is
evidence, however, that the addition of corticosteroids to calcium
hydroxide enhances tissue receptivity.

The biological action of pulp dressing materials cannot be fully evaluated by histological methods using animal material. In the last resort, materials must be tested in humans under conditions which normally apply in everyday clinical situations. Where histological assessment cannot be undertaken, sufficient numbers of patients with the appropriate clinical condition must be obtained to minimize the histological variation of pulp response. A long-term follow-up is necessary. The assessment methods used should allow the results to be statistically analysed and allow valid comparisons to be made.

B. OBJECTIVES

The clinical trials were undertaken to assess wound healing in the human pulp following pulpotomy and to assess the comparative efficacy of calcium hydroxide (Calnex) and calcium hydroxide (Calnex) containing proprietary corticosteroid compounds (Ledermix paste or Endomethasone paste) in promoting bridge formation and maintaining pulp vitality following pulpotomy in human carious teeth in different age groups. Essentially the trials were an ongoing clinical study and can be subdivided into four separate investigations, each with its own objective and each specifically designed to test this objective. The objective of each investigation is listed.

Investigation 1.   To assess wound healing in young carious molars within four defined age groups, following pulpotomy (pulp dressing material: Calnex).

Investigation 2.   To verify the results of Investigation 1, using larger patient numbers, and to compare the efficacy of three different pulp dressing materials (Calnex; Calnex plus Ledermix paste; Calnex plus Endomethasone) in promoting wound healing following pulpotomy in three defined age groups.

<u>Investigation 3</u>.   To further compare the efficacy of Calnex and Calnex plus Ledermix paste using intra-oral comparisons in contra-lateral teeth in two defined age groups.

<u>Investigation 4</u>.   To assess the relationship between bridge formation and vitality over post-operative periods of two and five years.

C.   <u>MATERIALS</u>

<u>Pulp capping materials</u>

The following pulp capping materials were used.   They were prepared immediately prior to use and were applied directly to the wound surface subsequent to haemorrhage control.   Any excess blood clot was first removed using a pledget of cotton wool soaked in anaesthetic solution.

(1)   Calnex(Associated Dental Products Ltd., London).

Calnex is a brand of calcium hydroxide with added serum salts. Methyl cellulose is an added ingredient.   The manufacturers state that the addition of serum salts results in a pH of about 11. The powder was mixed with the liquid to produce a thin paste.

(2)   Calnex with added Ledermix paste.

Ledermix paste (Lederle Laboratories, Cyanamid of Gt. Britain Ltd., Hampshire) contains triamcinolone acetonide 1%, dimethylchlortetracycline 3%, a water soluble cream containing triethanolamine, calcium chloride, zinc oxide, sodium sulphite and polyethylene glycol.

Equal amounts by volume of Calnex paste and Ledermix paste were mixed together on a glass slab.   The mixture was applied immediately to the exposed radicular tissues.

(3)   Calnex with added Endomethasone paste.

Endomethasone (Septodont, Paris) contains Dexamethasone powder, a corticosteroid, Tetra-iodo-thymol (which is said to liberate traces of iodine) and Trioxymethylene (a powerful antiseptic).   The formula for Endomethasone (Septodont, Paris) · is given as:

Dexamethasone	q.s.	
Tetra-iodo-thymol	25	g
Trioxymethylene	2.2	g
Radio-opaque excipient q.s.	100	g

Endomethasone is mixed with eugenol to a firm paste.   In the present investigation, equal amounts of freshly prepared Endomethasone paste and Calnex paste were mixed together and applied to the radicular pulp.

Associated materials

Dycal (Caulk Co., Delaware).   This is a calcium hydroxide composition. It is a rigid, quick setting material indicated as a protective base-liner under dental cements.   In the present trials it was used to seal the pulp dressings over the radicular tissues prior to the insertion of the Kalzinol base material.

Kalzinol (De Trey Amalgamated Dental Co., London).   This is a quick setting resin-bonded zinc-oxide-eugenol cement used in the present trial to build up an ideal cavity base morphology.

Citanest (Astra Chemicals Ltd., Watford).   3% with Octapressin. Used as a local anaesthetic agent.   Also used to wash out cavities during cavity preparation and assessment for bridge formation.

Variation in use of pulp capping materials

Table 1 shows the pulp capping material used in each investigation.

Table 1.   Details of age groups and pulp capping materials in the
            four clinical investigations.

	Age groups	Dressing
Investigation 1.	< 7 years 6 months)	
	> 7 years 6 months < 9 years)	
	> 9 years < 10 years 6 months)	Calnex
	> 10 years 6 months)	
Investigation 2.	< 7 years 6 months	Calnex; Calnex + Ledermix; Calnex + Endomethasone
	> 7 years 6 months < 13 years	Calnex; Calnex + Ledermix
	> 13 years < 35 years	Calnex + Ledermix
Investigation 3.	< 7 years 6 months	Calnex; Calnex + Ledermix
	> 13 years	
Investigation 4.	> 8 years < 35 years	Calnex + Ledermix

Note: The four investigations are presented in the chronological order
       in which they were conducted.

       Age groups: In accordance with the results of Investigation 1,
       only three age groups were used in Investigation 2.

       Dressing materials: Calnex was used in Investigation 1.   In
       Investigation 2 the results of three medicaments were compared
       in the age group < 7 years 6 months.   In accordance with the
       results of this part of Investigation 2, only two dressings were
       used in the second age group and in Investigation 3.

D.   METHODS

Patient numbers

Sufficient numbers of patients with appropriate clinical condi-
tions were obtained during the routine administration of dental
services in general practice. The recent opening of a practice in
the centre of a large housing development predisposed to young
people attending for treatment. The majority attended for the first
time troubled with acute symptoms.   A very high proportion of these
patients had not previously received routine dental care.

Patients were excluded from the trial if they had a history of
heart disease or any chronic debilitating disease. Patients on
corticosteroid therapy and those who had taken aspirin for several
days prior to receiving dental treatment were also excluded.

Diagnosis

Patients were included in the trial if they presented with a
deep carious lesion and complained of acute pain, and following a
diagnosis of chronic partial pulpitis.   The criteria of Miller &
Massler (1962) were used to appraise whether the caries was active
or arrested.

Age groups

In Investigation 1 the following age groups were used:

Group	Patient's age
1	< 7 years 6 months
II	> 7 years 6 months < 9 years
III	> 9 years < 10 years 6 months
IV	> 10 years 6 months

These groupings were arbitrary but based on the previous unpublished clinical observations that pulpotomy was less successful when performed on teeth erupted less than one year. First permanent molar teeth erupt at approximately six years (patient's age). The first group includes molar teeth erupted less than $1\frac{1}{2}$ years. Further spans of $1\frac{1}{2}$ years determined the limits of succeeding groups.

In Investigation 2, three age groups were used in accordance with the results obtained in Investigation 1. These were: (1) patients aged less than 7 years 6 months at time of operation; (2) patients aged more than 7 years 6 months but less than 13 years at time of operation; (3) patients aged more than 13 years but less than 35 years at time of operation.

In Investigation 3, two age groups were used: less than 7 years 6 months and more than 13 years. This allowed the comparison of young teeth with immature root formation and teeth in which root formation had reached completion.

In Investigation 4, very young carious teeth erupted less than 18 months were excluded from the investigation, i.e. all patients were aged more than 7 years 6 months at time of operation.

Pulpotomy technique

Pulpotomy is defined as the extirpation of the vital coronal pulp at the physiological narrowing of the pulp chamber at the entrance to the root canals. A standardised clinical technique was used which involved the use of a tray system. A minimum number of selected instruments aided technique standardization and helped reduce operative variables. The sterilized instruments and burs were set out in the sequence in which they were used. Infiltration

or block anaesthesia was employed. The local anaesthetic agent used was Citanest. Rubber dam was not used. The region was kept relatively dry using cotton wool rolls and high volume aspiration.

(a) Cavity outline form was first established using high speed instrumentation with water coolant.

(b) Gross caries was removed and the cavity floor was created. No attempt was made at this stage to remove all the caries over the pulp chamber. Any exposures were immediately dressed with calcium hydroxide paste.

(c) Removal of all unsupported enamel using finishing burs was undertaken.

(d) The prepared cavity was thoroughly washed using anaesthetic solution from a syringe.

(e) Using a sterile excavator and a large round bur (No. 6), an opening was made into the pulp chamber. The instruments used at this stage were not those employed previously during cavity preparation. Adequate access was obtained by opening out the walls of the pulp chamber. The pulp was extirpated at the entrance to the root canal using a sharp excavator.

(f) Haemorrhage was controlled using a cotton wool pledget soaked in anaesthetic solution. Excess blood clot was removed before applying the pulp medicament. This was done using a dry cotton wool pledget.

(g) Freshly prepared pulp dressing medicament was applied directly to the pulp tissue. This was kept in place by flowing Dycal over the medicament.

(h) A lining of Kalzinol was used to cover the dressing and to build up correct cavity morphology.

(i)   An amalgam restoration was inserted immediately.   Initially,
      the tooth was left out of occlusion to ensure a period of
      rest.

Assessment methods

      Two direct inspection criteria were used:

(a)   probing to ascertain the formation of a hard mineralized
      bridge at the exposure site (Masterton, 1966a).

(b)   probing through this bridge to assess the vitality of the
      pulp (Nygaard-Ostby, 1962).

      At the recall visit, the amalgam restoration and Kalzinol base
were removed using an air turbine with water coolant.   The cavity
was washed thoroughly using local anaesthetic solution from a
syringe.   The removal of any Dycal lining and remaining debris was
accomplished using a fine probe.   The final assessment for bridge
formation and vitality was then undertaken.

E.   RESULTS

Investigation 1

The total number of teeth treated in the trial was 192, of which
135 were assessed after a six month period.   Table 2 details the
number of teeth treated and assessed in each of the four age groups
and the dressing used.   The numbers and percentages of vital and
non-vital teeth showing complete bridge formation or no bridging
formation are listed.

In this investigation only Calnex was used as a pulp dressing.
In the age group < 7 years 6 months only 3.77% of teeth showed both
bridging and vital pulp; 15.10% showed vital pulp without bridging,
while 84.9% were non-vital at six months.   This contrasted markedly
with the other three age groups where the percentage of teeth assessed
as having complete bridge formation and vitality at six months was
about ten times greater.   The null hypothesis, that there was no
correlation between bridge formation and maintenance of pulp vitality,
was tested in each group.   The results were not significant at the 5%
level when analysed by the χ^2 test in any of the four groups but did
approach the significant value in all but the very young group.

A further analysis was made.   The null hypothesis was set up
that there was no difference between the four groups with respect to
bridging or vitality.   The results confirm that group 1 is less
similar to groups 2, 3 and 4, both with respect to bridge formation
and vitality (Table 3).

Conclusions from Investigation 1

1.   No statistically significant association was found between bridge
      formation and vitality in group 1 at the 5% level when analysed

Table 2. A summary and analysis of the results obtained in
Investigation 1.

Patient's age at time of operation	Group 1 <7y 6m	Group 2 >7y 6m >9y	Group 3 >9y <10y 6m	Group 4 >10y 6m
No. of teeth treated	70 (M 33 (F 37	30 (M 16 (F 14	45 (M 24 (F 21	47 (M 21 (F 26
No. of teeth assessed	53 (M 29 (F 24	25 (M 12 (F 13	27 (M 11 (F 16	30 (M 15 (F 15
Analysis of results:				
Bridging: vital	2	9	11	14
non-vital	12	5	8	8
No bridging: vital	6	2	2	2
non-vital	33	9	6	6
% showing bridging	26.41	56	70.3	73.3
% showing vital pulp	15.10	44	48.1	53.3
% showing bridging + vital pulp	3.77	36	40.7	46.6
χ^2 test	1.04	3.49	2.99	3.72
	0.50>P>0.10	0.10>P>0.05	0.10>P>0.05	0.10>P>0.05
Null hypothesis: there is no correlation between bridge formation & maintenance of vitality	not disproved	not disproved	not disproved	not disproved
		but χ^2 values are approaching the significant value of 3.84 in groups 2, 3 and 4.		

Notes: 1. All teeth were 1st permanent molars (maxillary & mandibular)
        2. Assessment period: six months post-operatively
        3. All teeth were dressed with Calnex

Table 3.   Investigation 1:   Age group comparisons.   (The null hypothesis that there is no difference between the four groups either with respect to bridge formation or vitality is tested using the χ^2 test.)

	Groups 1 & 2		Groups 1 & 3		Groups 1 & 4		Groups 2 & 3		Groups 2 & 4		Groups 3 & 4	
	1	2	1	3	1	4	2	3	2	4	3	4
Bridging	14	11	14	23	14	22	14	23	14	22	23	22
No bridging	39	14	39	4	39	8	11	4	11	8	4	8
χ^2	6.34		24.7		21.79		5.21		1.37		1.16	
	0.02 > P > 0.01		0.001 > P		0.001 > P		0.02 > P > 0.01		0.50 > P > 0.10		0.50 > P > 0.10	
	significant		highly significant		highly significant		significant		not significant		not significant	

	Groups 1 & 2		Groups 1 & 3		Groups 1 & 4		Groups 2 & 3		Groups 2 & 4		Groups 3 & 4	
	1	2	1	3	1	4	2	3	2	4	3	4
Vital	8	11	8	21	8	16	11	21	11	16	21	16
Non-vital	45	14	45	6	45	14	14	6	14	14	6	14
χ^2	7.78		29.0		12.23		6.20		0.48		3.86	
	0.01 > P > 0.001		0.001 > P		0.001 > P		0.02 > P > 0.01		0.50 > P > 0.10		0.05 > P > 0.02	
	significant		highly significant		highly significant		significant		not significant		significant	

Age groups: 1: <7 years 6 months;   2: >7 years 6 months <9 years;   3: >9 years 6 months <10 years;   4: >10 years 6 months.

by the χ^2 test.

2. When the association between bridging and vitality was tested by the χ^2 test in groups 2, 3 and 4, the value of P approached the significant value.

3. Since the association between bridging and vitality was not statistically significant at the 5% level, the investigation can only be considered to demonstrate a trend.

4. The results support the notion that very young carious teeth heal less favourably than older teeth following pulpotomy dressed with Calnex. The reasons for this are not brought out in the clinical investigation. Further non-clinical investigations are required to elucidate if this is due to diagnostic factors, a reduced inherent healing potential, a variation in pulpal response to operative procedures, factors relating to the oral environment in those patients whose teeth decay so rapidly in the first instance or some other unknown factor(s).

Significance of results in terms of future investigations

(a) In groups 2, 3 and 4, the numbers assessed at recall were small. Further investigations are required in which larger numbers are assessed.

(b) In future investigations three age groups can be used: (1) newly erupted teeth (< 7 years 6 months); (2) more mature teeth with open apices and (3) mature teeth with closed apices.

(c) Both maxillary (three roots) and mandibular (two roots) first permanent molars were included in this preliminary investigation. This variable should be eliminated in future investigations.

Investigation 2

The total number of patients treated in three age groups was 421, of which 332 were assessed after a six month period.   Table 4 presents a summary and analysis of the results of Investigation 2.

A poor healing response was obtained in the age group < 7 years 6 months irrespective of the pulp dressing medicament when bridging plus vitality were used as the criteria of success.   It is of interest to note that the percentage of teeth showing bridge formation in this age group was almost identical with each of the three medicaments.   However, the percentage of teeth recorded as having bridging plus vitality was higher in the subgroups in which a form of corticosteroid had been added to the Calnex paste (Fig. 1). There was no significant correlation between bridge formation and vitality when tested by the χ^2 test.

In the age group > 7 years 6 months but < 13 years at time of operation, there was a much improved healing response independent of the type of applied dressing.   There was a statistically significant association between bridge formation and maintenance of vitality.

In the age group > 13 years, only one dressing was used (Calnex including Ledermix paste).   There was a very high association between bridge formation and vitality when these parameters were analysed by the χ^2 test.

Comparison of medicaments within different age groups

It is necessary to deduce from the results of Investigation 2 whether the different medicaments had different success rates within each age group.   For clarity, these inter-age group

Table 4. A summary and analysis of the results obtained in Investigation 2.

(* C = Calnex; L = Ledermix; E = Endomethasone; † 87(41:46) = 87 (41 male:46 female)).

Age Group	< 7 yrs 6 mths			> 7 yrs 6 mths < 13 yrs		> 13 yrs < 35 yrs
Dressing *	C	C + L	C + E	C	C + L	C + L
No. teeth treated†	87(41:46)	80(37:43)	91(42:49)	80(40:40)	78(36:42)	75(37:38)
No. teeth assessed†	62(37:25)	54(25:29)	66(30:36)	55(28:27)	51(21:30)	44(20:24)
% assessed	71.26	67.50	72.50	68.75	65.38	58.66
Analysis						
Complete bridging:						
vital †	4(1:3)	10(4:6)	13(6:7)	27(12:15)	25(15:10)	27(10:17)
non-vital †	15(7:8)	9(6:3)	11(5:6)	14(6:8)	9(4:5)	4(2:2)
Incomplete bridging:						
vital †	11(5:6)	13(6:7)	17(9:5)	5(4:1)	7(2:5)	2(2:0)
non-vital †	32(16:16)	22(10:12)	25(9:16)	9(4:5)	10(4:6)	11(5:6)
% showing complete bridging	30.64	35.18	36.36	74.54	66.66	70.47
% showing vitality	24.19	42.59	45.45	58.18	62.74	65.9
% bridge + vital	6.45	18.51	18.18	49.09	49.01	61.36
Null hypothesis: there is no correlation between complete bridge formation & vitality. χ²	0.30	1.37	1.21	4.02	5.14	20.38
P	P > .50	0.50 > P > .10	0.50 > P > 0.10	0.05 > P > 0.02	0.05 > P > 0.02	0.001 > P

Note: The distribution of patients by sex was similar in all groups; there was no statistical difference in success rates at the 5% with regard to sex when analysed by the χ² test.

129.

Fig. 1.   A comparison of the results obtained with Calnex,
Calnex plus Ledermix, and Calnex plus Endomethasone
in the age group < 7 years 6 months.

18·51%   18·18%

6·45%

Calnex   Calnex plus   Calnex plus
                Ledermix   Endomethasone
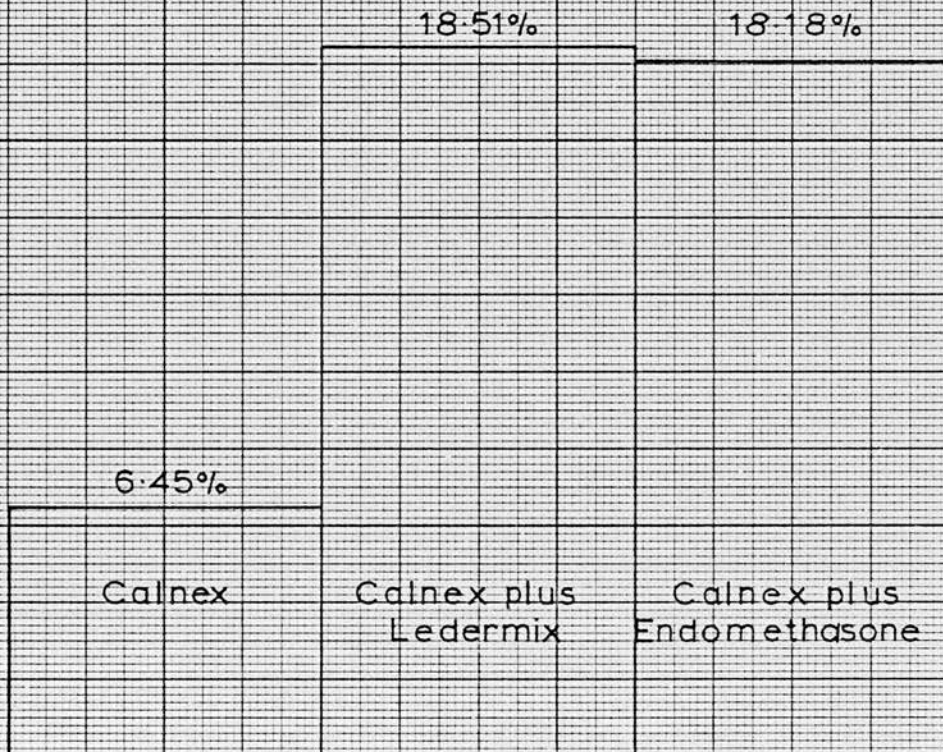
Note:

The histogram illustrates the percentage of teeth in each group

assessed as having complete bridge formation and a vital pulp,

at a six months post-operative period.

comparisons are discussed:

(1) in the age group < 7 years 6 months at time of operation (Table 5);

(2) in the age group > 7 years 6 months < 13 years at time of operation (Table 6).

(1) Age group < 7 years 6 months.   The addition of Ledermix or Endomethasone to Calnex paste resulted in an increase in the percentage of teeth recorded as having complete bridge formation and vitality at a six months post-operative period (see Table 5). There was a statistical difference between the group treated with Calnex and the group treated with Calnex plus Ledermix with respect to vitality when tested by the χ^2 test.   There was no statistical difference with respect to bridge formation when the two groups were similarly analysed.   Likewise, there was a statistical difference with respect to vitality but not with respect to bridging when the Calnex group was compared with the Calnex plus Endomethasone group using the χ^2 test.   Finally, the Calnex plus Ledermix group was compared with the Calnex plus Endomethasone group using the χ^2 test.   There was no significant statistical difference between the groups, either with respect to bridge formation or vitality.

(2) Age group > 7 years 6 months < 13 years.   In this age group, the Calnex group was compared with the Calnex plus Ledermix group with respect to complete bridge formation and maintenance of vitality using the χ^2 test.   There was no significant statistical difference between the groups treated with the different medicaments with respect to either of the parameters (Table 6).

Table 5.   Comparison of results obtained with the three different pulp dressings in the age group

<7 years 6 months at operation using the χ^2 test

Age group <7y 6m	Calnex v. Calnex plus Ledermix		Calnex v. Calnex plus Endomethasone		Calnex + Ledermix v. Calnex + Endomethasone	
Assessed 6m post-operatively	Calnex	Calnex plus Ledermix	Calnex	Calnex plus Endomethasone	Calnex plus Ledermix	Calnex plus Endomethasone
Bridging	19(30.6%)	19(35.2%)	19(30.6%)	24(36.4%)	19(35.2%)	24(36.4%)
No bridging	43(69.4%)	35(64.8%)	43(69.4%)	42(63.6%)	35(64.8%)	42(63.6%)
χ^2	0.31		0.59		0.1	
P	P>0.50		P>0.50		P>0.50	
Vital	15(24.2%)	23(42.6%)	15(24.2%)	30(45.5%)	23(42.6%)	30(45.5%)
Non-vital	47(75.8%)	31(57.4%)	47(75.8%)	36(54.5%)	31(57.4%)	36(54.5%)
χ^2	4.47		6.75		0.13	
P	0.05>P>0.02		0.01>P>0.001		P>0.50	

Note:

All teeth were 1st permanent molars.

Table 6. Comparison of results obtained with Calnex and Calnex plus Ledermix in the age group >7 years

6 months <13 years at operation using the χ^2 test

Age group >7y 6m <13y	Calnex	v.	Calnex plus Ledermix
Assessed 6m post-operatively	Calnex		Calnex plus Ledermix
Bridging	41(74.5%)		34(66.7%)
No bridging	14(25.5%)		17(33.3%)
χ^2		0.76	
P		0.50 > P > 0.10	
Vital	32(58.2%)		32(62.7%)
Non-vital	23(41.8%)		19(37.3%)
χ^2		<0.01	
P		Well above conventional significant level of 5% Null hypothesis not disproved	

Calnex alone was used as a pulp dressing in two age groups in Investigation 2: (1) < 7 years 6 months; (2) > 7 years 6 months < 13 years and, in Investigation 3 (vide infra) in one age group, > 13 years. Fig. 2 summarises the results obtained with Calnex alone in these three age groups. The null hypothesis was erected that there was no difference between the age groups either with respect to complete bridge formation or maintenance of vitality as assessed six months post-operatively. This was tested using the χ^2 test. The results indicated that there was a highly significant statistical difference between the < 7 years 6 months group and the other two groups with respect to both parameters. There was no significant difference between the > 7 years 6 months group and the > 13 years group. The results are presented in Table 7.

In Investigation 2, Calnex and Calnex plus Ledermix were used in two age groups: (1) < 7 years 6 months and (2) > 7 years 6 months < 13 years. Using the χ^2 test, the results were analysed and showed that there was a statistically significant difference at the 5% level between the age groups both with respect to bridge formation and vitality irrespective of the dressing. The results do indicate, however, that this difference was less pronounced when Ledermix was added, indicating the value of added corticosteroid in the treatment of very young teeth (Table 8).

Conclusions from Investigation 2

(1)   In the age group < 7 years 6 months, there was a poor healing response irrespective of the dressing used.

(2)   In the age groups > 7 years 6 months < 13 years and > 13 years, there was a good healing response independent of the dressing used.

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Fig. 2.   Summary of results obtained using Calnex in three age groups.
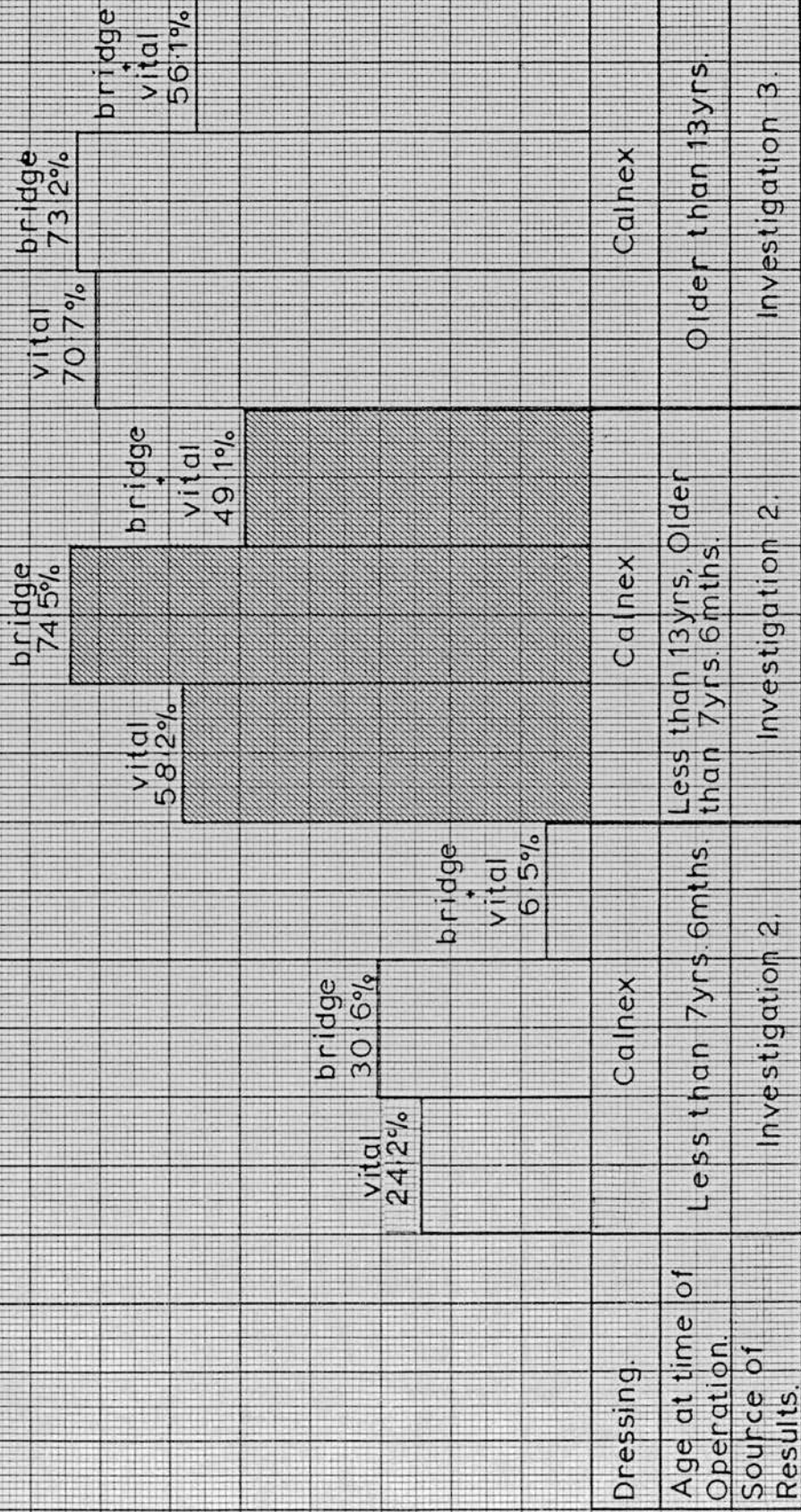
Dressing.	vital 24.2%	bridge 30.6%	bridge + vital 6.5%	vital 58.2%	bridge 74.5%	bridge + vital 49.1%	vital 70.7%	bridge 73.2%	bridge + vital 56.1%
Age at time of Operation.	Less than 7yrs. 6mths.			Less than 13yrs, Older than 7yrs. 6mths.			Older than 13yrs.		
Source of Results.	Investigation 2.			Investigation 2.			Investigation 3.		
	Calnex			Calnex			Calnex		

Table 7. Comparison of results obtained with Calnex in the age groups < 7 years 6 months; > 7 years 6 months < 13 years; > 13 years at operation, using the χ^2 test.

Age at time of operation	<7y 6m v. >7y 6m <13y		<7y 6m v. >13y		>7y 6m <13y v. >13y	
Assessment 6m post-operatively	<7y 6m	>7y 6m <13y	<7y 6m	>13y	>7y 6m <13y	>13y
Bridging	19(30.6%)	41(74.5%)	19(30.6%)	30(51.7%)	41	30
No bridging	43(69.4%)	14(69.4%)	43(69.4%)	28(48.3%)	14	28
χ^2	23.1		16.2		0.51	
P values	0.001>P>		0.001>P>		0.50>P>0.10	
Vital	15(24.2%)	32(58.2%)	15(24.2%)	29(70.7%)	32(58.2%)	29(70.7%)
Non-vital	47(75.8%)	23(41.8%)	47(75.8%)	12(29.3%)	23(41.8%)	12(29.3%)
χ^2	13.7		19.8		1.71	
P values	0.001>P>		0.001>P>		0.50>P>0.10	

Notes: 1. All teeth were dressed with Calnex only.
2. The results of the <7y 6m and >7y 6m <13y age groups were those obtained in Investigation 2.
3. The results of the >13y age group were those obtained in Investigation 3.

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Table <u>8.</u> Comparison of results obtained with Calnex and Calnex plus Ledermix in the two age groups

<7 years 6 months and >7 years 6 months <13 years using the χ^2 test

All results assessed 6m post-operatively	Calnex		Calnex plus Ledermix	
	<7y 6m	>7y 6m <13y	<7y 6m	>7y 6m <13y
Bridging	19(30.6%)	41(74.5%)	19(35.2%)	34(66.6%)
No bridging	43(69.4%)	14(25.5%)	35(64.8%)	17(33.3%)
χ^2	23		9.5	
P values	0.001>P		0.01>P>0.001	
Vital	15(24.2%)	32(58.2%)	23(42.6%)	32(62.7%)
Non-vital	47(75.8%)	23(41.8%)	31(57.4%)	19(37.3%)
χ^2	14.93		3.84	
P values	0.001>P>		0.05>P>0.02	

(3)   Analysis of results showed that there was a statistically signi-
        ficant difference in healing response in the age group < 7 years
        6 months compared to the age groups > 7 years 6 months < 13 years
        and > 13 years when Calnex alone was used as a pulp dressing.

(4)   Analysis of results showed that there was no significant differ-
        ence in healing response between the age groups > 7 years 6 months
        < 13 years and > 13 years when Calnex alone was used as a pulp
        dressing.

(5)   The addition of Ledermix or Endomethasone to Calnex aided mainten-
        ance of vitality in the age group < 7 years 6 months.

(6)   The addition of Ledermix or Endomethasone to Calnex had no
        significant effect on bridge formation in the age group < 7 years
        6 months.

(7)   The results confirm the notion that very young deeply carious
        teeth respond less favourably to pulpotomy than teeth in older
        age groups.

Significance of results in terms of future investigations

The results of Investigation 2 further support the notion that
very young deeply carious first permanent molars, especially in the
age group < 7 years 6 months, respond less favourable to pulpotomy
than do correspondingly older teeth. The analysis of results indicates
that the age groups > 7 years 6 months < 13 years are similar in their
healing response.   In order to eliminate any bias which may result from
the inclusion of "marginals", Investigation 3 was designed to compare
the efficacy of Calnex and Calnex plus Ledermix in two age groups, i.e.
< 7 years 6 months and > 13 years at time of operation.

Investigation 3

In this investigation, intra oral comparisons were made in two age groups: (1) < 7 years 6 months and (2) > 13 years. Calnex was used as a dressing in one tooth, the contra-lateral tooth being dressed with Calnex plus Ledermix. Included in the trial were 130 patients (260 teeth), of which 87 patients (174 teeth) were assessed after a six month period. The results and analysis are presented in Table 9. For clarity, the results are stated under two headings: (1) < 7 years 6 months and (2) > 13 years. Fig. 3 shows the percentage of teeth recorded as being vital and with bridging with respect to each medicament in both age groups.

(1) Age group < 7 years 6 months. There was no significant difference between the Calnex treated group and the Calnex plus Ledermix group at a 5% level using the χ^2 test. The results approached the significant level however and, on a percentage basis, better results were obtained both in terms of complete bridge formation and maintenance of vitality when Ledermix was added to Calnex. The percentage of teeth recorded as being vital was smaller than the percentage recorded as having complete bridge formation in both subgroups.

(2) Age group > 13 years. There was no significant statistical difference between the Calnex group and the Calnex plus Ledermix group at a 5% level using the χ^2 test. The percentage of teeth recorded as vital compared very closely with the percentage observed to have complete bridge formation in both subgroups.

Table 9. Summary and analysis of results obtained in Investigation 3 (intra-oral comparisons).

Age group	<7y 6m at operation		>13y at operation	
Tooth type	1st permanent mandibular molars		1st permanent mandibular molars	
No. of patients included in trial	70 (M34 (F36)		60 (M32 (F28)	
No. assessed	46 (M21 (F25)		41 (M21 (F20)	
No. assessed as % of total	65.7%		68.33%	
Dressing	Calnex	Calnex plus Ledermix	Calnex	Calnex plus Ledermix
Bridging	14(30.4%)	22(47.8%)	30(73.2%)	29(68.3%)
No bridging	32(69.6%)	24(52.2%)	11(26.8%)	13(31.7%)
χ^2	2.97		0.41	
P	0.10>P>0.05		not significant	
Vital	8(17.4%)	14(30.4%)	29(70.7%)	31(75.6%)
Non-vital	38(92.6%)	32(69.6%)	12(29.3%)	10(24.4%)
χ^2	2.1		0.11	
P	0.50>P>0.10		not significant	

Fig. 3. A comparison of the results obtained with Calnex and Calnex plus Ledermix in the age groups < 7 years 6 months and > 13 years.
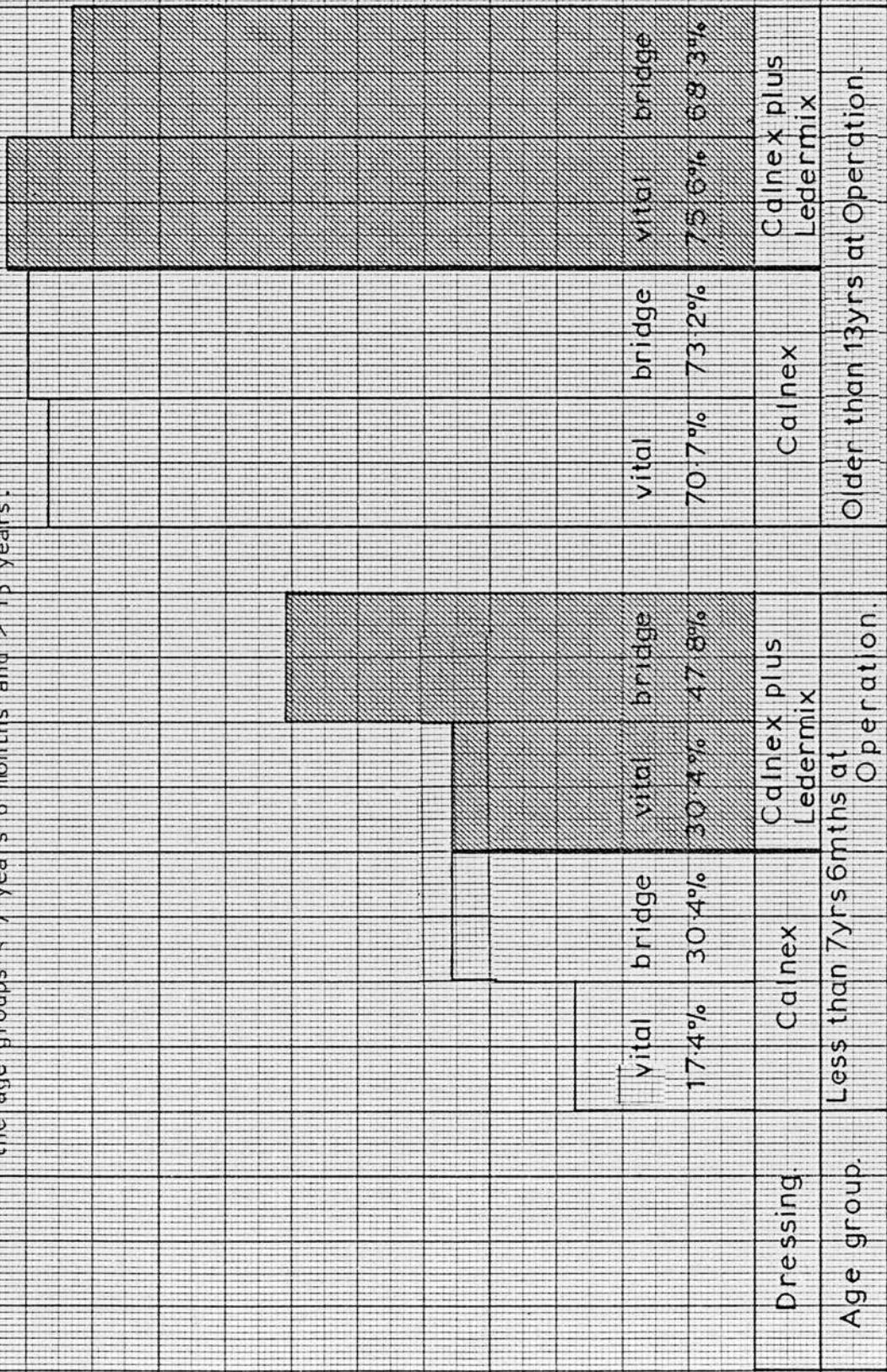
Dressing.	vital	bridge	vital	bridge	vital	bridge	vital	bridge
	17·4%	30·4%	30·4%	47·8%	70·7%	73·2%	75·6%	68·3%
	Calnex		Calnex plus Ledermix		Calnex		Calnex plus Ledermix	
Age group.	Less than 7yrs 6mths at Operation.				Older than 13yrs at Operation.			

Conclusions from Investigation 3

(1) The intra-oral comparison results obtained in Investigation 3
     confirm the results of previous investigations.

(2) The results confirmed that pulp necrosis can occur in spite
     of complete bridge formation in all age groups.

(3) Bridge formation in connection with a symptomless tooth is
     not a good indicator of pulp vitality in the age group < 7 years
     6 months.

(4) Bridge formation is a better indicator of pulp vitality in the
     age group > 13 years.

(5) The statements of Masterton (1966a) and Schroeder (1972)
     concerning bridge formation being an indicator of pulp vitality
     require to be modified in the light of these results.

(6) The results indicate that, in the age group < 7 years 6 months,
     the addition of Ledermix to Calnex tends to promote maintenance
     of tissue vitality.

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Investigation 4

Table 10 summarises the results of a retrospective, intra-oral comparison investigation. All teeth were dressed with Calnex plus Ledermix. At six months post-operatively one tooth was randomly selected for assessment; the contra-lateral tooth was then assessed at a later date.

The null hypothesis, that there was no statistical difference between the groups assessed at six months and two years, was tested by the χ^2 test. There was no statistical difference between the two groups at a 5% level with respect either to bridge formation or maintenance of vitality. The percentage of teeth recorded as having complete bridge formation at two years was 77.1% compared to 70.9% recorded at six months, suggesting a tendency for bridge formation to continue beyond a six month period. The percentage of teeth recorded as being vital at two years was 64.6% compared to 72.7% at six months (Fig. 4).

In the subgroups assessed at six months and five years, there was no significant difference either with respect to bridge formation or maintenance of vitality at the 5% level when analysed by the χ^2 test. This five year assessment again pointed to bridge formation continuing beyond the six month period and to a reduction in vitality in spite of complete bridge formation (Fig. 4).

Conclusions from Investigation 4

(1) There was no statistical difference between the groups assessed at six months and two years at the 5% level using the χ^2 test, either with respect to bridge formation or vitality.

Table 10. Summary and analysis of results obtained in Investigation 4 (intra-oral comparisons).

Post-operative assessment period	6 months	2 years	6 months	5 years
Tooth type	permanent premolars & molars		permanent premolars & molars	
No. of patients included in trial	69 (M 32)(F 37)	69 (M 32)(F 37)	31	31
No. assessed	55 (79.7%)	48 (69.6%)	27 (87.1%)	20 (64.5%)
Dressing	Calnex plus Ledermix		Calnex plus Ledermix	
Bridged	39 (70.9%)	37 (77.1%)	19 (70.37%)	15 (75%)
Vital	40 (72.7%)	31 (64.6%)	19 (70.37%)	13 (65%)
Bridged plus vital	35 (63.6%)	31 (64.6%	16 (59.3%	13 (65%)
Age group	All teeth erupted more than 18 months		All teeth erupted more than 18 months	

Note: The results are compiled from cases routinely treated in the surgery. The teeth were not originally included in any particular trial. The six month assessment was undertaken on one tooth of the pair to assess the success of the original treatment in 100 cases. From this pool of patients, recalls were sent out after 2 and 5 years.

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bridge+vital 65%
vital 65%
5 yrs.
bridge+ 75%
bridge+vital 59.3%
vital 70.4%
6 mths.
bridge 70.4%
Calnex plus Ledermix.

bridge+vital 54.6%
vital 54.6%
2 yrs.
bridge 77.1%
bridge+vital 63.6%
vital 72.7%
6 mths.
bridge 70.9%
Calnex plus Ledermix.

Age Range 8 - 35years.
Post-Operative period.
Dressing.
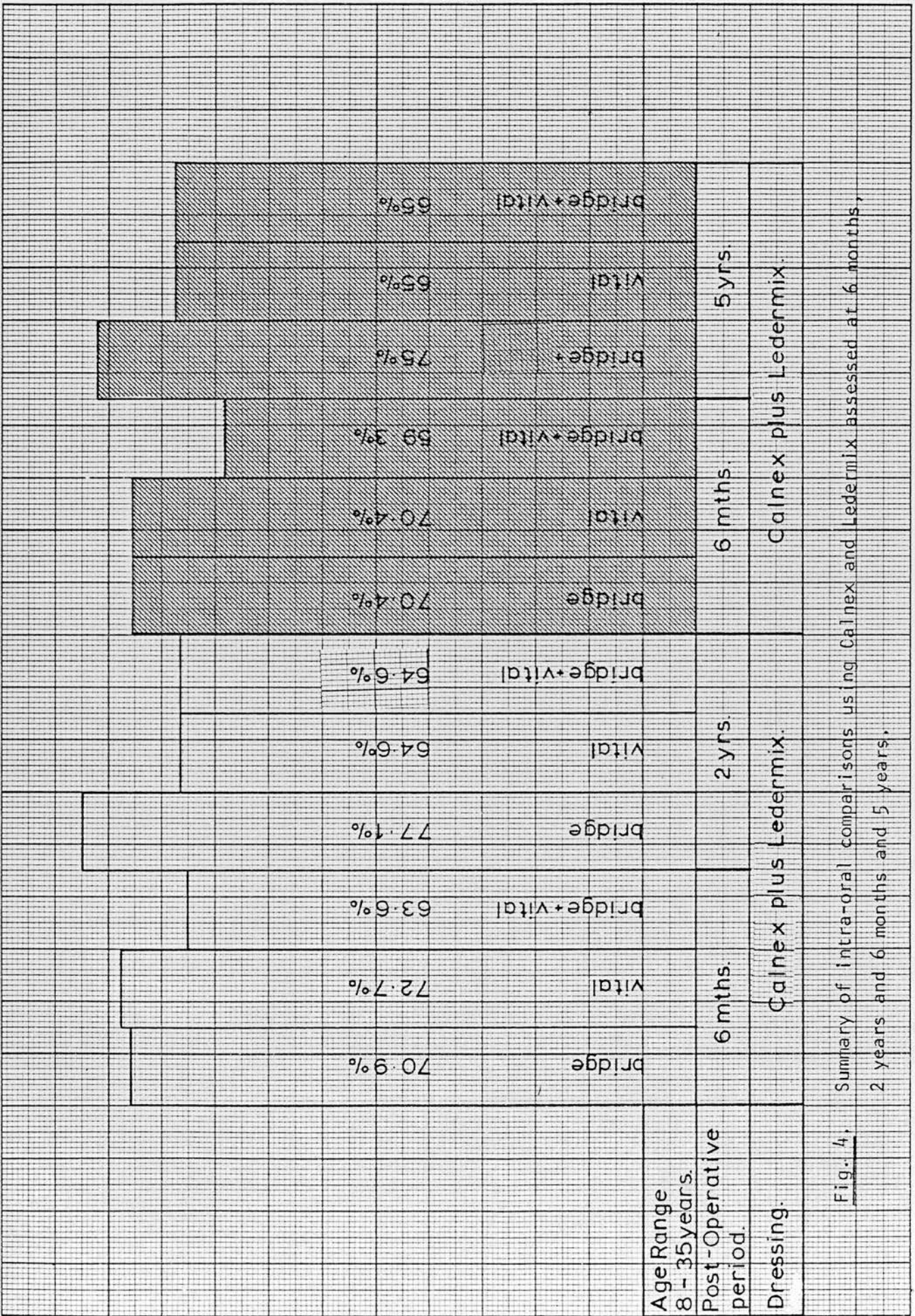
Fig. 4. Summary of intra-oral comparisons using Calnex and Ledermix assessed at 6 months, 2 years and 6 months and 5 years.

(2) The results suggest a tendency for bridge formation to continue in some cases beyond a six month period.

(3) The results suggest a tendency for pulp necrosis to occur in spite of complete bridge formation.

(4) There was no statistical difference between the groups assessed at six months and five years at the 5% level using the χ^2 test, either with respect to bridge formation or vitality.

(5) As in the six months/two years investigation, the results suggest a tendency for a continuation of bridging and a reduction in vitality to occur with time.

(6) Many non-vital teeth were clinically symptomless at the two and five year periods.

(7) The results show that painful, deeply carious teeth, which in many cases would be considered for extraction, can be treated by pulpotomy with the expectation of at least a 65% clinical success rate over a two to five year period.

The following points can be drawn from the investigations as a whole:

1. Following pulpotomy in human teeth, attempts at healing occur in all age groups. This is clinically evidenced by the formation of a hard tissue barrier at the exposure site.

2. The hard tissue barrier may be completely or incompletely formed.

3. Clinical assessment does not permit a qualitative assessment of the formed barrier.

4. Clinical assessment gives no direct information concerning the mechanisms involved in barrier formation.

5. At a six month post-operative period, the following states were observed in all age groups:

   (a) complete barriers associated with vital pulps.

   (b) complete barriers associated with non-vital pulps.

   (c) incomplete barriers associated with vital pulps.

   (d) incomplete barriers associated with non-vital pulps.

6. Post-operative discomfort is frequent following the use of calcium hydroxide paste as a pulp wound dressing.

7. The addition of equal amounts by volume of a proprietary corticosteroid/antibiotic medicament such as Ledermix paste or Endomethasone paste reduces post-operative discomfort.

8. Very young carious teeth respond less well to pulpotomy procedures as judged by two direct inspection criteria at a six month post-operative period.

9. The reasons for (8) are not brought out in the present trial. Further investigations are required to elucidate this point.

10. The addition of proprietary corticosteroid/antibiotic prepara-
    tions to Calnex paste improves healing in all age groups.

11. The improvement in healing is greatest^ in the age group
    containing teeth erupted less than 18 months.

12. The reasons for the above point cannot be deduced from the
    present investigation.

13. It is not known by which mechanisms calcium hydroxide compounds
    stimulate repair in the exposed pulp.   Further studies are
    required to investigate this fundamental issue as well as the
    possible variations in effect which may occur when cortico-
    steroid preparations are added to calcium hydroxide.

14. In all age groups (other than very young newly erupted teeth),
    there was no statistical difference with respect to bridge
    formation or vitality at six months, two years and five years.

15. Bridging was noted to continue in some cases beyond a six
    month period.

16. Long-term clinical evaluation indicated a reduction in vitality
    in spite of complete bridge formation.

17. Complete bridge formation cannot therefore be taken as a
    guarantee of maintenance of pulp vitality.

18. Bridge formation as assessed by direct clinical inspection is
    likely to indicate maintenance of pulp vitality in symptomless
    teeth with no radiographic evidence of periapical pathology.

19. Pulpotomy has been shown to be an effective clinical procedure
    in the treatment of permanent teeth in patients ranging from
    6 - 35 years of age, where less conservative techniques are
    ruled out.

20. Pulpotomy is a simple, quick, pain relieving procedure. It is especially useful in the treatment of young apprehensive patients. It may be the treatment of choice in the introduction of these patients (and their parents) to preventive dentistry when the patient presents for the first time with a painful deep carious lesion.

21. Bacterial infection of the radicular pulp tissue may be due to diagnostic errors or to errors in technique. It is not known to what extent, if any, the antibiotic contained in proprietary corticosteroid compounds contributed to the observed increase in healing.

F.   DISCUSSION

Design of studies on wound healing in the carious tooth

The clinical trial is the ultimate step in the evaluation of
any medical or dental technique.   It should be conducted only after
initial and confirmatory biological testing has been carried out on
laboratory animals.   It has the great advantage over purely experi-
mental studies in that it is undertaken under conditions which will
always exist when the procedures are adopted as routine clinical
measures.

Problems inherent in designing clinical trials include (1) the
obtaining of sufficient patient numbers with the appropriate condi-
tion;   (2) the problem of obtaining material for histological
investigation;   (3) the problem of following up patients for a
reasonable period of time when histological assessment is not
possible;   (4) the problem of assessment when histological investi-
gation is impossible.

Criteria and guidelines for the design of studies on the
pulpal response to materials placed in deep cavities have been
suggested by Stanley (1968a,b).   Assessment of results is normally
based on the method suggested by Kramer & McLean (1952).   This includes
inflammatory and calcific indices.   Controls are usually afforded by
using contra-lateral teeth prepared in a similar manner and filled with
zinc oxide eugenol.   Stanley (1968a) suggested that, if a sufficient
and comparable number of teeth are used (50-75), the biological
variation of human pulp response is minimized.   The amount of dentine
remaining beneath the cavity plays the most important role in the

incidence of pulp response. Where the effect of any material is being assessed, all specimens with a remaining dentine thickness greater than 2mm should be eliminated. It is of interest to the present study that the response to cutting occurs only beneath freshly cut (virgin) dentinal tubules not lined by reparative or irregular dentine. An investigator can bias the results of an operative technique by involving a preponderance of specimens with large or small average thicknesses of remaining dentine or teeth with more or less reparative or irritation dentine.

These factors illustrate the difficulty in eliminating variables when investigating pulp healing in human teeth which have been affected by caries. This difficulty is compounded when the investigated teeth are exposed either by caries or by surgical procedures, accidental or intentional.

Al Rubayi (1971) described a method for producing controlled exposures both in diameter and depth, in non-carious ferret teeth. Controlled exposure-size experiments are virtually impossible in human trials. At best, human trials should be designed to reduce existing variables to a minimum by the use of a standardized technique.

The importance of human pulp studies has been reduced by two factors. Most studies have been made on small sample numbers. Secondly, most studies have been concerned with sound teeth destined for orthodontic extraction. In young teeth, especially newly erupted, non-functional teeth, dentinal irritation may be transmitted to the pulp in a stronger form than when reparative dentine has been laid down. On the other hand, the open apex and good blood supply

in these young teeth should predispose to an excellent healing
potential.

In the present clinical study, all teeth were treated during
the provision of routine dental care in general practice. All the
treated teeth were carious. In these circumstances, prediction of
the response of the wounded pulp must be made from the results of
histologically assessed experiments conducted on both animal and
human material. The review of the literature includes the more
important of these experiments.

Appraisal of present study

Essentially, the present clinical trial assessed wound healing
following a standardized pulpotomy procedure in teeth affected by
deep carious lesions, and the assessment of any variations in success
rates following the pulpotomy procedure in different age groups and
with different pulp dressing materials.

Though there may be dispute about the efficacy of pulpotomy in
the preservation of pulp vitality, and also in the results obtained
when corticosteroid/antibiotic preparations are applied to the
exposed pulp, neither the technique nor materials can be considered
experimental. They have all been screened and are accepted treat-
ment methods. The present trial attempts to compare the efficacy
of certain procedures under defined clinical conditions by assessing
large numbers of patients over periods ranging from six months to
five years.

(a) The operative technique. Every effort was made to standardize
the operative technique. This included the use of a minimum number

of selected instruments in a tray system.

All cavity preparation was done using copious water spray.
Caries removal was done with a very slow revolving bur without
water spray.

Infiltration or block anaesthesia was employed.   There was
seldom difficulty in achieving the required degree of anaesthesia
which allowed the painless extirpation of the coronal pulp.

Rubber dam was not used.   The abandonment of this procedure
must not be taken as a suggestion that the avoidance of infection is
unnecessary.   The importance of bacterial contamination as a factor
influencing the response of the wounded pulp is firmly acknowledged.
Aseptic procedures should be adopted whenever possible.   Sterile
procedures are not possible in the oral cavity.   Treatment involving
a reduction in bacterial population is possible only by using sterile
instruments in a saliva-free environment.

Rubber dam is virtually impossible to apply to an erupting tooth.
Some patients objected to the protracted operative procedure.   If
rubber dam had been used in some cases and not in others, this would
have resulted in the subdivision of the assessed cases.   The region
was kept relatively free from salivary contamination by using cotton
wool rolls and high volume aspiration.

(b) Diagnosis.   The prime objective in the treatment of the deep
carious lesion is to preserve or restore the integrity and function
of the pulpo-dentinal organ.   This objective can only be fulfilled
if the pulp pathology is of a reversible nature.   Treatment
planning and the choice of clinical technique depends on an

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accurate diagnosis of the existing pulp pathology.

The clinical diagnosis of the pulp pathology in relationship
to the deep carious lesion is acknowledged to be a difficult
problem. A lack of correlation exists between the clinical
symptoms and signs and the histopathology of the pulp. Seltzer,
Bender & Ziontz (1963) and Baume (1970b) illustrate that a single
affected pulp may show two or more histological pictures if serial
sections are studied.

In order to formulate clinical guidelines for the treatment of
the deep carious lesion, a thorough knowledge is required of the
dynamics of the carious lesion, the relationship of the bacteria to
softened dentine and the protective properties of the remaining
dentine (see review section). There exists a complex relationship
between the carious process and the resultant pulpal changes. The
carious process is not a single entity. Pulp reactions have been
described as a result of early incipient caries (Langeland & Langeland,
1968) whereas Reeves & Stanley (1966) and Shovelton (1970) found
little pulpal inflammation unless bacteria were within 0.8 to 1.1mm
from the pulp. Irreversible pulp pathology was considered a late
event in the progress of caries.

Miller & Massler (1962), Barber & Massler (1964) and Baume
(1970b) differentiate between active and arrested caries and the
variation in permeability of the associated dentine. Corbett (1963)
noted that reparative dentine was only laid down in approximately 50%
of cases and that this did not always prevent pulpal inflammation.

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It is by no means clear what agent or agents produce pulp
damage. Massler (1972) differentiated between the infected pulp
and the pulp affected by toxins and irritants emanating from the
carious lesion. Opinions differ as to the extent to which bacteria
penetrate dentinal tubules (Olgart et al., 1974). It is acknow-
ledged that the vital pulp has a great resistance to the penetration
of bacteria (Bradford, 1960; Seltzer et al., 1977). Johnson et al.
(1973) considered that the outward flow of fluid under pulpal
pressure was one factor offering resistance to bacterial penetra-
tion.

Seltzer et al. (1977) and Torneck (1978) suggested that immuno-
logical phenomena play a significant role in the production of pulp
disease and that delayed hypersensitivity reactions may be mediated
by the diffusion of bacterial products through dentine. The thick-
ness and the physico-chemical properties of the dentine covering the
pulp offer protection against external irritants. It is not always
possible to judge the remaining dentine thickness clinically.
Imperfect mineralized reparative dentine is not always detectable
radiologically.

(c) Materials. Calcium hydroxide is regarded as the preferred
material in the treatment of the exposed pulp. Histological
studies have shown that it is not without certain drawbacks. It
is said to exert a continuous stimulatory effect on the pulp tissues
and this can lead to massive reparative dentine formation and
blockage of root canals (Seltzer & Bender, 1965) or to internal
resorption of dentine (Masterton, 1965b). Study of serial sections
is said to show that bridge formation is seldom complete (Paterson,

1974; Langeland, 1979) and this leads to a chronic inflammatory process and the eventual necrosis of the pulp. The mode of action of calcium hydroxide is not known. Severâl investigations have suggested that the dental pulp has an inherent healing potential (Torneck et al., 1973). Reparative dentinogenesis occurs without the use of therapeutic agents in germ free animals according to Retzlaff & Castaldi (1969) and in normal rats, whether wax or therapeutic agents were used (Luostarinen, 1971).

It is known that a minimal inflammatory stimulus is required for normal healing (Brunius & Zederfeldt, 1965). Several authors (Nyborg, 1955; Masterton, 1964a; Schroder, 1972) have suggested that calcium hydroxide exerts such a minimal inflammatory stimulus via the necrobiotic zone rather than by a direct participation of the chemical in bridge formation (Sciaky & Pisanti, 1960; Pisanti & Sciaky, 1964).

During the last two decades, corticosteroid preparations alone or in combination with antibiotics have been advocated for use in the treatment of the inflamed pulp. They have been tested clinically by Kiryati, 1958; Kozlov & Massler, 1960; Cowan, 1966; Fiore-Donno & Baume, 1966 and Clarke, 1968, 1970). Only Kozlov & Massler reported calcific repair following the placement of steroids on the pulp tissue.

The adverse effects of corticosteroids are listed as: suppression of the inflammatory defence reaction (Sinkford & Harris, 1964); suppression of connective tissue formation (Sinkford & Harris, 1964);

suppression of cellular differentiation (Brosch, 1966); effect on normal odontoblast function (Mjor & Ostby, 1966; Ulmansky & Langer, 1967) and the dissemination of bacteria (Klotz et al., 1965). Paterson (1976) conducted tests in which Ledermix cement, Ledermix paste and Pulpomixine were tested using Albino rats. In a follow-up study, the components of Ledermix were tested, i.e. the carrier paste alone without steroid or antibiotic, the carrier paste plus steroid and the carrier paste plus antibiotic. The results of both studies were assessed histologically and compared with the effects of Dycal. All corticosteroid preparations gave poor results in conventional animals and little difference was observed in the results obtained with the carrier alone and with those obtained when the complete formula was used. The results confirm the responses of the histologically assessed trials conducted on human material (Cowan, 1966; Clarke, 1968; Barker & Ehrman, 1969; Sykaras, 1972). These trials cast some doubt on the validity of clinically assessed trials reporting the successful use of corticosteroid preparations. However, excellent results were obtained when corticosteroid cement was applied to germ-free pulps. Paterson (1976) concluded that the poor results found in conventional animals cannot be entirely attributable to the drug. He considered that the possibility exists that the drug alters the response of pulp tissue to bacterial contamination.

The reluctance of the dental profession to forsake the advantageous qualities of the steroids is reflected in the continuous inclusion of corticosteroids in both clinical and histologically assessed trials.

The most frequent complication of pulp capping is that involving the inflammatory response, oedema and ensuing pulp degeneration. The rationale behind the use of corticosteroids is the belief that the dental pulp in mature teeth is unable to survive the inflammatory response without therapeutic aid.

Knowledge concerning the mode of action and the involved cellular mechanisms of corticosteroids is scant (Sinkford & Harris, 1964). Schroeder (1965) cautioned against leaving corticosteroids in contact with pulp tissue because of the possibility of continuous depression of connective tissue formation and inhibition of cellular differentiation. Anti-inflammatory drugs such as cortisone have, however, been shown to delay healing only during the first few days (Sandberg, 1966). Once healing is established, cortisone is without effect. In practice, wounds do heal in patients on long-term steroid therapy but healing is slower (Forrester, 1976).

Many of the conclusions formed from histologically assessed trials could be questioned on account of the short post-operative periods. Where necrosis has occurred, this could be due as much to the toxicity of non-active carrier ingredients or to leakage around fillings as to the action of steroids per se.

There is histological evidence (Sinkford & Harris, 1964) that corticosteroids increase vascular tone when applied to the exposed pulp. Tissue vitality is dependent on a functional capillary network. Tissue oxygen tensions have been suggested as being of importance in wound healing and may affect fibroblast proliferation, migration and ground substance synthesis (Niinikoski, 1969; Hunt, 1972), as well as cellular differentiation (Niinikoski, 1969).

The review of the general literature has suggested that there seems to exist an optimum relationship between the inflammatory response and fibroblastic proliferation, i.e. between the inflammatory phase and the proliferative phase. Several different approaches have been taken to utilize the advantageous effects of calcium hydroxide and corticosteroids in achieving such an optimum relationship.

Bhaskar et al. (1969) undertook a study to determine if the addition of corticosteroids could enhance the tissue tolerance to calcium hydroxide. Using rats, pellets of calcium hydroxide and calcium hydroxide containing steroids were subcutaneously implanted on either side of the midline. It was found that the addition of steroids to calcium hydroxide reduced the intensity and duration of oedema, reduced the cellular infiltrate, markedly reduced or eliminated tissue necrosis and markedly reduced dystrophic calcification. Bhaskar suggested the addition of small amounts of corticosteroid should improve patient comfort and enhance the success of calcium hydroxide pulp capping procedures and approbated its clinical use (personal communication). Following this, the present author reported on the clinical use of Calnex containing small quantities of Ledermix paste (Quintessenz Essay Competition, 1970).

Schroeder (1972) described a similar technique in which calcium hydroxide containing Ledermix paste was applied as a permanent pulp capping agent with good results. Shovelton et al. (1971) reported on the clinical trial of several materials. Included was a technique in which Ledermix paste was applied for three days followed by the application of calcium hydroxide. Best results appeared to be

obtained by this two stage method when pain had been present prior to treatment. The following points were made by the authors: the varying success rates should be viewed with caution and considered as trends since the differences between the medicaments were not statistically significant at the 5% level (χ^2 test). The analysis may have been affected by the number of patients who failed to attend for follow up; it was even suggested that the success was more related to the technique per se than to the materials placed in contact with the pulp.

On the basis of the evidence presented in the literature, the combination of calcium hydroxide and corticosteroids is a promising combination. It is equally evident that detailed biological testing of the components is still required together with long-term clinical trials. The present investigation was undertaken to fulfil this latter need.

Maeglin et al. (1966) and Retzlaff & Castaldi (1969) stated that the only positive aspect of corticosteroid therapy is the dramatic reduction in pulpal pain. Paterson (1976) stated that, on the basis of current evidence, routine use of corticosteroids as pulp capping agents cannot be supported. These authors believe corticosteroids inhibit wound healing.

On the other hand, several authors report maintenance of pulp vitality following the use of corticosteroids (Cowan, 1966; Clarke, 1968; Barker & Ehrman, 1969; Sykaras, 1972). Their work can be criticised on the grounds that they accepted as successful treatment those pulps which remained vital but where there was lack of odonto-blast differentiation.

160.

Future investigations must be directed towards an examination
of the fundamental aspects of pulp wound healing. The action effect
of each ingredient included in proprietary materials should be
screened for their individual effect on the processes of wound
healing.

According to Fremont-Smith (1968), when a cell is differenti-
ated, this is a result of environmental interaction with the gene
potential. It is becoming apparent (see review of literature)
that oxygen plays a critical role in normal healing. Steps have
been taken by the author to develop platinum micro-electrodes which,
in conjunction with pulpotomy, can be used to determine oxygen
tensions in healing pulp wounds. Such an approach, coupled with
histological assessment, would allow a more fundamental approach to
the investigation of pulp capping materials and their effect on
pulp tissues.

In the present investigations, calcium hydroxide and calcium
hydroxide plus proprietary corticosteroids were used. All materials
used were commercially available preparations. The combination of
corticosteroids and calcium hydroxide was undertaken in an attempt to
attain better tissue receptivity. Further detailed histological
assessment is required.

(d) Patient numbers. Large numbers of appropriate cases were
obtained during the routine administration of general dental
services. The recent opening of the practice in the centre of a
large housing area predisposed to young people attending for
treatment; the majority had not previously received routine dental
care.

Reasonable follow-up numbers were obtained. This is in contrast with the experience of Shovelton et al. (1971) and it is difficult to make firm conclusions why this was so but the following differences in approach may have been contributory.

1.   Patients attending the practice were always treated by one dentist who also provided all other necessary dental care.

2.   Patients were not told they had received a particular type of procedure. The prognosis was not discussed.

3.   All patients were recalled whether they were included in a particular trial or not.

This was an intended policy. It is the author's opinion that the public will more readily respond to a request which offers them a service than one which involves their co-operation in a scheme (seemingly) not directly beneficial to them.

There seems to be certain advantages in conducting properly designed trials within the field of general dental practice.

(e) Age of patients. Acute and rapid caries is common in children and teenagers. The primary dentition is highly susceptible to acute caries activity from 4 - 8 years. Teenagers are most susceptible from 11 - 18 years. Rampant caries in children is characterized by frequent periods of acute caries activity in which there is rapid destruction of calcified tooth structure.

Wound healing in young teeth, especially those with open apices, is considered to have a better prognosis than in older teeth where atrophy may have resulted from previous disease episodes. Alterations in wound healing response in association with the deep carious cavity

have not been fully investigated. The purpose of the present investi-gation was not to advocate a particular technique or medicament. A notion had been formulated from clinical experience that a poorer wound healing potential existed in very young carious molars. The investigation fulfilled the first step in verifying this notion by assessing wound healing in carious involved teeth in different age groups. Subsequent investigations are required to ascertain the cause of this altered response. It is known that, in caries, the dead tract response is more frequently found than the translucent zone response. An exception is occlusal caries in molars which inexplicably is more frequently associated with a translucent zone than with a dead tract. Further information is required on the variation in dentinal responses with respect to age. In the second section of this thesis, experiments are detailed which attempt to quantify permeability characteristics of dentine.

(f) Assessment methods. Shovelton et al. (1971) investigated the efficacy of materials for direct capping of the exposed pulp. Assessment of the pre-operative condition was undertaken by percussion, electric pulp testing and apical radiographs were exposed. Post-operative success was determined using similar methods at 6, 12 and 24 months. The authors were aware of the limitations of these criteria especially the difficulty in correlating clinical findings with the histological state of the pulp. A tooth free of symptoms is not in itself a guarantee of success. There is also a difficulty in correlating the results of electric pulp test readings with the state of the pulp. Radiography can only be of

positive value in demonstrating failure of treatment.

The two direct inspection criteria used were advocated by Nygaard-Ostby (1962) and Masterton (1966a). Bridge formation was considered by Masterton (1966a) and Schroder (1972) to indicate a restoration of odontoblast function. Masterton (1966a) verified this histologically and stressed the direct post-operative examination of the wound site to confirm complete healing.

Shovelton et al. (1971) noted a fall in success rate over a two year period in pulp capping experiments. The present author's experience has shown that pulp vitality is not always maintained in spite of clinically verified bridge formation. It was felt necessary to include a test which would correlate bridge formation with pulp vitality. Histological assessment was ruled out because of the nature of the trial. The statement by Nygaard-Ostby (1962) that "the best way to ascertain the vitality of the pulp tissue is to see the bleeding pulp" indicated the second direct assessment method.

In the assessment of pulp healing, clinical and histological parameters are recorded and related to tissue function. Vitality tests relate subjective findings to continuation of pulp function; radiographs and histological assessments relate anatomical and morphological variations to cellular biology. No currently used assessment method measures tissue function per se.

It is essential to have a clear understanding of what is being assessed; it is essential to appreciate the limitations of any assessment method. Moreover, in the assessment of wound healing, a

definition of what is meant by healing is mandatory "lest we fall into the mire of fruitless argument" (Massler, 1972).

Healing takes place within the connective tissue of the pulp in a similar manner to other connective tissues. An essential difference is that cellular differentiation must occur to replace lost odontoblasts. Healing occurs in the pulp when a functional odontoblast palisade is reformed with ensuing dentinogenesis. A spatial and functional re-organisation of subodontoblast pulp elements should occur at the same time.

There is a variation in the morphology of the odontoblast layer and the subodontoblast zones at different stages of dentinogenic activity (Scott & Symons, 1961). These temporal variations are also seen at an ultrastructural and histochemical level (Ten Cate, 1962, 1966; Reith, 1968). The relationship between the various pulp tissue elements and their involvement in pulp physiology is not fully appreciated. This adds to the complexity of assessing the restoration of functional tissue homeostasis on purely histological grounds.

Cellular synthetic activity, i.e. the production of collagen and ground substance synthesis, including sulphated glyco amino glycans (Weinstock & Young, 1972), seems fundamental to odontoblast function. These substances are considered to be involved in the mineralization process (Taves, 1965). There is evidence which suggests that the odontoblasts play a further role as a "functional membrane" similar to that attributed to osteoblasts (Talmage, 1969). The fluid compartment associated with the mineralized dentine will have calcium and phosphate concentrations controlled by the solubility product of this

tissue. This is lower than the extracellular fluid of the pulp tissue which is in equilibrium with the extracellular fluid of the body in general. The two compartments are separated by a continuous odonto-blast palisade and is suggestive of odontoblast participation in calcium homeostasis. Further evidence in support of this notion is the report of calcium containing granules in odontoblast processes (Kashiwa & Sigman, 1966) and the suggestion by Frank (1968) that the odontoblasts engage in catabolic as well as anabolic processes as shown by the ultrastructural visualization of lysosome-like bodies in the perikaryon (Frank, 1966) and odontoblast process (Ten Cate, 1967).

Calcium is known to play a major role in physiologic processes; the maintenance of appropriate concentrations and distributions are essential for cellular function.

The influence of a functional odontoblast layer (engaged in calcium ion regulation) on the vitality of subjacent cells remains speculative in the light of present knowledge concerning pulp physi-ology. It is, however, highly probable and fits the known facts.

It is considered by the present author that the only hopeful prognostic sign of continued pulp homeostasis and tissue vitality is the identification of restoration of a pulpo-dentinal organ. Moreover, any criterion which ignores the inter-relationship between the odontoblast cell function and reparative dentinogenesis must be considered as inadequate. Cowan (1966) regarded maintenance of vital pulp tissue and not reparative dentinogenesis as his criterion of success. This ignores the pulpo-dentinal organ as one of the innate immune non-specific body defence mechanisms involved in maintenance of

tissue homeostasis.   It cannot be regarded as an adequate assess-
ment of healing.

Paterson (1976) states that the significance of the dentine
bridge in assessing the future prognosis for the exposed pulp is
difficult to evaluate.   Careful examination of serial sections
showed defects in every bridge seen when exposed rat molars were
treated with various medicaments.   If long-term studies showed
that "bridge formation" was defective in the majority of cases
treated by either direct pulp capping or pulpotomy, then the
efficacy of these procedures would be in doubt.

The two direct inspection criteria used in the present
investigations do not directly assess the restoration of a
functional pulpo-dentinal organ.   Their limitations are acknow-
ledged.   They are considered as having a valid clinical use and to
be superior to vitality and radiographic tests which are open to a
greater latitude of interpretation.   It is hoped to assess the
effects of the procedures histologically and verify the restoration
of the pulpo-dentinal organ on morphological grounds.

(g) Assessment period.   In view of the anticipated difficulty in
obtaining patients for recall, a six month assessment period was
used initially.   Further investigations were undertaken when
assessment was carried out at two and five years.   Mitchell (1968)
reported a five year follow up in a clinical trial of a
corticosteroid/antibiotic mixture.   He found that at two years the
corticosteroid/antibiotic mixture appeared superior to the control
which consisted of the starch vehicle.   After five years there

appeared to be no difference between the groups. Shovelton (1970) reported a reduction in success rates after 12 and 24 months compared to results obtained at six months.

Materials such as calcium hydroxide may display excellent qualities when assessed histologically in man and animals over 30 - 60 days (Glass & Zander, 1949; Nyborg, 1955). Their efficacy in the long term may be less favourable (i.e. due to continuous irritation and pulp obliteration). Contrarywise, materials may show poor qualities in terms of stimulating cellular differentiation, fibrilogenesis and calcified matrix formation when assessed histologically over short periods. Only after long-term follow-up periods can their true efficacy be assessed. The need for long-term post-operative periods is stressed both for histologically assessed investigations and clinical trials.

General comments

The present investigation is unique in several respects. One operator/investigator conducted the trial in general practice over a ten year period. It was a comparative study in which the results of pulpotomy and several pulp dressing materials were compared in patients of different age groups. Only permanent molar teeth were used in the main trials. Two direct inspection methods were used to assess wound healing in the pulp. These two methods had not previously been used in combination. These criteria, in certain respects, are superior to criteria (e.g. subjective symptoms, vitality tests, radiographs) previously utilised in the assessment of clinical trials of this nature.

Such criteria allow the statistical analysis of sub-groups by the χ^2 test. They are not, however, beyond criticism. "Bridging" must be viewed as an "end-point" measurement which is the result of sequential and perhaps opposing mechanisms. Clinical trials offer no information concerning the involved mechanisms or variations in the mechanisms brought about by different conditions. The validity of the criteria is based on previously reported histological investigations. It is considered that the post-operative association of vitality of the pulp tissue and "bridge formation" at the exposure site is an expression of re-establishment of odontoblast function following cellular differentiation. It had been intended to examine the pulps of successfully treated teeth at six months and two years post-operatively by histological methods. A histological assessment would have established the extent of restoration of the pulpo-dentinal organ following pulpotomy.

In a trial of this nature, there is the inherent difficulty of making an accurate diagnosis of the pulp pathology pre-operatively. The inclusion of incorrectly diagnosed pulp conditions will lead to incorrect conclusions concerning the efficacy of the pulpotomy technique. Standardized diagnostic procedures were used and every attempt was made to eliminate pulps which could have been treated by more conservative techniques.

It is accepted that pulpal inflammations and, especially pulp infection, occurs as a very late consequence of a carious attack in most cases. This statement is frequently made in support of the use of a conservative approach in the treatment of teeth affected by a

deep carious lesion.   Careful cavity preparation and the avoidance of exposure followed by the placement of an appropriate lining and restoration are considered to reverse any early pulpal pathology. However, it must also be accepted that the deep carious lesion is a variable entity.   It cannot be defined in precise clinical terms. It is true that one of the most important criteria affecting the pathology of the pulp is the remaining dentine thickness (effective depth of Shroff).   The activity and rate of penetration of the lesion are also important as are the physico-chemical properties of the dentine.   The purpose of the investigation was to assess the efficacy of pulpotomy in the treatment of teeth affected by active rapidly penetrating caries and an associated chronic partial pulpitis. The present investigations have shown that pulpotomy is a procedure which can justifiably be used in certain clinical cases.

The question arising from the present trials is whether the dentinal bridge is the same in those teeth which remain vital compared to those teeth whose pulps become necrotic; the quality or properties of the formed dentine bridge is taken as a fine indicator of odontoblast function.   It is reasonable to conclude that odonto-blast function has been restored when tubular dentine is formed in association with cells morphologically similar to the original odontoblasts.   It is equally unreasonable to assume that cellular function will not be restored because a fibrous tissue is first formed at the exposure site and odontoblast cells are not present. A temporal factor is involved.   Full functional and morphological expression may vary depending on such factors as the applied stimulus and environmental conditions.   Non-clinical investigations into pulp

wound healing have, in the main, defined wound healing as a resolution of pathological conditions. They have dealt mainly with morphological changes while studies of functional disturbances are rare. It has been shown by several authors that there is little correlation between clinical symptoms and the histopathology of the pulp either pre-operatively or post-operatively. This indicates that morphological data provides little information about the functional state of the pulp. The light-microscopic observations relating to the sequential events of wound healing are well documented. A major difficulty remains that investigators tend to work with inadequate definitions. Massler (1972), in an article on the therapy conducive to healing of the human pulp, starts by saying, "Before attempting to answer the questions implied in the title, it is profitable to examine the parameters of the problem. What is meant by healing of the pulp?" It is essential to define in precise terms what is meant by wound healing. A wound is a disruption of cellular and anatomic continuity. Wound healing is a restoration of continuity. In the present author's opinion, pulp healing cannot be considered to have occurred until the exposure is covered by a dentine layer associated with an intact odonto-blast palisade, and until function has been restored for a period of time.

Kramer & McLean (1952) established criteria for assessing pulp responses to operative procedures. Based on these, Stanley (1968b) suggested a histopathological criterion for use in human pulp study. Cellular displacement, inflammatory cellular infiltrate and reparative dentine formation were noted. Estimates of the degrees of response

are purely subjective exercises.   Histological assessments of
exposed pulp wounds use a variation of the inflammatory index
together with a histomorphological classification of the formed
bridge (Sayegh, 1969).   It is normally accepted that the classifi-
cation of bridges on a histomorphological basis is arbitrary.
"Bridges" classified as fibrillar or globular may become tubular if
allowed more time.

In brief, these criteria measure end-points which are in them-
selves important.   They, however, tell little about the involved
mechanisms.   End-point measurements of wounds are the sum of
sequential and often opposing mechanisms.   In the assessment of
techniques and pulp capping medicaments, more precise information is
required concerning the effects of the materials on the involved
mechanisms.   In support of this appeal is the report by Spangberg
& Langeland (1973).   Twelve different root canal filling materials
in general use were tested for toxicity in vitro and in vivo using
quantitative methods.   All materials were highly toxic to Hela
cells.   A correlation of the in vivo and in vitro methods of evalua-
tion were made and it was concluded that the biologic properties of
these materials were not acceptable.   Healing obviously occurs in
some cases in spite of the treatment.

In the assessment of pulp wound healing, it is necessary to
distinguish between the properties of repair and the components of
the reparative process.

The formation of a dentine bridge is a property of repair
supposedly indicative of a healed pulp. The components or mechanisms
of pulp wound healing include: platelet aggregation, blood

clotting, formation of fibrin, an inflammatory response to injury, foreign bodies (including pulp dressing medicaments), bacteria and necrotic tissue, fibroblastic proliferation, cellular differentiation, ground substance synthesis, collagen synthesis, mineralization, the spatial organisation of various cell types, and capillary proliferation.

There is much to be learned about the process of dentinogenesis and reparative dentinogenesis following pulp exposure. The present clinical investigation indicates that the success of wound healing may vary in different age groups and with different medicaments. Studies should be designed to test the cytotoxicity of different materials and assess their effect on the various mechanisms. Information regarding pulp wound healing has been only casually reported. Methods are available for the measurement of blood flow (Edwall & Kindlova, 1971; Scott et al., 1972); vascular damage and permeability (Majno et al., 1961; Cotran, 1965); collagen synthesis (Ross & Benditt, 1962a); oxygen tensions (Brighton et al., 1969); cellular differentiation using labelling indices (Tonna & Cronkite, 1961). These are quantitative methods which measure not only end-points but mechanisms. In spite of the acknowledged difficulties, 'pulp healing' should be regarded as a set of sequences of definable components which should be isolated for study. It is important to know ultimately all the precise mechanisms of wound healing so as to be able to control the variables which retard or complicate healing.

It is of interest to discuss the pulpotomy procedure and the various medicaments used in the clinical trial. It is emphasised that present knowledge regarding their mode of action is mainly

speculative.

The following serves to introduce headings under which the subject can be conveniently discussed.

Wound healing has been defined as a restoration of anatomical and functional continuity of living tissue, brought about by a ' series of sequential mechanisms. Few wounded tissues reduplicate the precise structure of the pre-existing tissue. Bone is an exception. Its unique regenerative and plastic qualities result from the ability of unspecialised cells of mesenchymal origin to differentiate. Osteogenesis has three fundamental prerequisites: namely, the 'proper' or 'competent' cell, the proper nutrition and the proper stimulus. The latter two factors, in the main, consti-tute the cells' environment. Cell differentiation is a result of environmental interaction with gene potential. Fremont-Smith (1968) stated that, if enough were known about the environment, and if it were appropriately modified, the manifestations would also be modified. There is no genic determination which does not involve a crucial aspect of environment.

The injured pulp was once considered to have no ability to heal because of its peculiar environment and because it was thought that odontoblasts could not differentiate from wounded pulp tissue. A vast literature exists, based on well designed investigations, which clearly shows that the pulp can and does heal given the proper support and environment. Following trauma, there is cellular differentiation and a spatial reorganisation of pattern dependent on the existence of the three aforementioned prerequisites, i.e. the

proper cell, the proper stimulus and the proper nutrition.

(a) The proper cell. During tooth development, at the 'Bell' stage, the cells of the inner dental epithelium exert an organizing influence upon the underlying mesenchymal cells which differentiate into odontoblasts (Orban, 1962). This statement illustrates the basic concept of the cellular interaction. Recent investigations using labelled isotopes have confirmed the views of early light microscopists that odontoblasts are replaced by migration and differentiation of mesenchymal (perivascular) cells deep in the pulp. The formulation of clinical procedures on a non-empirical basis requires knowledge concerning the biological status of these cells and whether any regional variation occurs. It has been implied that the cells giving rise to regenerated odontoblasts vary numerically and qualitatively within different regions of the pulp. The evidence is circumstantial; the biological status and potential numbers of cells available remain unknown.

In the course of development or physiological aging, there is an observed decrease in the relative numbers of cellular elements of the pulp. Moreover, the coronal pulp tissue is much more cellular and less fibrous than the apical pulp tissue. Injury, pathological or iatrogenic, hastens physiological aging. Seltzer & Bender (1965) and Hesse (1967) are among those who state the view that pulpotomy operations, in the adult, are fraught with hazard because, in removing coronal pulp tissue, the greatest number of undifferentiated mesenchymal cells are extirpated. Their view is that there is a diminution in competent cell numbers along the corono-apical axis.

This is a physiological phenomenon. Aging and trauma further reduce the numbers of competent cells.

There is evidence that odontogenic pulp cells also vary qualitatively along a corono-apical axis. In the developed tooth, the form and arrangement of odontoblasts are not uniform throughout the pulp. They are more cylindrical and longer in the crown and become cuboid in the middle root and flat near the apex. In the area close to the apical foramen the dentine is irregular (Orban, 1962). Unknown factors exist which regulate the morphology and function of the odontoblasts in the intact tooth. These factors seem to persist and regulate the morphology and function of the regenerated cells following trauma. Nyborg (1955); Nyborg & Tullin (1965); Stromberg (1968) reported on and reviewed the histological studies relating to pulpectomy procedures. They all reported a non-canalized hard tissue barrier formation near the apex in association with flattish cells. Thus, both in the intact tooth and subsequent to trauma, there exists a variation in histo-differentiation of odontoblasts. It is not known to what extent this variation depends on the acquired developmental fate of the cells giving rise to odontoblasts or on purely environmental conditions. Glasstone (1936) was one of the first to observe that neither the odontoblast layer or ameloblast layer could complete differentiation alone in vitro. Briefly, these experiments showed that either layer, isolated in vitro, cannot undergo normal differentiation. These findings have since been confirmed by other investigators including Koch (1967) and Slavkin et al. (1969).

As a result of ectodermal-mesodermal interactions or inductions, a particular developmental fate is acquired by the cells. The cells are said to become 'determined'. It is further known that cells so induced may influence and determine by contact, uncommitted cells of their own type (Deuchar, 1971). Kollar & Baird (1969) gave examples of cells receiving some type of induction stimulus from their own cell type. The enamel organ of mouse incisors was readily induced to form non-dental structures when cultured with various types of foreign mesoderm, i.e. mesoderm determined the kind of structures formed. Reciprocal combinations were also tried. Foot-plate ectoderm was cultivated with dental papilla mesoderm producing normal teeth. However, snout ectoderm cultured with dental papilla mesoderm produced bristles. These results illustrate the phenomena of axial gradient and apical dominance and indicate that snout ectoderm was 'determined' to form hair, having already received some induction stimulus from its own mesoderm. Embryonic studies have confirmed that determination and differentiation proceed cranio-caudally in time sequence. This has been shown to occur within the developing tooth. Koch et al. (1970) isolated various parts of tooth rudiment and tested the ability of the various parts to reconstitute a whole tooth. It was found that the proximal third of incisor tooth rudiments differentiated more successfully than either the middle or distal thirds.

In the newly erupted tooth, root formation is continuing and involves the mechanisms of induction and differentiation of various cell types. The rapid carious process and the extirpation of the coronal pulp by pulpotomy may well, independently or in combination,

remove functional elements necessary to induce normal cell differen-
tiation and normal pattern formation. The reduced regenerative
potential of the very young tooth (indicated in the present study)
may therefore be due to the removal of functional elements and is
therefore of a qualitative nature as suggested by Wolpert (1969)
rather than of a quantitative nature as suggested by Seltzer &
Bender (1965).

(b) The stimulus. The wound healing process is a complex integrated
cellular response. Cell production must be balanced by cell death;
collagen production by hydrolysis, degradation and absorption.
Capillary formation must be balanced by capillary obliteration. In
the absence of this homeostasis, an uncontrolled overproduction of
cells and their products occurs. A brief review of these general
processes was given in the section on wound healing. It should be
made clear that the large volume of literature on this subject is
often speculative. The stimuli initiating and inhibiting these
cellular mechanisms are really unknown. This must constantly be
borne in mind when considering the effect of pulp wound dressings.

Since the initial work of Glasstone (1936), many investigators
have believed that reparative dentinogenesis following pulp exposure
is impossible. Their views are summed up in the statement of Fish
(1948): "In the absence of organising epithlium, pulp healing by
regenerated odontoblast-mediated dentine is impossible." Masterton
(1966b) published the results of his investigations showing that pulp
exposures in human and monkey teeth were repaired by tubular dentine
bridges and regenerated odontoblasts. His statement: "It would seem
logical to assume that the necrotic layer associated with a calcium

hydroxide dressing possesses the essential induction factor for reparative dentinogenesis", indicates that he considered the stimulus for pulp cell differentiation as occurring in terms of ectodermal-mesenchymal interactions. He further stated: "It would seem to be logical to assume that ameloblasts and the necrotic layer associated with a calcium hydroxide dressing possess the essential induction factor for dentinogenesis, a factor which is not possessed by gingival epithelium."

It is erroneous to think in terms of a single factor diffusing from the ameloblasts or necrotic layer and inducing cellular differentiation within the uncommitted mesenchymal cell pool. Slavkin & Bavetta (1968) have suggested that morphogenetic expression during odontogenesis occurs as a function of a series of reciprocal epithelio-mesenchymal interactions. Organogenesis proceeds as a function of the interactions between specific tissues, cells, structural proteins, informational macromolecules and is influenced by micro-environmental humoral factors.

The work of Kollar & Baird (1969) suggests the underlying mechanisms involved in the regeneration of odontoblasts from undifferentiated cells. They illustrated that cells, once "determined" by the original induction interactions, can pass on some type of induction stimulus to their own cell types or to less differentiated cell types.

There is growing evidence that the differentiation of stem cells in the healing pulp occurs due to a non-specific stimulation (Sveen & Hawes, 1968) and that wound infliction per se provokes a mesenchymal blastema available for renewed dentinogenesis (Waldhart & Linares, 1972).

Rowe (1967) debated whether the material applied to the exposed pulp actively stimulated healing or merely allowed the pulp to undergo repair.   Reparative dentinogenesis in the rat pulp was shown to occur beneath a variety of materials.   No therapeutic material is required in germ free animals for the stimulation of reparative dentinogenesis.   Current wound healing research suggests that materials such as calcium hydroxide are effective by predisposing to advantageous environmental conditions.   Calcium hydroxide plus corticosteroid/antibiotic medicaments (as used in the present clinical trial) have been shown to be superior to calcium hydroxide alone in maintaining pulp vitality beneath formed dentinal bridges.   This is probably due to the attainment of proper environmental conditions rather than any direct involvement of the constituents.

The presence of the proper stem cell without the proper environmental conditions would be ineffective in producing reparative dentinogenesis.   The addition of corticosteroid/antibiotic preparations to calcium hydroxide as a pulp capping medicament was reported by the present author (Quintessenz Essay Competition, 1970). Similar preparations were reported by Schroeder (1972).   The rationale behind the incorporation of corticosteroids to calcium hydroxide has already been discussed.   The results of the present investigation indicate that better tissue healing as measured by 'bridge' formation and 'vitality' is obtained with these preparations.   The results support the contentions of Bhaskar et al. (1969) that these materials in combination improve pulp tissue receptivity.

An optimal inflammatory stimulus is required for healing (Ehrlich & Hunt, 1968).   Cortisone is known to delay fibroblastic

activity during the first few days of wound healing. This is not its only effect. Capillary function is regulated by the corticosteroids. In view of the recent understanding of oxygen requirements in the healing wound, investigations of the type already suggested are required. A fall in pO_2 appears to be critical with respect to wound infection. A striking reduction in infection rate is observed following inspiration of increased oxygen levels (Hunt et al., 1972a,b). The deleterious effects of pulp infection on wound healing in this tissue are well documented. Tissue oxygen tension measurements within pulpotomy wounds by means of microelectrodes would indicate whether or not there were variations in capillary function following the use of calcium hydroxide and calcium hydroxide plus corticosteroid/antibiotic preparations.

(c) Proper nutrition. Nutritional factors include proteins, amino acids, ions and oxygen. The structure and physical characteristics of a cell's micro environment (essentially the ground substance), will determine whether nutrients reach the plasma membrane in adequate amounts, cross it and serve a useful function inside the cell. The general nutritional status of the organism is obviously important. A number of local conditions are important especially with respect to wound healing. These include (1) the distance of the cell from its source of supply in adjacent vessels; (2) diffusion rates in the extracellular space (a function of the state of aggregation and hydration of the ground substance components) and (3) the interposition of barriers to diffusion (i.e. other cells) between the source of nutrition and the cell.

The local circulation is disrupted when a tissue is wounded. Subsequent haemostasis devascularizes the wound edge. In the case of pulp wounds dressed with calcium hydroxide, this essential devascularization penetrates into the wound tissue. The wound's nutrition must come from the normal tissue on the uninjured side. The nutritional demands of the injured tissue are greatest at a time when local circulation is least competent to supply them. In the otherwise healthy organism, of all the nutritional requirements, it is becoming apparent that oxygen plays a critical role in normal healing (Forrester, 1976).

Oxygen is essential for healing and wound metabolism: (1) for energy production; (2) for collagen synthesis (hydroxylation of proline and lysine) and (3) cell proliferation. Hunt (1970) proposed the following hypothesis to explain the growth of granulation tissue and its dependence on oxygen tensions within the wound. The distance which a fibroblast can migrate from the nearest capillary is limited by the pO_2 in the extracellular fluid. When the pO_2 falls to less than 10mm Hg, the cell can probably no longer divide, synthesise collagen or migrate. The adjacent fibroblasts nearer to the capillary have an environment in which these processes can occur. Reproduction in cells near the capillaries is probably limited by contact inhibition. Capillary growth can take place in the previously formed ground substance. As each new capillary establishes a continuous circulation, the supply of oxygen to the most distant fibroblast is increased and thus the fibroblast can reproduce itself and migrate further into the tissue defect.

Mineralization may be related to local low tissue oxygen tensions. The uptake and retention of calcium by mitochondria is an active process requiring energy (Lehninger, 1970). In the growing epiphyseal plate, glycogen is absent from those areas of the hypertrophic cells adjacent to the calcification zone. Since there is no other source of energy available for the uptake and retention of calcium in that region of low pO_2, the mitochondria lose calcium to the external environment. A similar zone of low pO_2 and low glycogen content exists subjacent to the necro-biotic zone in wounded pulp tissue dressed with calcium hydroxide (Cabrini et al., 1960).

The foregoing discussion, based on the results of the present clinical trials and the literary review, is designed to increase the perspective of pulp wound healing. Despite the difficulties associated with pulp studies, opportunities exist for relevant fundamental research. A few of the avenues open to experimentation have been highlighted and experiments suggested which would increase understanding of pulp physiology.

SECTION 3

POTENTIOMETRIC INVESTIGATIONS

A.   INTRODUCTION

The permeability of dentine has been a subject of interest for some time.   Experiments with dyes and radio isotopes have shown that the penetration of dentine can occur from both the pulpal and oral aspects, in vivo and in vitro.   In the main these experiments have centred on proving that permeability exists and on demonstrating the paths along which penetration occurs by diffusion.

A second type of investigation has studied the hydrodynamics of dentine and bulk fluid movement through the dentinal tubules (Brannstrom, 1960a,b;   Merchant et al., 1977).   Invariably, unerupted molar teeth have been used in these experiments and interpretation of results must be made with this in mind.

It is important to investigate how changes in the structure of the tooth may oppose the passage of dyes, isotopes or solutions by the closure of physiological channels before the onset of caries, or by the active production of barriers to diffusion and fluid flow after caries has affected the tooth substance.

Klein & Amberson (1929) investigated the ionic-sieve behaviour of enamel.   In the course of their experiments, they briefly examined the physico-chemical properties of dentine.   In the light of the accumulating evidence that dentine is permeable to various ions and molecules, Klein & Amberson's observations on dentine require to be re-examined.   If the diffusion of ions and molecules through dentine is dependent on the physico-chemical properties of the structure, the factors controlling this diffusion must be investigated for a fuller understanding of dentine nutrition and the function of dentine as part

of the body's innate defence mechanisms.

The present preliminary investigations study the selectivity of dentine caps and discs to ionic transport and interpret the results in the light of ion-exchange theory.   A review of the pertinent literature and a resumé of ion-exchange theory is presented.

B.   OBJECTIVES

A series of potentiometric experiments was conducted to investigate the permselectivity of human dentine to ionic transport using concentration cells.

Experiment 1.   To measure the potential difference across dentine caps and discs prepared by various methods and from various types of teeth, when these caps or discs separated two unbuffered electrolyte solutions of different concentrations.

Experiment 2.   To measure the potential difference across dentine discs prepared from intact teeth separating two buffered solutions of different concentrations over a range of pH values and to estimate the iso-electric point of dentine.

Experiment 3.   To measure the membrane potentials across dentine discs prepared from carious teeth and to calculate the approximate fixed net charge within dentine.   To compare this experimentally obtained value of fixed charge density with values of fixed charge density derived from chemical analysis data.

A subsidiary experiment 4 was undertaken to ascertain the pH at the base of deep carious lesions after the excavation of all soft

carious dentine, in teeth diagnosed as suitable for pulpotomy and
to compare this with the iso-electric point of dentine obtained in
experiment 2.

C.   THEORY

Ion-exchange membranes

The term 'membrane' implies a material of whatever shape or
form which, when used to separate two solutions, restricts the
diffusion of both ions and solvent molecules.   Two main classes of
ion-exchange materials are recognised: (1) the zeolites are
naturally occurring alumino-silicates which possess lattice struc-
tures with inter-communicating pores large enough to allow the
diffusion of certain ions;   (2) the second class of ion-exchangers are
gel-like materials having a three-dimensional network of hydrocarbon
chains carrying fixed ionic groups.   Both types of ion-exchanger can
be described as having a framework carrying positive or negative
surplus charges which are compensated by mobile counter ions of opposite
sign.   Cation exchangers contain cations, and anion exchangers
contain anions, as counter ions.   These counter ions can be
exchanged for other ions of the same sign.   Ion exchange is
essentially a diffusion process and has little, if any relation to
chemical reaction kinetics in the usual sense (Helfferich, 1962).

The fixed charge theory

Teorell (1935) and Meyer & Sievers (1936) independently
postulated the "fixed charge theory" which accounts for the behaviour
of ion-exchange membranes in general.   The main features of the theory

are: (1) the membrane itself is regarded as having a charge due to either adsorption, dissociation or polar character but it is not necessary to make any further assumption as to the nature of the charge. The effect of the membrane can be regarded as that of an "added ion" of fixed homogeneous concentration. All ions in solution are regarded as permeable through the membrane with given mobilities. (The ionic mobilities may or may not differ from their mobilities in free water.) (2) there exists permanent Donnan equilibria between the external solutions and the membrane surfaces. These regulate the passage of ions into the membrane.

Fig. 5 is a schematic illustration of the structure of a typical ion exchange resin.

Fig. 6 is a schematic illustration of an ion exchanger in a solution.

Membrane potentials

Many phenomena can be qualitatively explained using this model. Membrane potentials are one of the most important membrane phenomena. It is important to realise that the membrane potentials are little affected by the geometrical configuration of the membrane. Within wide limits the membrane potentials are the same for discs, plugs, ribbons, etc. if they are prepared from identical ion-exchanger materials. If an ion-exchange material is used to separate the same salt solution at different concentrations, an electrical potential develops across the membrane. Using the above treatment, a description of this membrane potential can be given in terms of ionic concentrations, ionic mobilities and membrane charge. The potential which
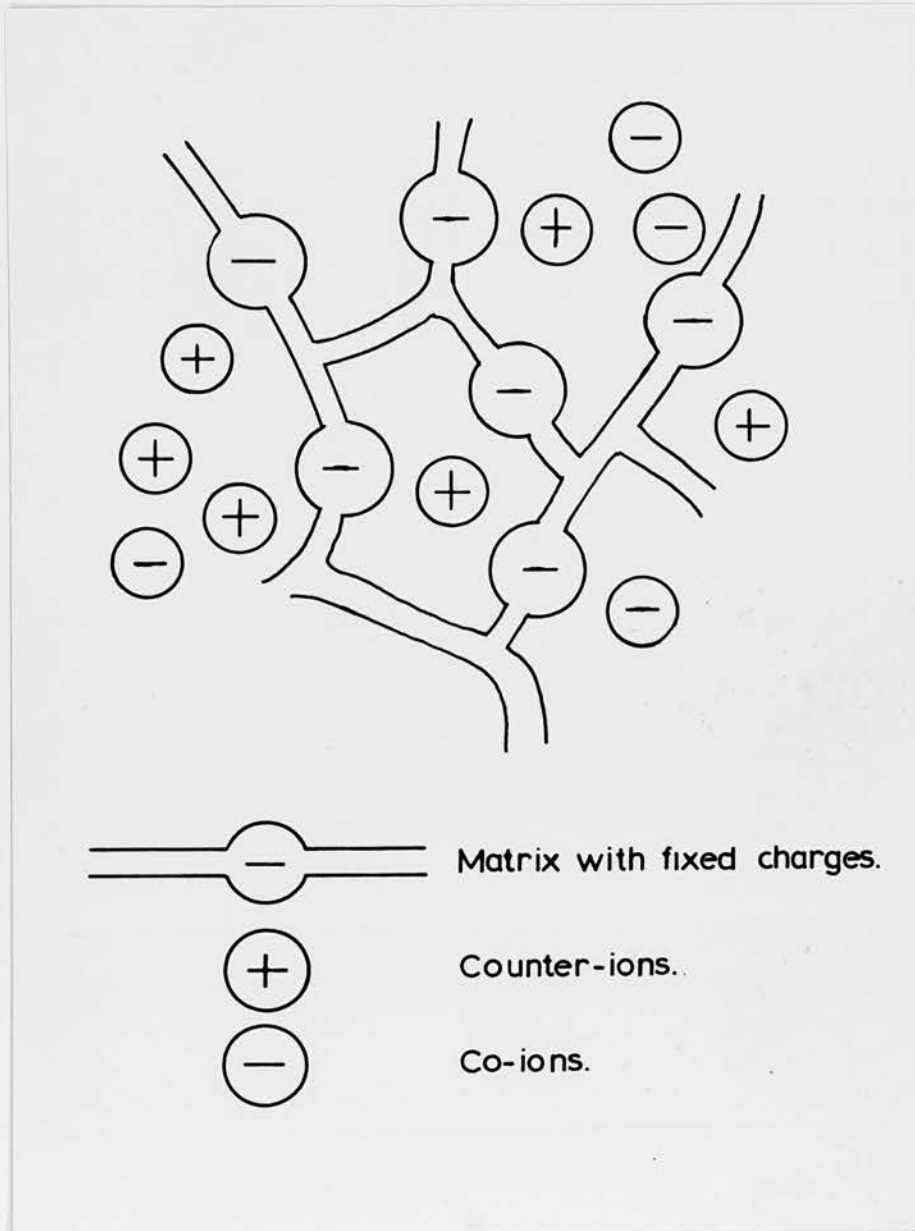
Fig. 5. Schematic illustration of the structure of a typical ion exchange resin.

188.
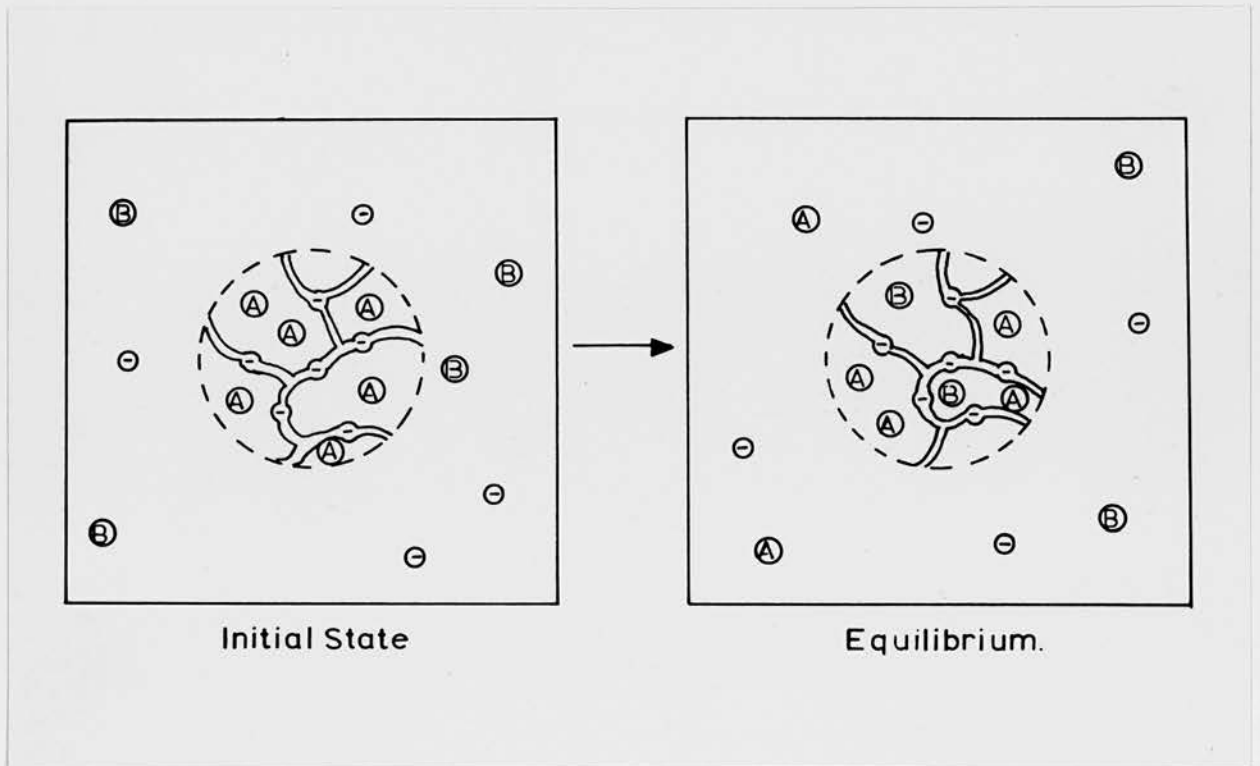
Fig. 6.   Schematic illustration of an ion exchanger in a solution.

The initial state represents a cation exchanger containing
counter ions (A) in a solution containing counter ions (B).
The counter ions are redistributed by diffusion until
equilibrium is attained.

arises across an ion-exchange membrane is essentially the same as the liquid junction potential between two monovalent solutions of different concentrations. The potentials originate because the more concentrated of the two solutions tends to diffuse into the more dilute and depends on the relative mobility of the cations and the anions. The potential is given by the ideal formula (Henderson, 1908)

$$E_j = \frac{RT}{nF} \cdot \frac{U_+ - U_-}{U_+ + U_-} \cdot \log_e \frac{a_1}{a_2} - - - - \text{(equation 1)}$$

where E_j is the junction potential; U_+ and U_- the mobilities of the cation and anion respectively; a_1 and a_2 are the activities of the two salt solutions; R is the gas constant and T the absolute temperature. This equation reduces to the so-called Nernst equation:

$$E_j = \frac{RT}{nF} \log_e \frac{a_1}{a_2} (2t_+ - 1) - - - - \text{(equation 2)}$$

where t_+ is the transport number of the cation.

The formula, with appropriate modifications, is applicable to junctions between any two salt solutions.

When one solution is a 1/10 dilution of the other, the activities can be taken as being equivalent to the concentrations and $a_1/a_2 = 10$. From standard conductance data, the ratio of the ionic conductance of chloride to that of sodium is taken as 1.5 at infinite dilution. Substituting in equation 1

$$E_j = -(0.60 - 0.40) \frac{RT}{F} \log_n 10$$

at 25^0C $E_j = -11.8$ mV

When an ideal cation exchange membrane is interposed between the two salt solutions, cations are allowed to freely diffuse through the

membrane but anions are excluded.   In this case all the current is
transported by the cation and equation 2 reduces to

$$E_j = \frac{RT}{nF} \log_e \frac{a_1}{a_2} \; - \; - \; - \; - \; (\text{equation } 3)$$

Again considering a 10:1 univalent electrolyte activity gradient,
the maximum potential which can arise at 25^0C is +59 mV for an ideal
cation exchange membrane.   Non-ideal membranes exhibit lower
potentials than predicted by the above equation.

   Donnan equilibrium.   Equations 1 and 2 express the potential in
terms of ionic concentration and ionic mobilities.   When two salt
solutions are separated by a membrane containing a charged immobile
colloid containing a net charge of x equivalents/litre, the concentra-
tions of cations and anions at equilibrium are predicted by the Donnan
theory of membrane equilibrium.   Such equilibria can be very
complicated.   The following simple example illustrates the theory.

   Consider a membrane separating a solution of NaCl and a solution
of Na^+R^-, in which R^- is an ion that cannot diffuse through the
membrane, initially in solution 1, the concentration of sodium ions
equals the concentration of chloride ions.   In solution 2, the
concentration of sodium ions equals the sum of the concentration of
the anions.   Both the Na^+Cl^- and the Na^+R^- are considered to be
completely dissociated.   Both the Na^+ and Cl^- can diffuse through
the membrane and tend to do so.   Their rate of diffusion is propor-
tional to the activities of the ions.   The rate of diffusion from
solution 1 to solution 2 is given by the expression

$$\text{Rate } 1 \; \alpha \; \{Na^+\}_1 \{Cl^-\}_1$$
$$\text{or Rate } 1 = k_1 \{Na^+\}_1 \{Cl^-\}_1$$

191.

Similarly, the rate of diffusion from solution 2 to solution 1 is given by the expression

$$\text{Rate 2} \; \alpha \; \{Na^+\}_2 \{Cl^-\}_1$$

$$\text{or Rate 2} = k_2 \{Na^+\}_2 \{Cl^-\}_2$$

At equilibrium the rates of diffusion are equal, i.e.

$$\{Na^+\}_1 \{Cl^-\}_1 = \{Na^+\}_2 \{Cl\}_2$$

$$\text{or} \quad \frac{\{Na^+\}_1}{\{Na^+\}_2} = \frac{\{Cl^-\}_2}{\{Cl^-\}_1} = r \; \text{(the Donnan ratio)}$$

At equilibrium the two solutions must be electrically neutral. It follows from the above Donnan ratio that the activity of sodium in solution 1 must be greater than the activity in solution 2. Also, the greater the concentration of the non-diffusible ion, the greater the difference between the activities of the diffusible ions on the two sides of the membrane, and hence the greater the potential difference between the two solutions.

Based on Henderson's theory of liquid junction potentials and the Donnan theory of membrane equilibrium, Frieden & Hisaw (1953) derived an equation from the ideal equation 1 which related the membrane potential to the immobile charge within the membrane. They gave this as

$$E = - 11.8 + 215x \; - \; - \; - \; - \; \text{(equation 4)}$$

where x is the concentration of net charge in equivalents/litre; and -11.8 represents the liquid junction potential at 25^0C; E is the observed potential in mV, between the two liquids separated by the membrane.

Measurement of membrane potentials

The membrane potential cannot be measured directly. The system existing when two solutions are separated by a membrane can be designated thus:

solution 1 | membrane | solution 2
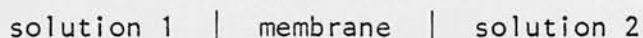
The membrane potential is usually measured by incorporating two electrodes into the solution

electrode | solution 1 | membrane | solution 2 | electrode
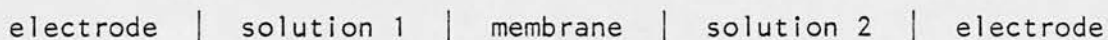
The emf of such a cell consists of the membrane potential and the electrode potentials. The standard procedure is to use calomel electrodes and assume that the electrode potentials balance one another exactly. Thus the emf of the complete cell equals the membrane potential.

Factors affecting membrane potentials

The ion-exchange behaviour of membranes is chiefly determined by the fixed ionic groups. The chemical nature of the groups affects the ion-exchange equilibria. An important factor is the acid or base strength of these groups. Weak acid groups are ionized at only high pH. At low pH they form undissociated groups and no longer act as fixed charges. Strong acid groups remain ionized even at low pH.

The ionized groups are neutralized by mobile cations or counter ions. Usually the membrane shows selective permeability, i.e. takes up certain counter ions in preference to others. A membrane with fixed ionic basic groups, neutralized by mobile anionic counter ions, is permeable for anions. A membrane consisting of amphoteric

molecules is not selective at the iso-electric point; it is permeable for anions on the acid side and permeable for cations on the alkaline side of the iso-electric point.

The concentration of fixed ions can be expressed in milli-equivalents per litre of tissue water. This is an excellent way of characterizing an ion-exchanger. The concentration of fixed ionic groups refers to a particular state under given experimental conditions. It is a variable rather than a characteristic constant. Among the factors that determine the selectivity constant for a membrane, and therefore the potential difference across it, are:

(1)   the pH of the solutions and the pK of the ionogenic groups

(2)   the concentrations or more precisely the activities of the solutions

(3)   the physical arrangement of the molecular network

D.   MATERIALS AND METHODS

Apparatus

The membrane potential of cells of the type

| reference electrode | solution 1 (molar concentration) m_1 | membrane | solution 2 (molar concentration) m_2 | reference electrode |
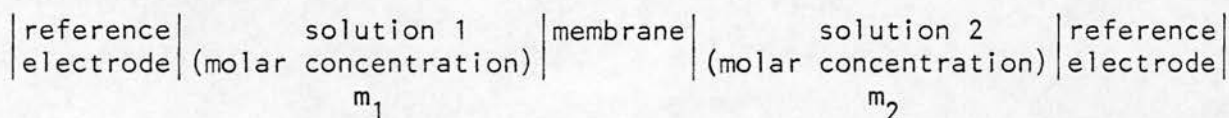
were measured using a Pye junior potentiometer with a range of 250 mV. A Pye galvonometer (nominal resistance 22 ohms) was used as a null instrument. The reference electrodes were of the mercury-calomel type. They were designed, constructed and supplied by Probion Ltd. (Leslie, Scotland). Connections to the cell solutions were made through KCl

salt bridges.   The electrodes were of the low-leak type and dilute KCl solutions were used in the salt bridges to minimize contamination of the cell solutions from leakage.   Low impedence screened leads were used for all connections.   The cells were contained in a thermostatically controlled water bath.   All readings were taken at a temperature of $25^0C \pm 1^0C$.   To minimize 'film' control, agitation of the layers of solution adjacent to the membrane was undertaken using a mechanical agitator.

Fig. 7 shows the electric circuit for potential measurements and Fig. 8 shows the apparatus set up.

The pH at the base of carious cavities was measured using a micro-antimony electrode and reference electrode designed, constructed and supplied by Probion Ltd. (Leslie, Scotland) in conjunction with an E.I.L. Ltd. (England) pH meter, model 7020 with temperature adjustment.

Solutions

Analytical reagent grade chemicals (Analar, B.D.H. Chemicals Ltd., Poole, England) and de-ionized distilled water (Analar, B.D.H. Chemicals Ltd., Poole, England) were used to prepare all solutions. Buffered NaCl solutions (0.10M and 0.010M) were prepared from mixtures containing a calculated ratio of sodium acetate to acetic acid.

The pH values of the two series of NaCl solutions were 4.0, 4.6, 5.0, 5.3 and 5.7.   The pH of each buffered solution was measured using a pH meter and glass electrode (E.I.L. 7020).   The solutions were kept in stoppered bottles and the pH re-checked prior to use.
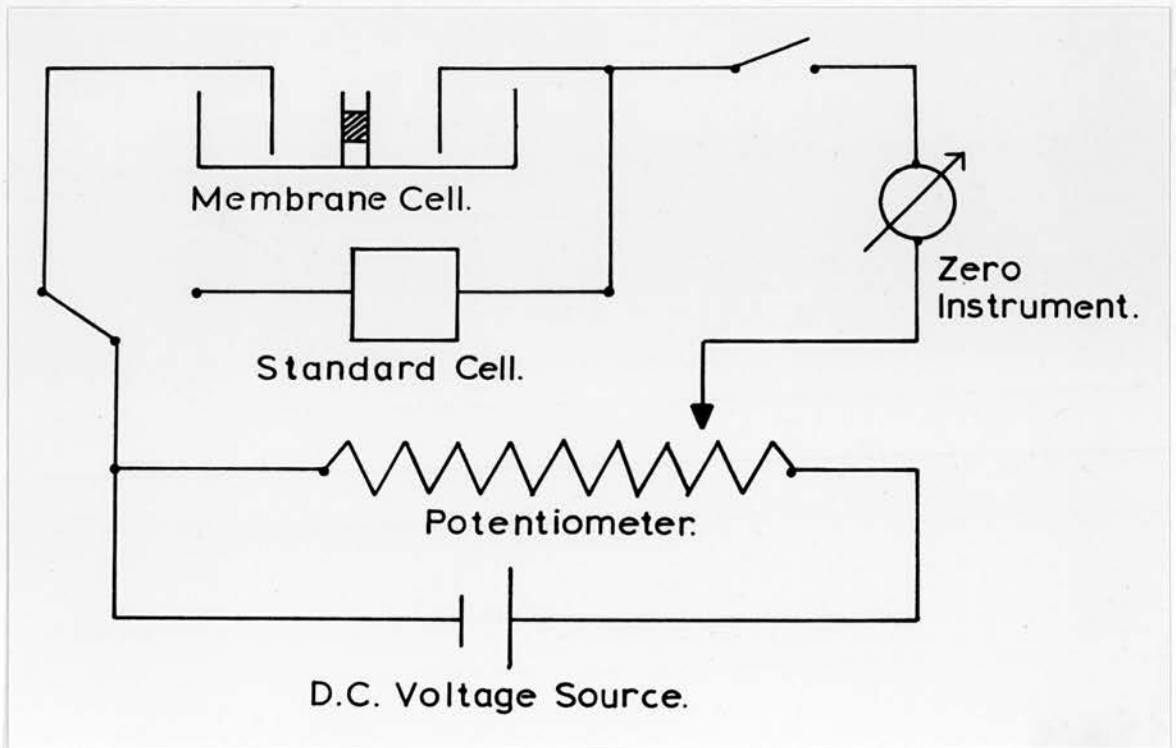
Fig. 7.   Electric circuit for potential measurements.

Fig. 8. Apparatus set up showing the electrodes on the extreme
         left, the potentiometer in the middle foreground and the
         galvanometer above and to the left.   To the right of
         the galvanometer is a DC voltage source.

The mobility of the acetate ions is similar to that of the chloride ion. Longworth (1935) stated that the transport number of sodium in a pure NaCl solution is 0.40. In a sodium acetate solution this is said to approach a value of 0.55 and, in a mixture of 95 parts of NaCl to 5 parts sodium acetate, the transport number is said to be 0.408. According to Joseph et al. (1957) this will result in an error or approximately 1 mV in the estimation of dilution potentials. The calculation is applicable to the acetate buffer at pH 4.6.

Source of dentine

Dentine was obtained from human teeth extracted in the course of routine treatment of patients in general practice. Details of teeth used are given in Table 11.

Preparation of cells

Two types of cell were used: (a) cell type 1 (dentine caps) and (b) cell type 2 (dentine discs).

Cell type 1. Intact canine teeth were obtained from four patients aged 16 to 31 years. All teeth were in occlusion. Immediately after extraction under local anaesthetic (Citanest), all the enamel was removed from the tooth using a high speed handpiece and a 557 carbide fissure bur with copious water spray. The denuded dentine cap was examined under a hand magnifying glass (x10 magnification) to verify the removal of all enamel. The root was then sectioned at the amelo-cemental junction using a diamond disc. All traces of pulp tissue were removed using a number 6 round bur in a conventional speed handpiece.

<u>Table 11.</u>   Details of teeth used in potentiometric investigations.

Experiment 1:

Cell number	Tooth type	Status	Patient's age (yrs) ($\overline{X} \pm SD$)	Preparation method
1 - 4	Canine	Non-carious functional	22.5 ± 6.65	Dentine cap (cell type 1)
5 - 8	Canine	Non-carious functional	26.25± 7.5	Standard disc (cell type 2)
9 -12	Canine	Carious functional	29.5 ± 6.1	Standard disc (cell type 2)
13,14 17	Canine	Unerupted	12.6 ± 6.4	Standard disc (cell type 2)
15,16	Premolar	Unerupted	14.0	Standard disc (cell type 2)
18-21	Canine	Non-carious functional	14.5 ± 3.0	Modified disc (cell type 2)
22-25	Canine	Carious functional	28.5 ±10.47	Standard disc boiled in ethelene glycol (cell type 2)

Experiment 2:   Cell Nos. 5 - 8 were used.

Experiment 3:   Cell Nos. 9 -12 were used.

The prepared cap was connected to a glass tube using 'Araldite'. A concentration cell was then constructed by filling the glass tube with 0.100M KCl or NaCl and bringing the tip of the dentine into a container of more dilute electrolyte solution. Details of the mounting of this type of cell are given in Figs. 9 and 10.

Cell type 2. A second type of cell was prepared using dentine discs. These discs were prepared from three types of teeth: (1) unerupted canines and premolars; (2) erupted, functional canines having no detectable caries or restorations and (3) erupted canines with large arrested carious cavities.

Two methods of preparation were employed in cutting the discs, a standard method and a modified method (see Fig. 11).

(a) Standard disc preparation. Immediately on extraction, using a high speed handpiece and a 557 carbide fissure bur, all enamel was removed from the tooth. This was done under a water spray. A disc was then prepared by creating a flat plane parallel to the amelo-cemental junction on both the enamel and pulpal aspects. All pulp remnants were removed but only minimal grinding was undertaken on the pulpal side. Initially the prepared disc was about 1.50mm thick. Using a silicone-oxide sandpaper, the disc was reduced from the enamel side only until a uniform disc approximately 1mm thick was obtained.

The prepared disc was then sandwiched between two plastic 'O' rings to ensure a uniform exposed surface area. The outer

Fig. 9.   Schematic illustration of the mounting of cell type 1

(dentine cap).

Solution a_1 was always 0.100M KCl or NaCl.   The test

solution a_2 was progressively diluted.

Fig. 10. Photograph of cell type 1 (dentine cap).

The dentine cap is attached to the glass tube

filled with solution a_1 (0.100M KCl or NaCl).

The plastic container holds the test solution a_2.
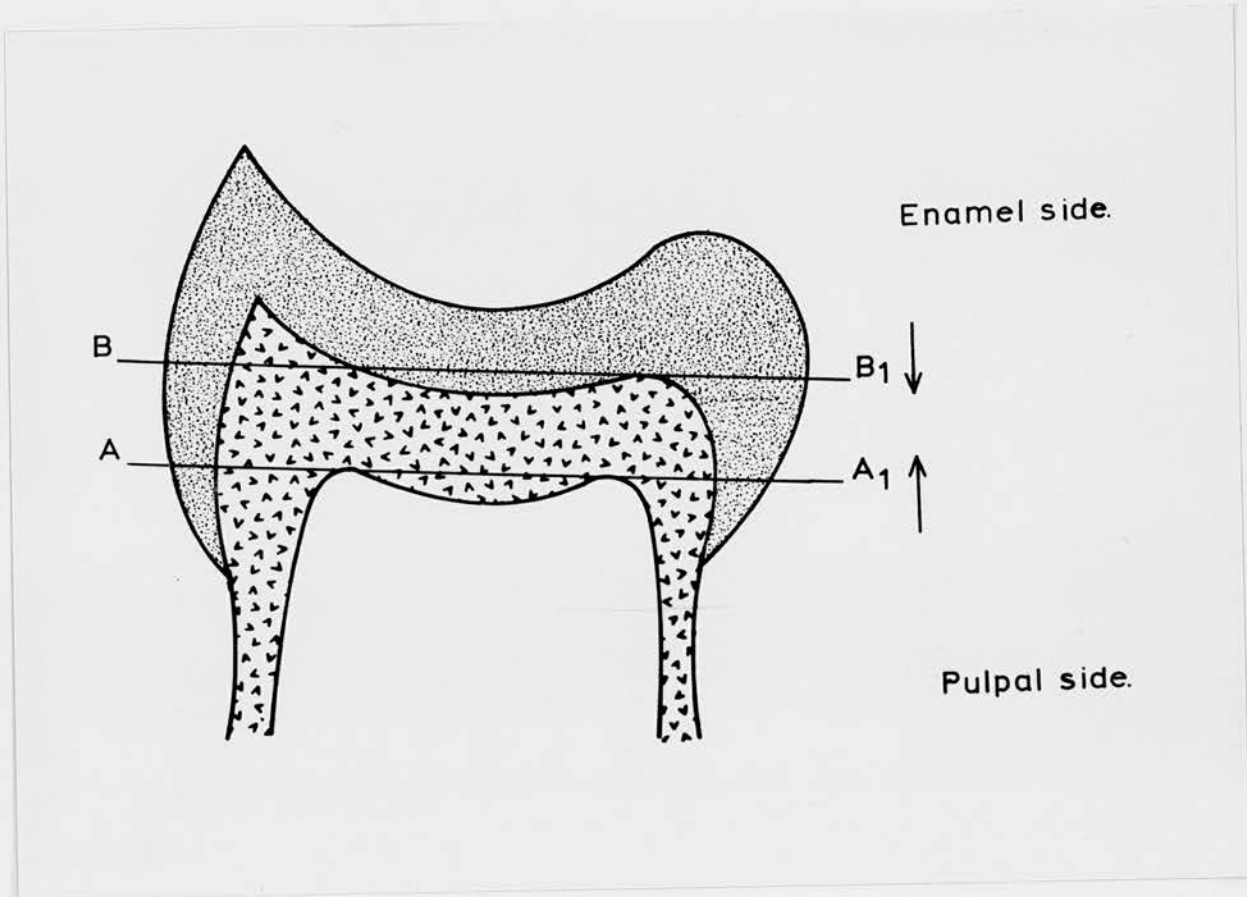
Fig. 11.   Diagram indicating location of dentine discs relative

to the pulp and enamel.

Standard discs were prepared by preparing a flat plane $A-A_1$
parallel to the amelo-cemental junction.   Only minimal grinding
was undertaken on the pulpal side commensurate with the removal
of all pulp remnants.   Further reduction was undertaken from
the enamel side along the plane $B-B_1$.

Modified disc preparations were prepared in a similar manner but
a reduction of at least 1mm was undertaken from the pulpal side
along the plane $A-A_1$.   The arrows indicate the direction of
reduction from both the enamel and pulpal aspects.

surface of these rings was then covered by coloured adhesive tape. The ring/dentine/ring sandwich was then embedded in Araldite. When set, the outer surfaces were ground clear of Araldite until the colour tape was just removed. This left the exposed dentine surfaces.

(b) Modified disc preparation. Dentine discs were prepared as in the standard disc preparation until a disc 1.5mm thick was obtained free of pulp tissue. Using a silicone-oxide sandpaper, the disc was then reduced from the pulpal side by at least 1mm. Further grinding was done on the enamel side until a disc approximately 1mm thick was obtained.

In addition, four dentine discs prepared by the standard method were boiled in 3% KOH to remove organic constituents.

Each disc was then incorporated into a cell by sealing it between two plastic containers using Araldite (see Figs. 12 and 13).

Procedure for measuring membrane potentials

The cells were allowed to reach equilibrium with either a KCl or NaCl concentration of 0.100/0.010M. The pH of these solutions varied depending on the experiment. Equilibrium was considered to have been reached when the emf was constant to within 0.5 mV on three consecutive days. The solution on the pulpal side of the dentine cap or disc was always 0.100M KCl or 0.100M NaCl. This is referred to as solution a_1. The test solution varied with the experimental conditions and was brought into contact with the 'enamel' aspect of the dentine cap or disc. This is referred to as solution a_2. The solutions a_1 and a_2
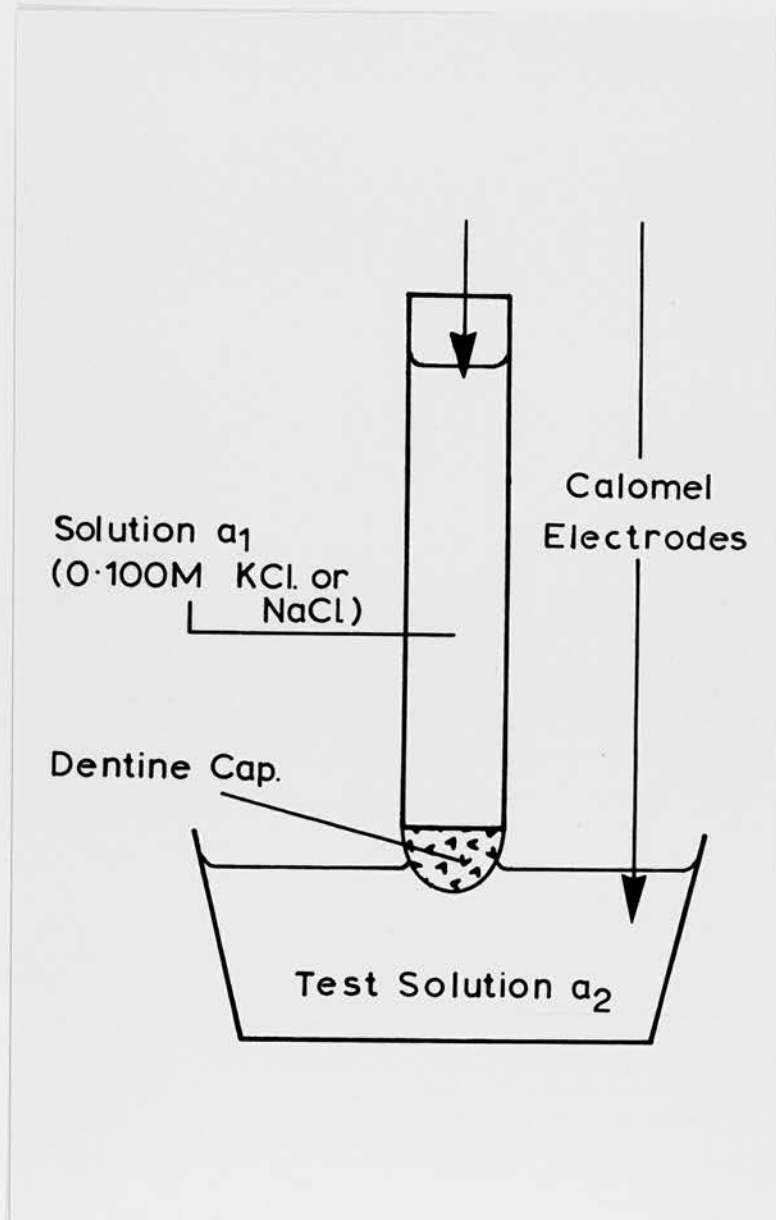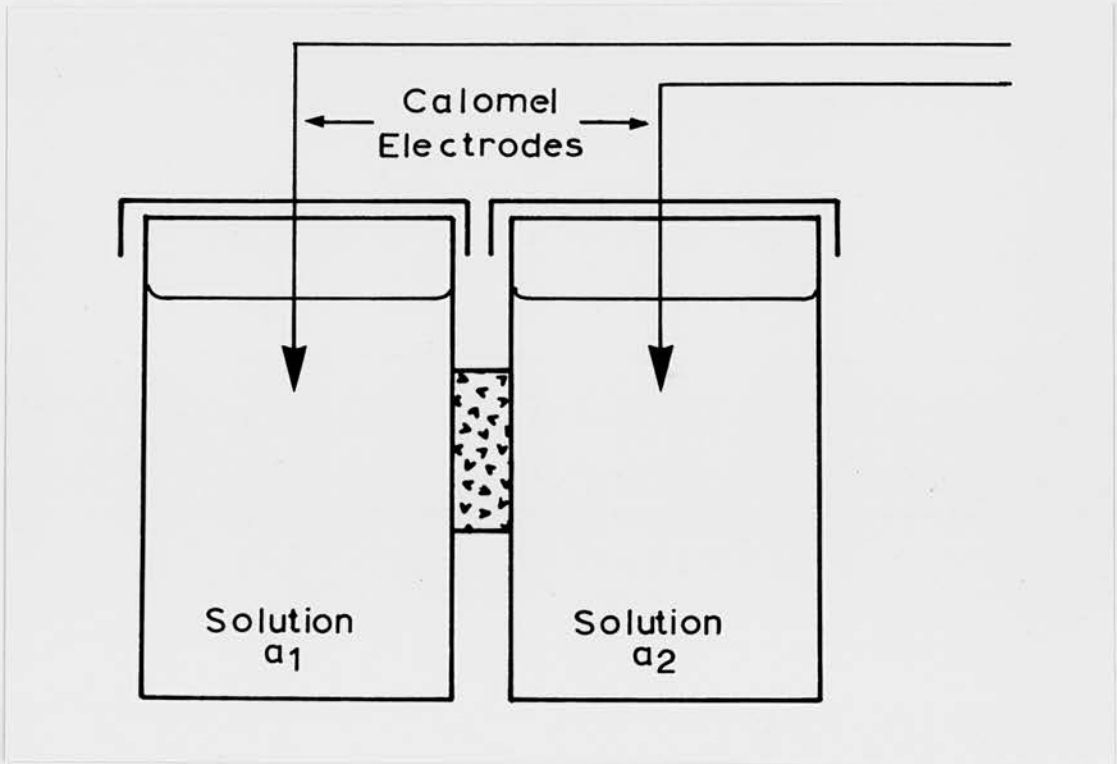
Fig. 12. Schematic illustration of mounting of cell type 2

(dentine disc).

Solution a_1 was always 0.100M KCl or NaCl. The test

solution a_2 was progressively diluted.

Fig. 13. Photograph of cell type 2 (dentine disc).
The dentine disc is incorporated into a cell by sealing
between two plastic containers.   The container on the
left held solution a_1 (0.100M KCl or NaCl), and the
container on the right held the test solution a_2.
Both containers are shown capped, to minimize evaporation.

were changed daily and changed prior to measuring the membrane potential. Both containers were provided with caps to minimize evaporation.

The electrodes were placed in solutions a_1 and a_2 only long enough for the emf of the cell to be measured. This was done to reduce contamination of the solutions by leakage from the electrodes. To minimize the absorption of CO_2 from the atmosphere, a small quantity of soda lime, held in a piece of dental gauze, was attached to the lid of each container.

Experiment 1: all cells were allowed to attain equilibrium with a KCl concentration gradient of 0.100/0.010M. The solutions were unbuffered. Equilibrium was considered to have been attained if the measured emf's were consistent within 0.5 mV on three consecutive days.

The electrolyte concentration (solution a_1) on the pulpal side was maintained at 0.100M KCl. On the enamel aspect (solution a_2), the concentration was varied to 0.02M, 0.004M and 0.0008M. With each change in concentration gradient, equilibrium was allowed to be reached. The solutions on both sides of the membrane were changed daily and changed before and after the measurement of potentials.

On completion of measuring the series of potentials with KCl solutions, the experiment was repeated using NaCl solutions.

In effect, each dental cap or disc was used to construct four different concentration cells with KCl solutions and four different concentration cells with NaCl solutions. All measurements were taken at $25^0 C \pm 1^0 C$.

Experiment 2: Four concentration cells were prepared using dentine discs from intact canines (standard disc preparation procedure). The salt solutions used to construct the concentration cells were buffered sodium chloride solutions. These solutions were kept in stoppered bottles. The concentration-cell electrolytes were changed three times daily. The pH of the solutions was checked prior to each change using a glass electrode and the E.I.L. pH meter.

The concentration gradient across each membrane was always 10:1; the NaCl was maintained at 0.100M on the pulpal side and 0.010M on the enamel side. Equilibrium was reached and the stabilized membrane potential measured for each cell at each pH. When changing from one buffered solution to another, the cells were washed out and allowed to stabilize with unbuffered NaCl solution for at least seven days before being reconstituted with a buffered solution at a new pH. The complete sequence of events for any one cell was:-

(1) Equilibrate with buffered NaCl (pH 5.7): solutions changed three times daily and before and after each potential reading.

(2) Wash out with unbuffered NaCl solution: solutions changed three times daily for seven days.

(3) Re-equilibrate with buffered NaCl (pH 5.4): as in (1).

(4) Repeat (2) and continue the cycle for each buffered solution.

The measurements were taken at $25^0C \pm 1^0C$, temperature control being maintained using a thermostatically controlled water bath.

Experiment 3: Four cells (Nos. 9 - 12) were used. These had previously been used in experiment 1. A concentration gradient of 10:1 was established across the cell membrane using unbuffered NaCl.

The NaCl was maintained at 0.100M on the pulpal side and 0.010M on the enamel side. The solutions were changed frequently and equilibrium was allowed to take place. The steady state membrane potentials were observed at $25^{\circ}C \pm 1^{\circ}C$ as in previous experiments. The approximate net charge within dentine was calculated using the formula $E = -11.8 + 215X$.

Experiment 4: Prior to the experiment proper, the pH of three standard buffer solutions was measured using the antimony micro-electrode and also with the glass electrode supplied with the E.I.L. pH meter (model 7020).

The pH at the base of active carious lesions was determined in the following manner using the antimony micro-electrode. Teeth from young patients aged from 6 to 12 years were extracted in the course of general dental services. All teeth were grossly carious molars categorised as having an active type of carious lesion (Miller & Massler, 1962). The teeth were extracted under general anaesthetic and all patients had fasted from food and liquids for more than four hours prior to the extractions. Immediately prior to extracting the teeth, the cavities were opened out using a high speed instrument with water spray. This was to allow access when measuring the pH. No attempt was made to remove caries at this stage. The teeth were then extracted and washed in de-ionized water to remove saliva and any salivary buffering effect. The extracted teeth were mounted on a bench in some soft dental wax. Using sharp spoon excavators, all soft caries was removed until hard dentine remained. The hardness was judged by using a sharp probe and the hardness of a human finger

nail as the standard.   Care was taken not to expose the pulp.
Three teeth in which pulp exposure occurred, were excluded from the
experiment.   When the cavity was prepared to hard dentine, it was
carefully washed again with de-ionized water and dried with cotton
wool.   A small drop of de-ionized water was then placed on the
cavity floor and allowed to equilibrate with the dentine.   The
cavity pH was measured using the E.I.L. pH meter (model 7020 with
temperature adjustment), the antimony micro-electrode (Probion Ltd.,
Leslie, Scotland) with a calomel reference electrode.   The pH of the
cavity was taken as the mean of three consecutive readings which
were within ± 20 mV of each other.   The time taken to obtain a set
of pH readings for any one tooth never exceeded ten minutes from the
time of extraction.

E.   RESULTS

Experiment 1

(a) Dentine caps (cell type 1).   The results of concentration cell
measurements obtained with dentine caps are given in Table 12.   Figs.
14 and 15 show the variation in membrane potentials as a function of
the activity ratio (a_1/a_2) of the salt solutions on either side of the
dentine caps when the univalent electrolytes KC1 and NaCl were used
to construct concentration cells of varying gradients.   In each graph,
the theoretical prediction for an ideal cation-exchange membrane is
shown

The recorded membrane potentials for both the univalent electro-
lyte solutions were positive and lower than the calculated values for

Solution a_1	Solution a_2	a_1/a_2	$\log_e(a_1/a_2)$	Ideal ϕ (mV) (calculated)	Mean ϕ (mV) observed							
					Cell 1		Cell 2		Cell 3		Cell 4	
					KCl	NaCl	KCl	NaCl	KCl	NaCl	KCl	NaCl
0.1M	0.1M	1	0	0	0	0	0	0	0	0	1	0
0.1M	0.02M	5	1.609	41.33	12	10	12	9	10	8	11	9
0.1M	0.004M	25	3.218	83.41	22	21	21	18	19	17	22	17
0.1M	0.0008M	125	4.828	124.04	27	25	28	24	27	24	28	23

Table 12. Results obtained from dentine caps (cell type 1) and unbuffered KCl and NaCl solutions at 25^0C $\pm$ 1^0C.

Fig.14.

Fig. 15.

Cell 3.

Cell 4.
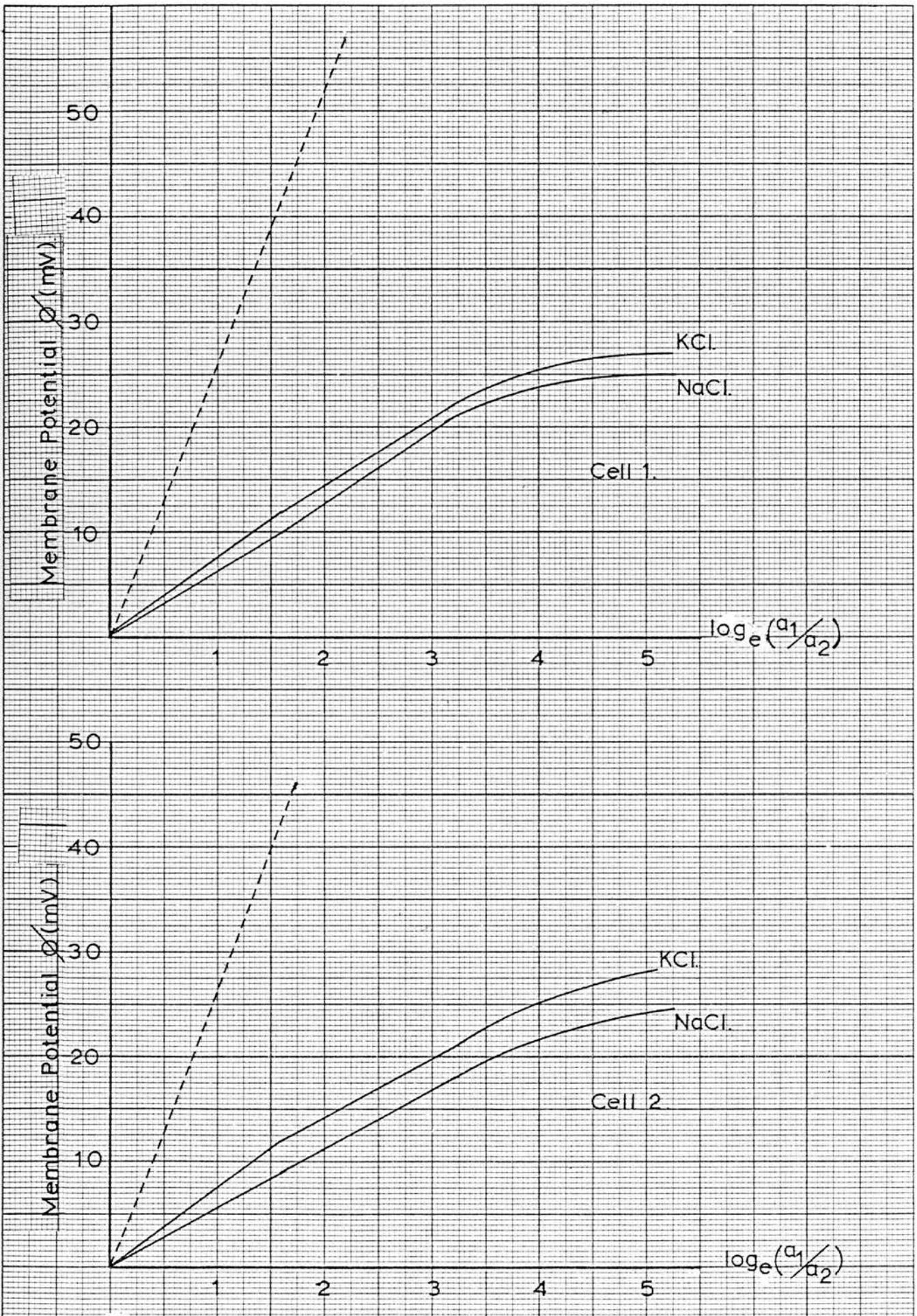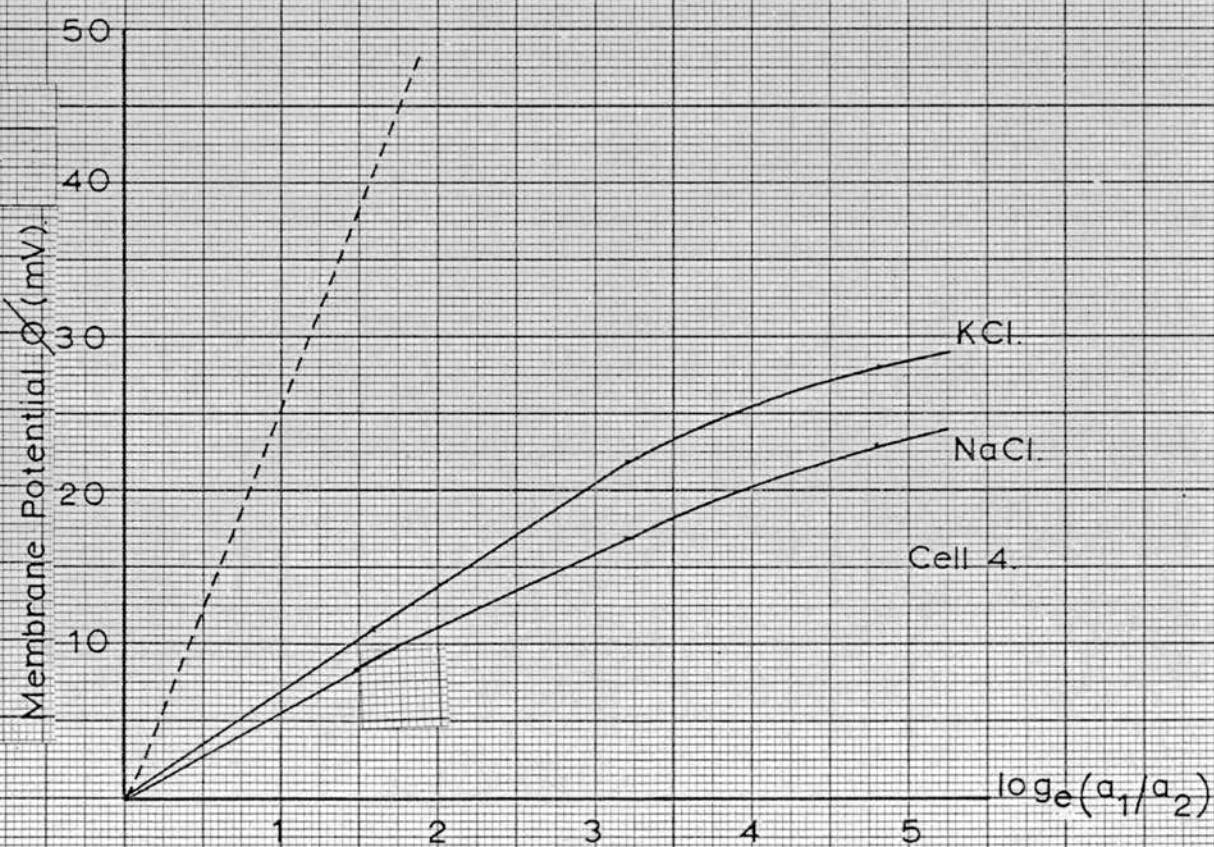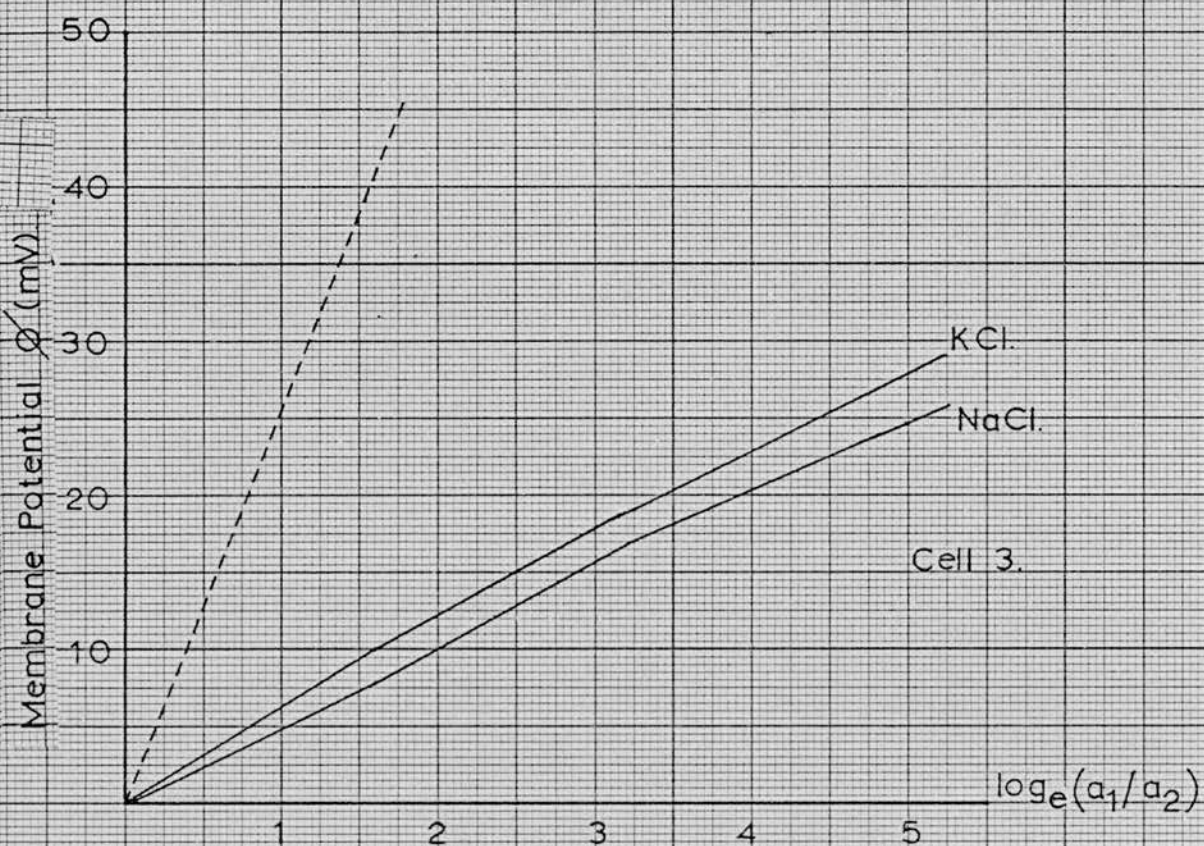
Figs. 14 and 15.

Results obtained from dentine caps and unbuffered KCl and NaCl solutions indicating the variation in membrane potential across the dentine caps as a function of the activity ratio (a_1/a_2) of the salt solutions. Also shown (dotted line) is the maximum potential gradient for an ideal cation exchange membrane according to the Nernst equation.

a perfect cation-exchange membrane. The membrane potential recorded with KCl was always larger than the potential recorded with NaCl at any given concentration gradient.

(b) Dentine discs (cell type 2). The results of concentration cell measurements obtained with dentine discs prepared by the standard and modified methods and after boiling standard discs in ethelene glycol are given in Tables 13, 14, 15, 16 and 17.

Figs. 16, 17, 18 and 19 show the variation in membrane potentials as a function of the activity ratio (a_1/a_2) of the salt solutions on either side of the dentine discs when the univalent electrolytes KCl and NaCl were used to construct concentration cells of varying gradients. In each case, the theoretical prediction for an ideal cation-exchange membrane is shown. In Figs. 20, 21 and 22, in addition to the theoretical slope for an ideal cation-exchange membrane, the slope for ideal liquid junction potentials is given.

For cell numbers 5 - 12 inclusive, the recorded membrane potentials for both the univalent electrolyte solutions were positive and lower than the calculated values for a perfect cation-exchange membrane. The membrane potential recorded with KCl was always larger than the potential recorded with NaCl at any given concentration gradient.

For cell numbers 13 - 25 inclusive, no measurable equilibrium potentials were obtained at any concentration gradient using KCl. When NaCl solutions were used, membrane potentials developed within 12 - 24 hours. These were unstable and fell off rapidly.

Solution a_1	Solution a_2	a_1/a_2	$\log_e (a_1/a_2)$	Ideal ϕ (mV) (calculated)	Mean ϕ (mV) observed							
					Cell 5		Cell 6		Cell 7		Cell 8	
					KCl	NaCl	KCl	NaCl	KCl	NaCl	KCl	NaCl
0.1M	0.1M	1	0	0	0	0	0	0	0	0	0	0
0.1M	0.02M	5	1.609	41.33	11	9	11	10	10	9	12	10
0.1M	0.004M	25	3.218	83.41	21	19	20	19	20	18	23	20
0.1M	0.0008M	125	4.828	124.04	26	23	27	25	25	22	27	23

Table 13. Results obtained from dentine discs (cell type 2; standard preparation procedure, non-carious functional canines) and unbuffered KCl and NaCl solutions at $25^{\circ}C \pm 1^{\circ}C$.

Solution a_1	Solution a_2	a_1/a_2	$\log_e (a_1/a_2)$	Ideal ϕ (mV) (calculated)	Mean ϕ (mV) observed							
					Cell 9		Cell 10		Cell 11		Cell 12	
					KCl	NaCl	KCl	NaCl	KCl	NaCl	KCl	NaCl
0.1M	0.1M	1	0	0	0	0	0	0	0	0	0	0
0.1M	0.02M	5	1.609	41.33	12	11	13	11	12	11	12	11
0.1M	0.004M	25	3.218	83.41	24	23	25	24	25	23	24	22
0.1M	0.0008M	125	4.828	124.04	27	25	29	26	28	27	28	27

Table 14. Results obtained from dentine discs (cell type 2; standard preparation procedure, carious canines) and unbuffered KCl and NaCl solutions at 25°C ± 1°C.

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Solution a_1	Solution a_2	a_1/a_2	$\log_e (a_1/a_2)$	Ideal ϕ (mV) (calculated)	Liquid junction potential E_j (calculated)	Mean ϕ (mV) observed									
						Cell 13		Cell 14		Cell 15		Cell 16		Cell 17	
						KCl	NaCl	KCl	NaCl	KCl	NaCl	KCl	NaCl	KCl	NaCl
0.1M	0.1M	1	0	0	0	0	0	0	0	*	0	*	0	*	0
0.1M	0.02M	5	1.609	41.33	-8.2	+3	+2	+2	+1	*	-2	*	-2	*	-1
0.1M	0.004M	25	3.218	83.41	-16.53	+6	+4	+7	+5	*	-3	*	-4	*	-3
0.1M	0.0008M	125	4.828	124.04	-24.08	+7	+5	+8	+6	*	-6	*	-6	*	-5

Table 15. Results obtained from dentine discs (cell type 2; standard preparation procedure, unerupted canines and premolars) and unbuffered KCl and NaCl solutions at 25°C ± 1°C.

*No stabilized readings obtained.

Solution a_1	Solution a_2	a_1/a_2	$\log_e (a_1/a_2)$	Ideal ϕ (mV) (calculated)	Liquid junction potential E_j (calculated)	Mean ϕ (mV) observed							
						Cell 18		Cell 19		Cell 20		Cell 21	
						KCl	NaCl	KCl	NaCl	KCl	NaCl	KCl	NaCl
0.1M	0.1M	1	0	0	0	*	0	0	0	*	0	*	0
0.1M	0.02M	5	1.609	41.33	-8.2	*	-2	+3	+1	*	-3	*	-3
0.1M	0.004M	25	3.218	83.41	-16.53	*	-5	+7	+4	*	-7	*	-6
0.1M	0.0008M	125	4.828	124.04	-24.08	*	-8	+11	+7	*	-8	*	-10

Table 16. Results obtained from dentine discs (cell type 2; modified grinding procedure, functional non-carious canines) and unbuffered KCl and NaCl solutions at $25^0C \pm 1^0C$.

* No stabilized readings obtained.

Solution a_1	Solution a_2	a_1/a_2	$\log_e (a_1/a_2)$	Ideal ϕ (mV) (calculated)	Liquid junction potential E_j (calculated)	Mean ϕ (mV) observed							
						Cell 22		Cell 23		Cell 24		Cell 25	
						KCl	NaCl	KCl	NaCl	KCl	NaCl	KCl	NaCl
0.1M	0.1M	1	0	0	0	*	0	*	0	*	0	*	0
0.1M	0.02M	5	1.609	41.33	-8.2	*	-5	*	-3	*	-3	*	-4
0.1M	0.004M	25	3.218	83.41	-16.53	*	-9	*	-11	*	-8	*	-7
0.1M	0.0008M	125	4.828	124.04	-24.08	*	-13	*	-15	*	-12	*	-11

Table 17. Results obtained from dentine discs (cell type 2; standard disc preparation boiled in ethelene glycol, carious canines) and unbuffered NaCl solutions at 25°C ± 1°C.

* No stabilized readings obtained.

Fig. 17.

Cell 7

Cell 8.

Fig.18.

Fig.19.

Cell 11.

Cell 12.
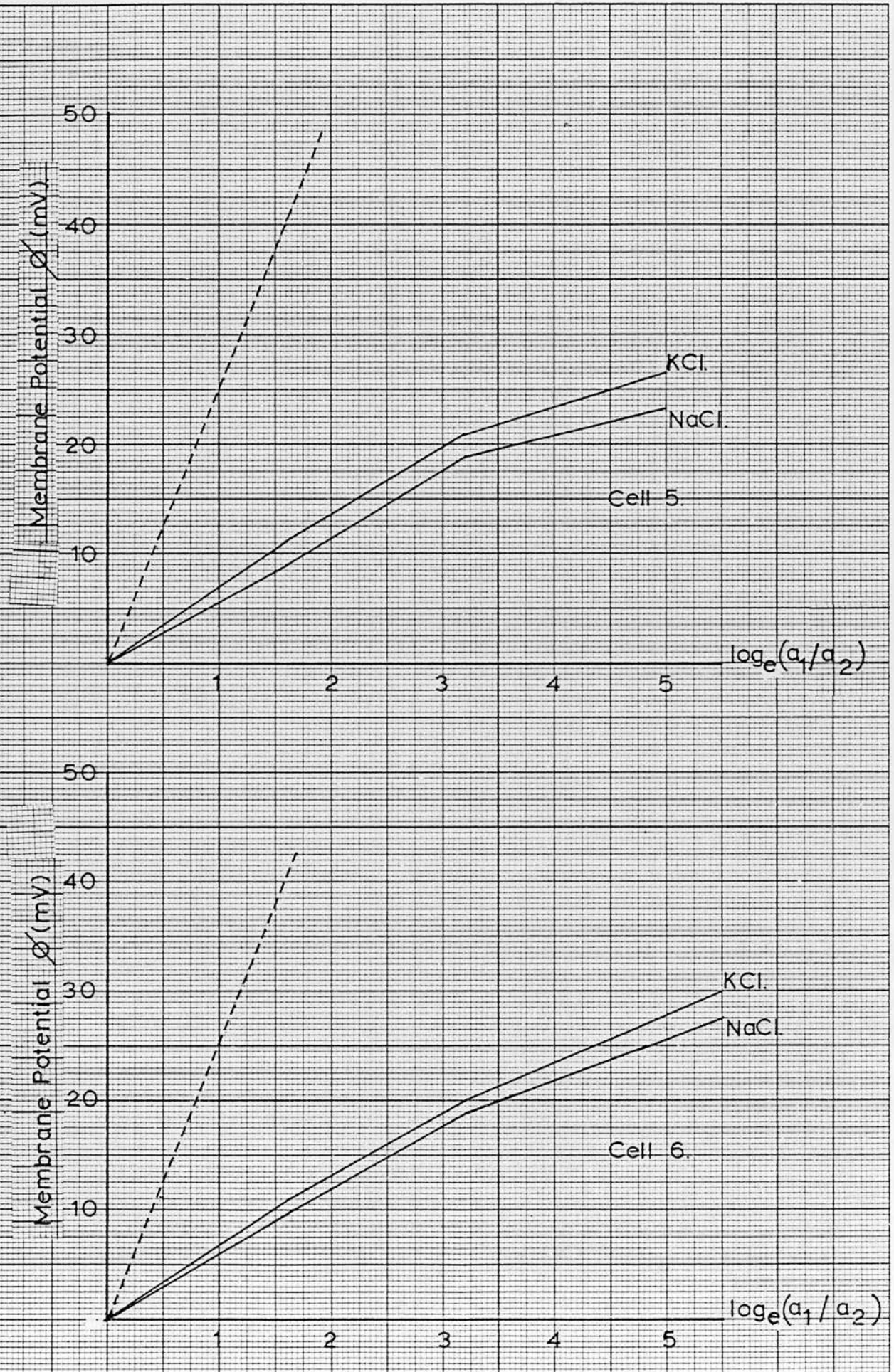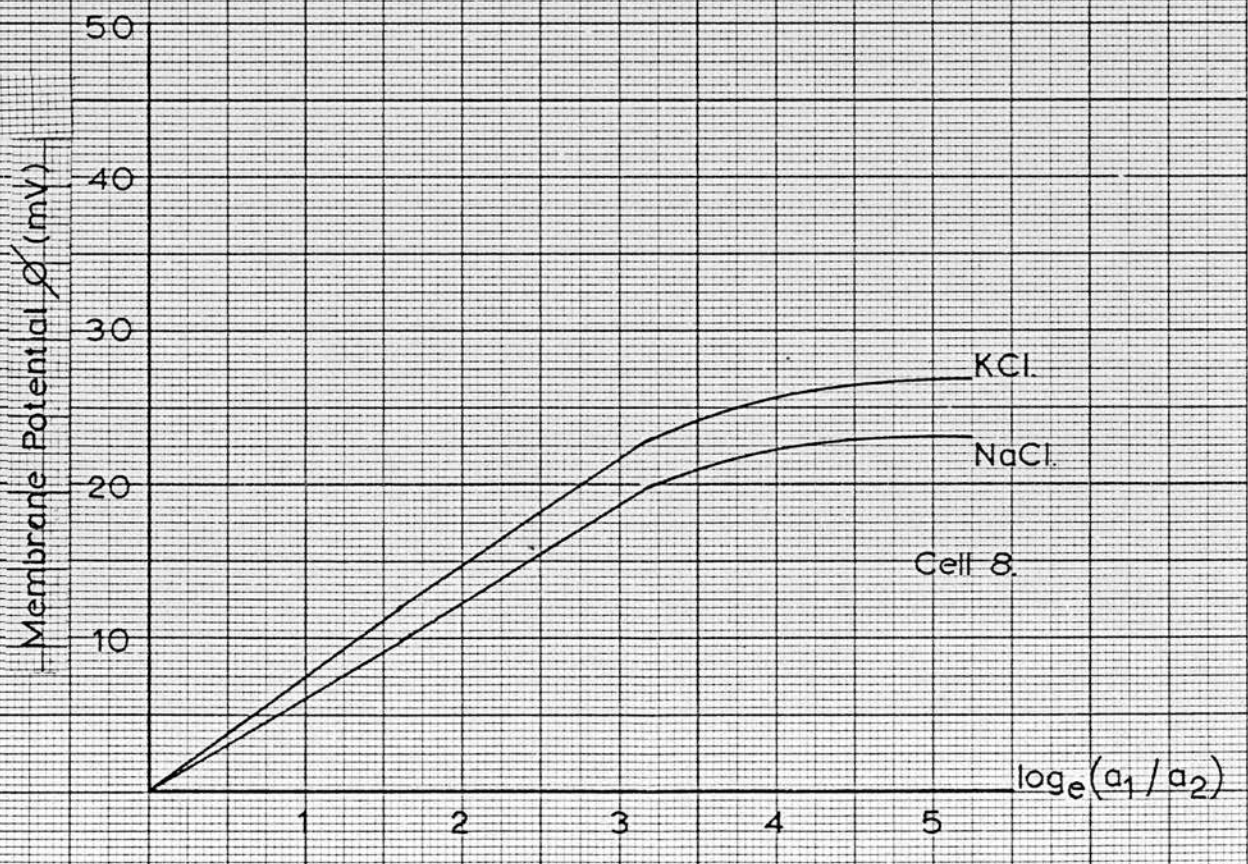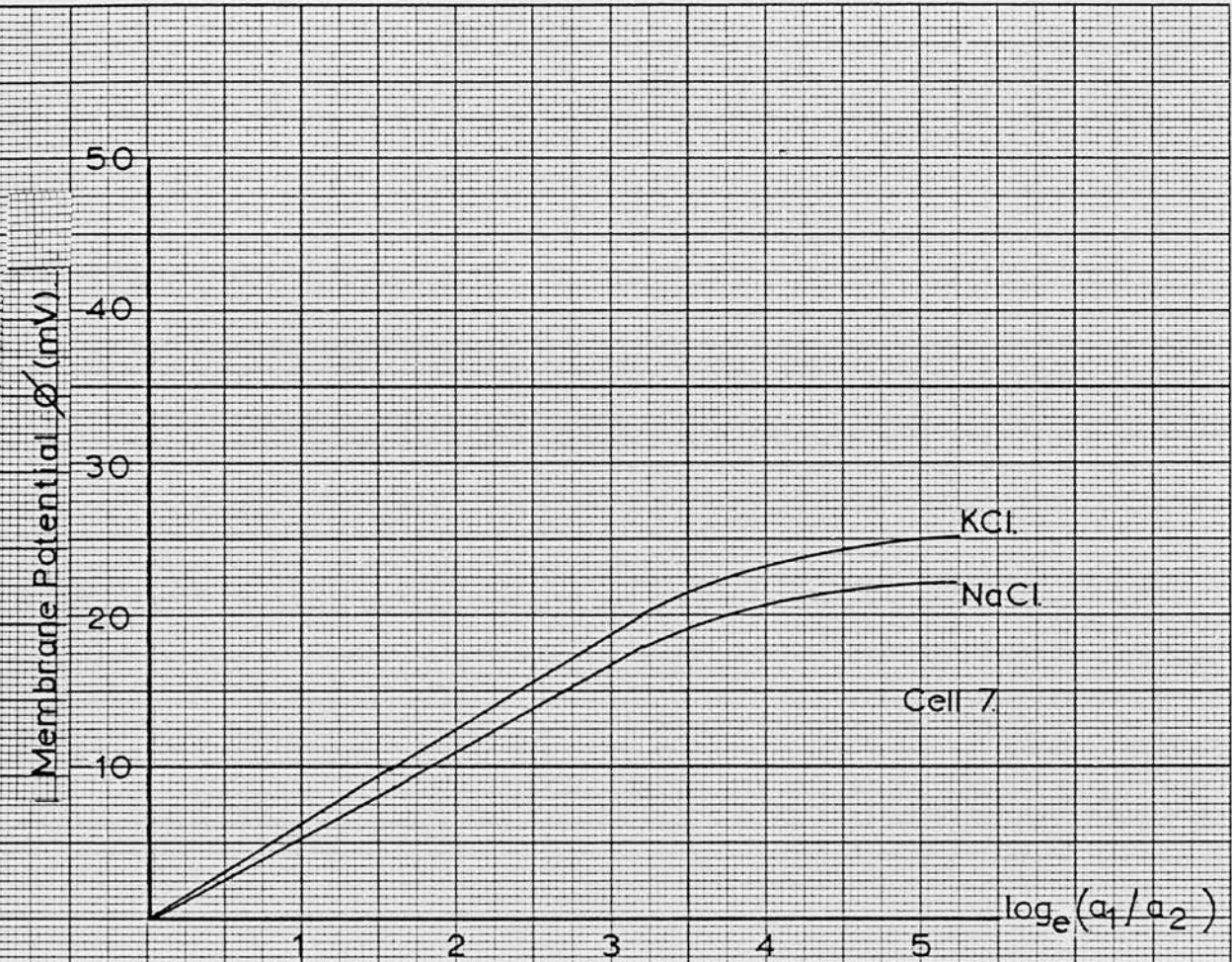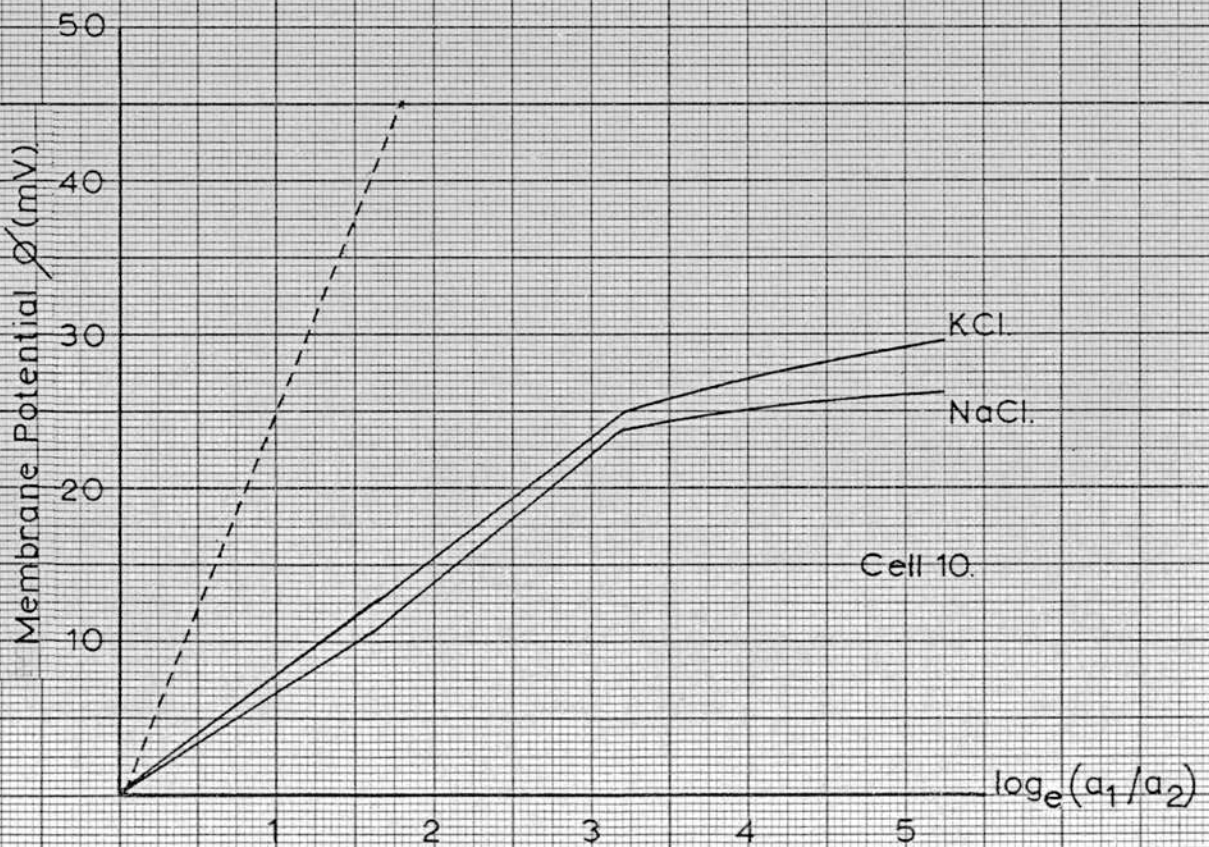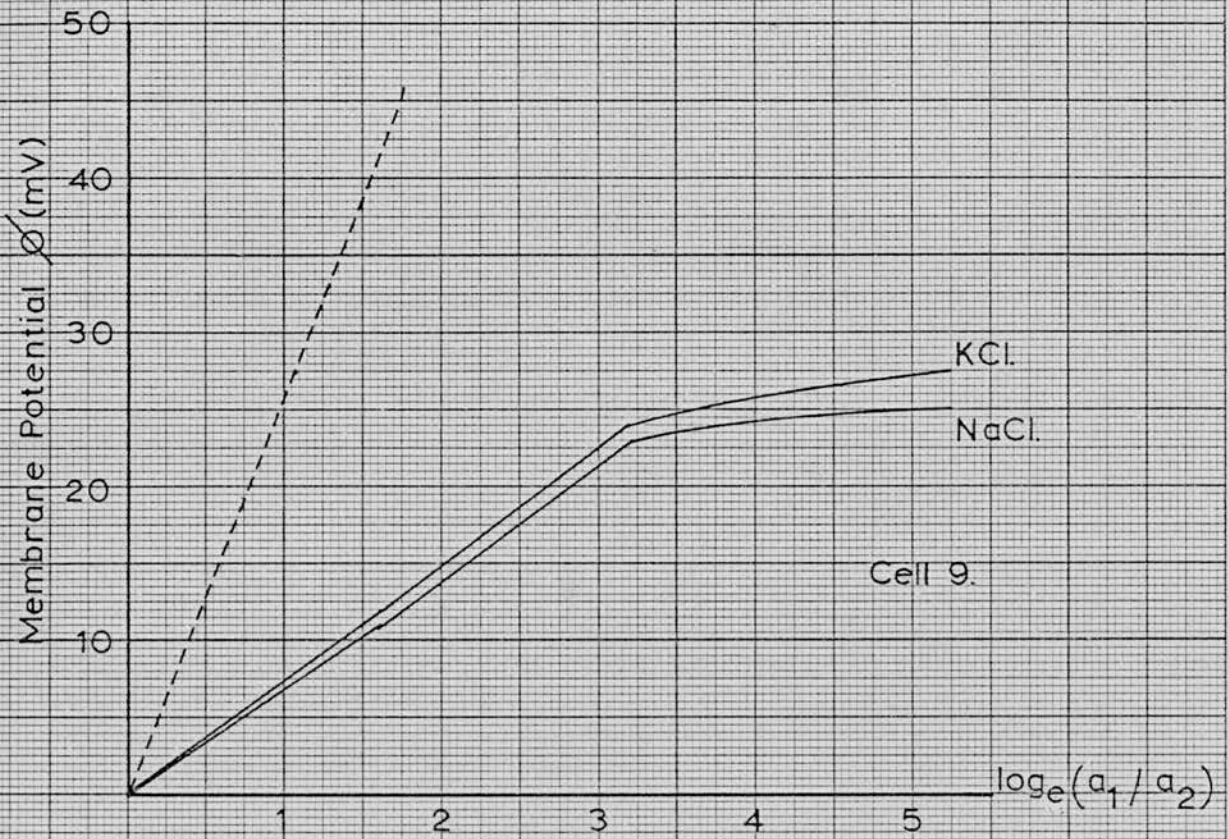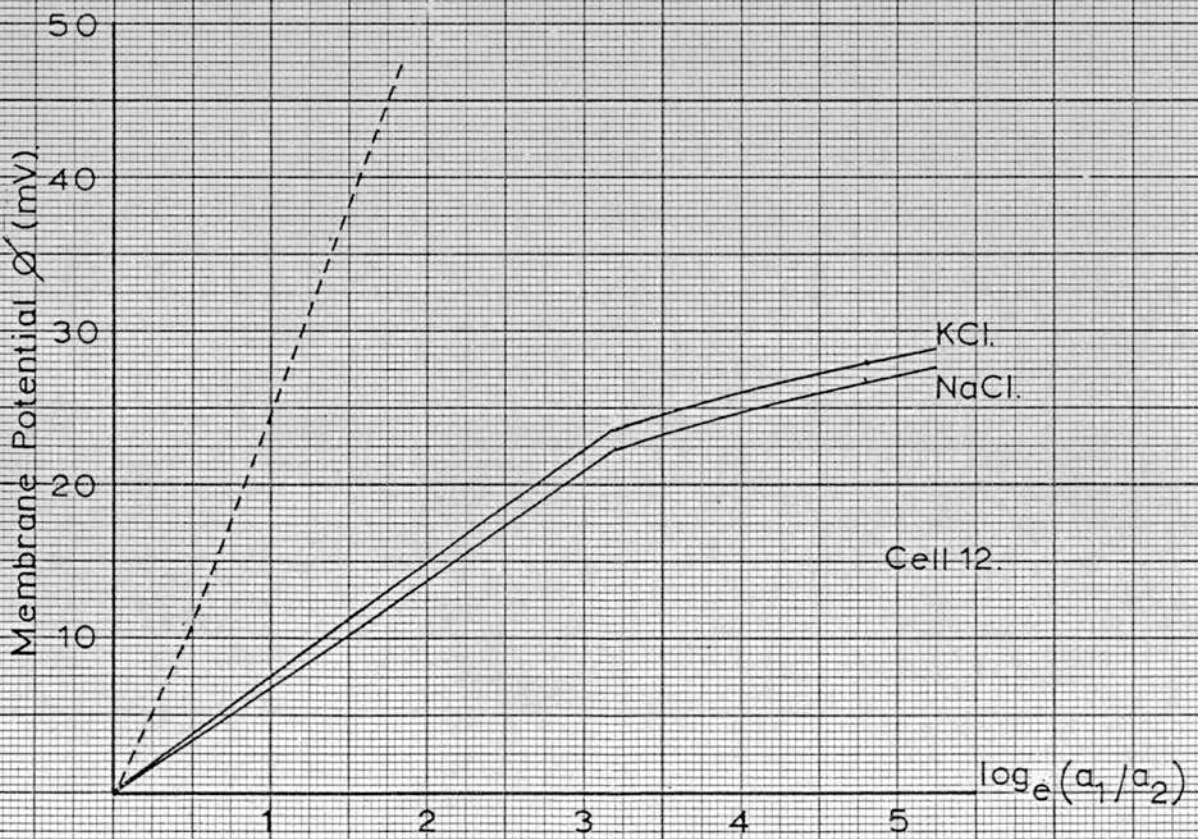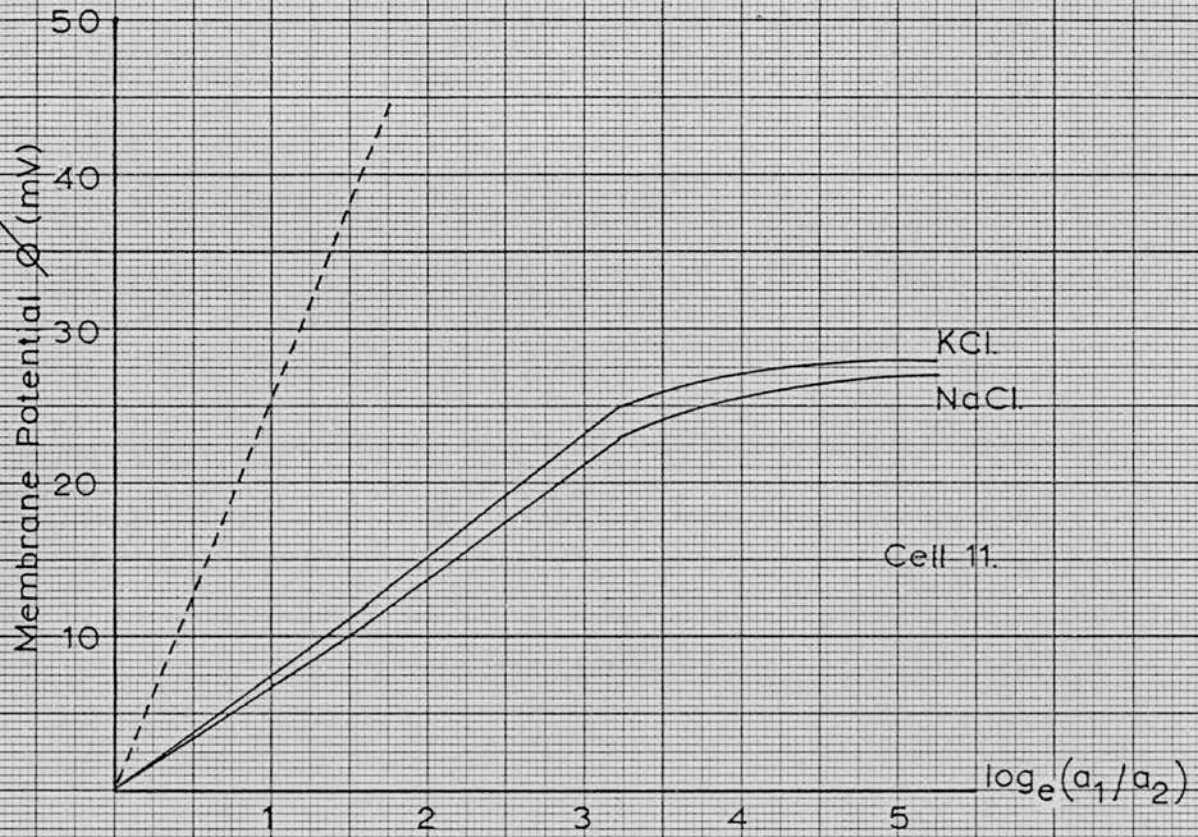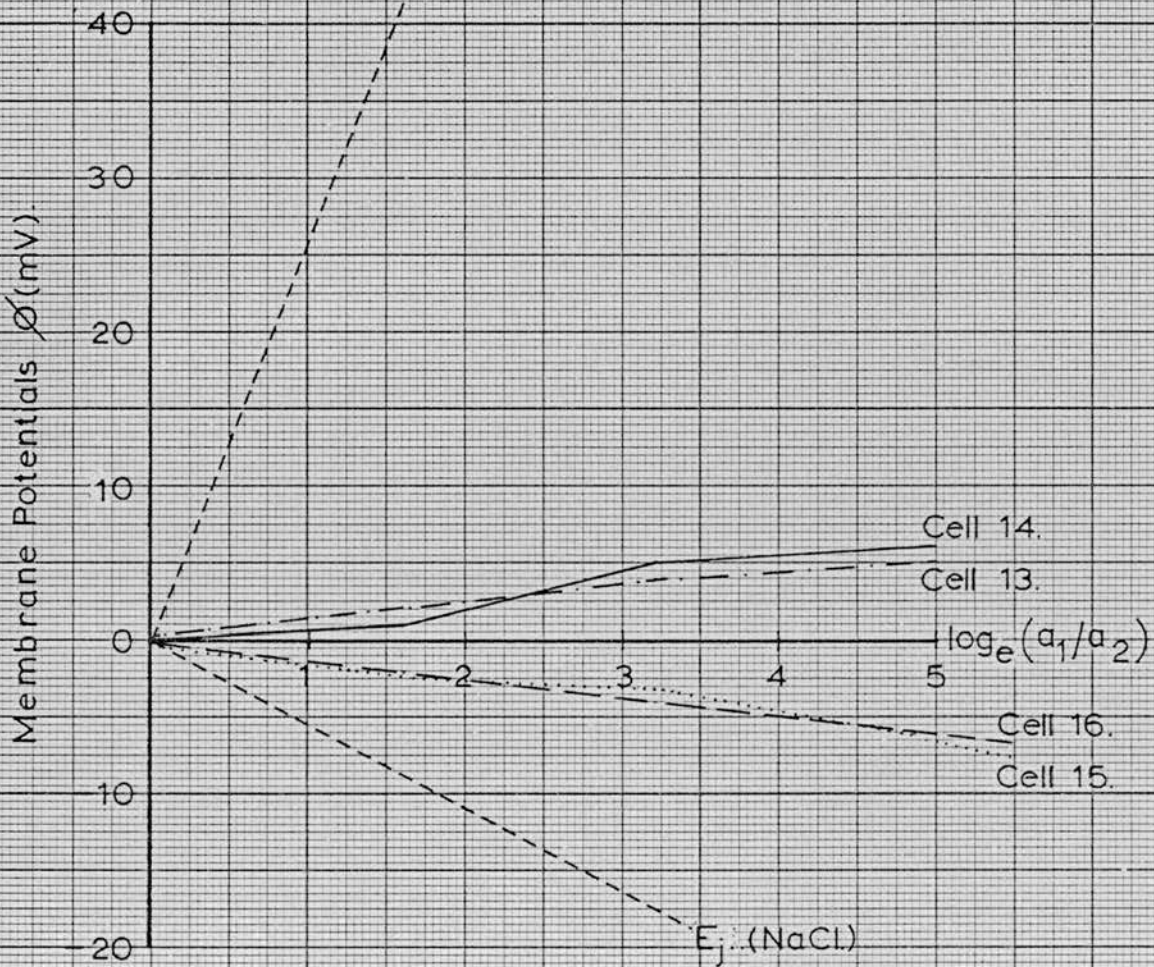
Fig. 20.   Results obtained from dentine discs and unbuffered KCl and
NaCl solutions indicating the variation in membrane
potential across dentine discs (standard preparation
procedure; unerupted teeth) as a function of the activity
ratio $(a_1 a_2)$ of the NaCl solutions.

Also shown is the maximum theoretical potential gradient
for an ideal cation exchange membrane and the theoretical
liquid junction potential gradient for NaCl.
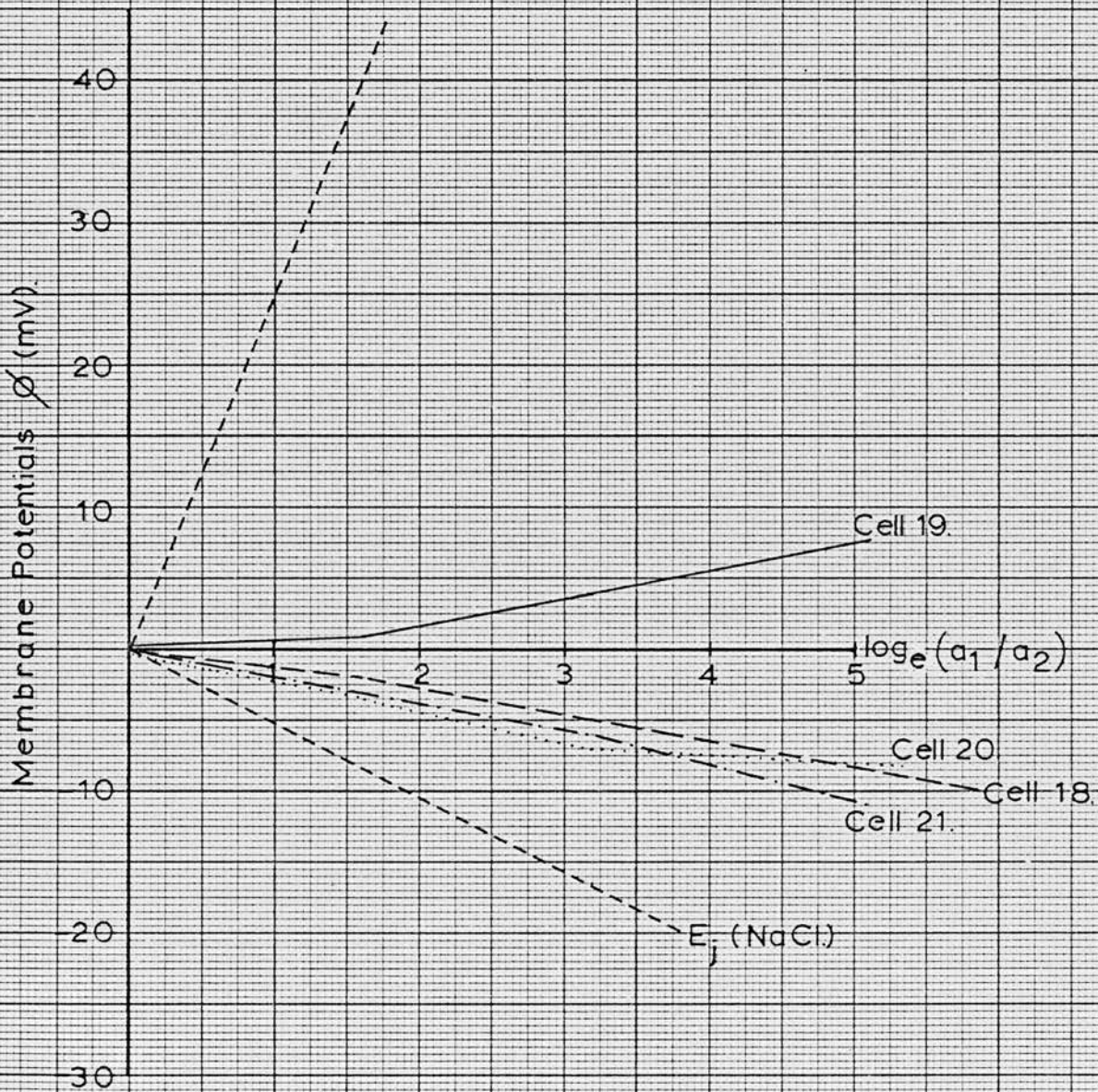
Fig. 21. Results obtained from dentine discs and unbuffered KCl and NaCl solutions indicating the variation in membrane potential across dentine discs (modified disc preparation; functional, non-carious canines) as a function of the activity ratio (a_1/a_2) of the NaCl solutions.

Also shown is the maximum theoretical potential gradient for an ideal cation exchange material and the theoretical liquid junction potential gradient for NaCl.

225.
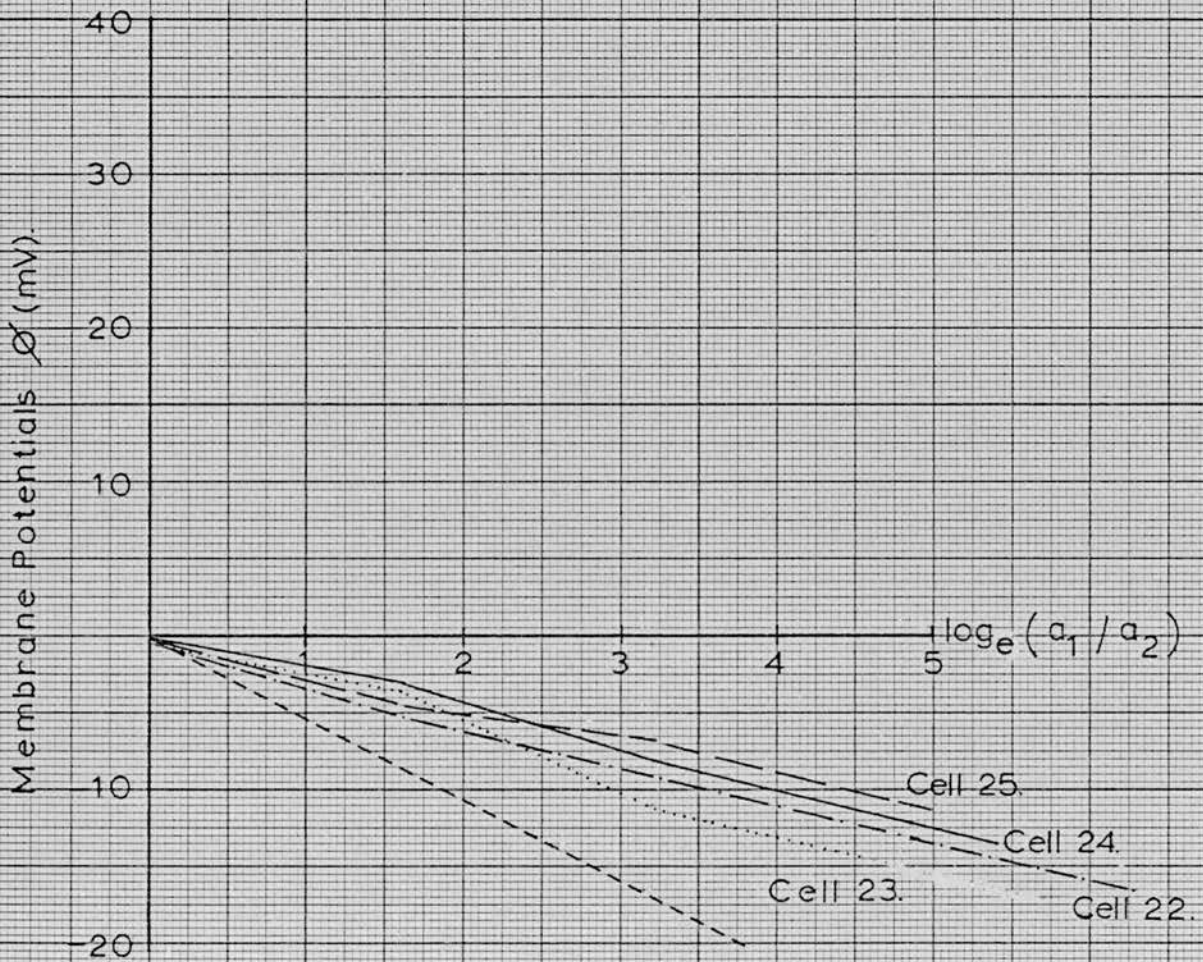
Fig. 22.   Results obtained from dentine discs (standard disc
preparation, boiled in ethelene glycol; carious canines)
indicating the variation in membrane potential across
dentine discs (standard disc preparation plus boiling
in ethelene glycol; carious functional canines) as a
function of the activity ratio (a_1/a_2) of the salt solutions.

In the group of teeth prepared from unerupted teeth, cells 13 and 14 gave low positive potentials at all concentration gradients. Cells 15, 16 and 17 gave negative potentials at all concentration gradients. Likewise, in the group of teeth prepared by the modified method of disc preparation from intact functional teeth (cells 18-21), cell 19 gave low positive potentials at all concentration gradients and cells 18, 20 and 21 gave negative potentials at all concentration gradients. In the group of teeth prepared by boiling discs in ethelene glycol (alkaline), all teeth gave negative potentials at all concentration gradients (Figs. 20, 21 and 22).

(c) Magnitude of membrane potentials. In general, the membrane potentials obtained for cells 1-12 inclusive were similar for both KCl and NaCl solutions at each concentration gradient. The results thus show that the magnitude of the membrane potentials is independent of the morphology of the dentine membrane.

(d) Equilibration rates. Equilibrium was defined as being obtained when the measured membrane potential remained steady to within 0.5 mV over a period of three consecutive days. The apparatus did not permit constant monitoring of potentials. These were generally read three times daily, at 10 a.m., 2 p.m. and 6 p.m.

   (i) Dentine caps: It generally took 3 - 7 days to establish a
        stabilized membrane potential across newly constructed dentine
        caps. Once this stabilized membrane potential had been
        obtained, the time taken to reach a new equilibrium, on
        changing the salt concentration gradient, was only 1 or 2 days.

   (ii) Dentine discs: For teeth numbers 5 - 12 inclusive, it gener-
        ally took 2 - 3 days to establish a stabilized membrane potential

across newly constructed cells.   Once this had been established
with any one salt, the time taken to reach a new equilibrium on
changing the salt concentration gradient was usually less than one
day.

Experiment 2

Table 18 shows the equilibration membrane potentials at each pH
value for four cells.   The cells gave rise to comparable membrane
potentials which were seen to diminish as the pH decreased.

The correlation coefficient (r) between the membrane potentials
of the pH was calculated as 0.969.   The standard error of the
correlation coefficient (t) is 12.50 (P < 0.001).   This indicates a
strong correlation between membrane potentials and pH.   The correla-
tion can be regarded as being highly significant.

The regression equation was calculated from the experimental
data and the regression line drawn on the scatter diagram (Fig. 23).
The iso-electric point of dentine lies between pH 4.0 and 4.8.

Experiment 3

Table 19 shows the observed membrane potentials obtained in
experiment 3.   Steady state potentials were obtained in 3-5 days.
All measurements were made at $25^0C \pm 1^0C$.   The results compared well
with those obtained in previous experiments under similar conditions.

(a) Calculation of fixed charge density from the experimental data.
In the Theory section, it has been shown that an approximate
value for the fixed charge density can be obtained using the formula

$$E = -11.8 + 215X$$
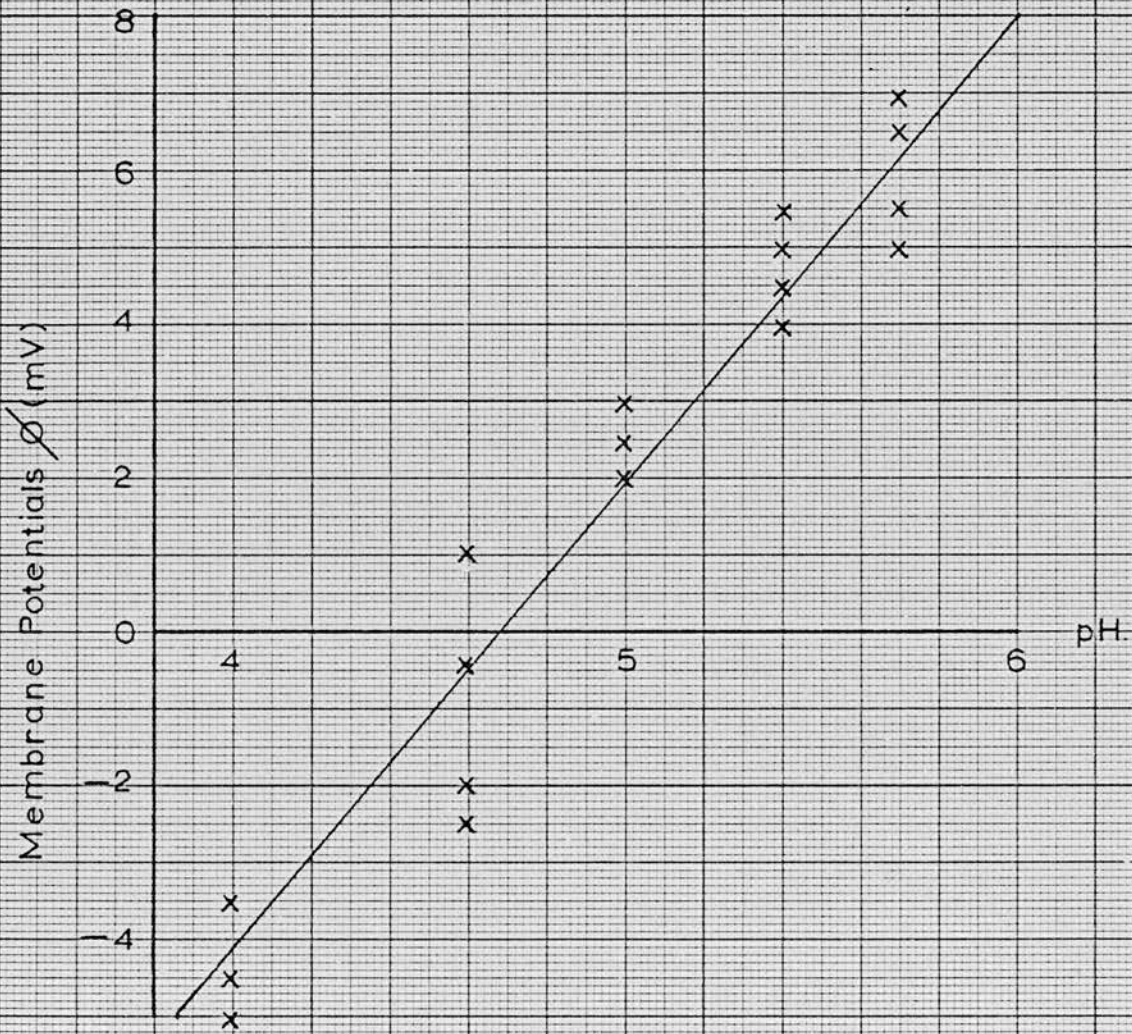
Fig. 23.   Graph illustrating the correlation between membrane
potential and pH as obtained in experiment 2.

Table 18.   Variation in membrane potential with pH in four
           different concentration cells (a_1/a_2 = 10: buffered
           NaCl: temperature $25^0C \pm 1^0C$).

pH	4.0	4.6	5.0	5.4	5.7
Sample 1	-3.5 mV	+1.0 mV	+3.0 mV	+5.0 mV	+7.0 mV
2	-4.5 mV	-2.0 mV	+2.0 mV	+4.0 mV	+5.5 mV
3	-5.0 mV	-2.5 mV	+2.0 mV	+4.5 mV	+5.0 mV
4	-3.5 mV	-0.5 mV	+2.5 mV	+5.5 mV	+6.5 mV
$\bar{X}$ ±SEM	-4.13 ±0.38	-1.0 ±0.79	2.38 ±0.24	4.75 ±0.32	6.0 ±0.45

Table 19. The net negative fixed charge in equivalents/litre tissue
water calculated from the mean membrane potential across
four dentine samples at $25^0C \pm 1^0C$ (unbuffered NaCl).

Cell	Solution a_1	Solution a_2	Mean membrane potential	Calculated fixed charge in equivalents/litre
9	0.1M	0.01M	18.3 ± 0.57 mV	0.140
10	0.1M	0.01M	18.3 ± 1.15 mV	0.140
11	0.1M	0.01M	17.6 ± 1.15 mV	0.136
12	0.1M	0.01M	18.0 ± 1.0 mV	0.138

Mean net negative fixed charge of dentine $= 0.139\pm0.001$ ($\overline{X} \pm$ SEM)
equivalents/litre.

where E is the observed potential in mV and X the fixed charge density in equivalents/litre.

Using the above formula and the results of experiment 3, an approximate value of the fixed net negative charge on dentine can be obtained.   The mean value so obtained is 0.139 ± 0.001 equivalents per litre tissue water ($\overline{X}$ ± SEM).

A value for the total negative charge of dentine was derived from chemical analysis data (Eastoe, 1967) by making the following approximation: chondroitin sulphate provides two negative groups per repeating disaccharide unit and dentine is 10% hydrated.

The approximate value for the fixed charge density of dentine was thus estimated to be 0.176 equivalents/litre tissue water.

Experiment 4

(a)   The emf/pH slope of the antimony micro-electrode.   Table 20 shows the values of the standard buffer solutions and the observed mean values obtained using the antimony micro-electrode and the glass electrode.   Between the pH of 3.5 and 7, the slope for both the glass electrode and the antimony electrode is linear and in agreement with each other.   These findings are in agreement with those of Kleinberg (1958) and Dirksen et al. (1962).

(b)   The pH of the base of carious cavities.   The pH within cavities varied considerably.   Every attempt was made to measure the pH of the cavity in the zone relating to the penetrating lesion.   Care was taken to return the antimony electrode to the same test area when observing the reproducibility of the results.   The reference electrode was maintained in the same position within the cavity through-out the readings.   Table 21 shows the mean pH of each cavity, the age

Table 20. Comparison of pH of standard buffer solutions measured
by glass electrode and antimony micro-electrode

pH of standard buffer (at 20°C)	pH using antimony electrode	pH using glass electrode
3.5 ± 0.01	3.6	3.5
4.0 ± 0.01	3.9	3.9
7.0 ± 0.01	7.0	6.9

Table 21.   The pH at the base of active carious lesions after removal of all carious dentine.

Tooth No.	Cavity pH (mean of 3 readings)	Patient's age		Type
		Years	Months	
12	3.4	11	7	/6̅
21	3.4	8	5	6/
1	3.5	7	2	/6̅
10	3.5	10	4	6̅/
9	3.6	6	10	6̅/
14	3.6	7	5	/6
17	3.6	9	11	/6̅
20	3.7	7	7	6/
22	3.7	6	11	/6
2	3.9	7	2	6̅/
13	3.9	7	5	6̅/
16	3.9	8	3	6/
18	4.0	6	4	6/
3	4.1	8	2	/6
11	4.1	11	7	6̅/
7	4.2	8	2	6/
19	4.2	6	4	/6
6	4.3	7	3	/6̅
4	4.4	9	10	/6̅
15	4.4	8	3	/6
5	4.5	7	6	6/
8	4.5	8	4	6̅/

mean   3.9

SD   ± 0.36

SEM   ± 0.077

of the patient and the type of tooth.

The average pH at the base of an active carious lesion after removal of all carious dentine was found to be 3.9 ± 0.077 (mean ± SEM). These values compared well with the results of Dirksen et al. (1963).

F.   DISCUSSION

Theory

The behaviour of ion-exchange membranes has been explained in terms of the "fixed-charge" theory of Teorell (1935) and Meyer & Sievers (1936). Prior to applying the theory to a biological substance like dentine, it is best to discuss some of the more obvious weaknesses.

(a) The structure and chemical nature of the membrane. The theory assumes that the membrane matrix contains water spaces and is homogeneous. The spatial distribution of the fixed, ionized groups is considered constant in every part. In heteroporous membranes, "fixed charge" control of ion migration may be confined to a few critical points. When a membrane consisting of layers of different permittivity, is bathed in two solutions of different concentrations and is allowed to equilibrate, the observed emf will be dependent on the properties of the most permselective layer (Meyer & Birnfeld, 1945).

(b) Concentration of fixed ionic groups in the membrane. The concentration of fixed ionic groups is a variable rather than a constant characteristic of any membrane. It refers to a particular

state under given experimental conditions. Only when ionogenic groups are ionized do they act as fixed charges. Strong-acid and strong-base groups are, by definition, practically completely ionized over a wide range of pH values. In contrast, weak-acid and weak-base groups are predominantly non-ionic at low and high pH respectively. The degree of ionization of the ionogenic groups depends on the pK value of the groups and on the pH within the membrane. The pH within the membrane depends on factors other than the pH of the external solutions. Suppose the external solution contains a strong electrolyte such as NaCl. Na^+ ions enter the membrane and displace H^+ ions. The internal pH is thus raised. This effect is increased when the concentration of Na^+ ions in the external solution is increased. In the present experiments, dilute external solutions were used.

The experimental technique

The potentiometric methods used in the present investigation allow a quantitative approach to the characterization of dentine in terms of fixed ionic groups and permittivity. The permittivity of a membrane to salts may be determined by the magnitude of the potentials developed across it. Conversely, a study of the potential difference that arises across a membrane, when it separates two salt solutions of different concentrations, can be used to characterize the membrane. From an experimental point of view, the method is easy to use. Only concentration (activity) and mobility quantities enter.

(a) <u>Membrane potential, electrodes and emf measurements</u>.   The emf
of a cell such as was constructed consists of a membrane potential
and the electrode potentials.   The membrane potential is defined
as the electric potential difference between the two bulk solutions.
It consists of the diffusion potential within the membrane, the
phase-boundary potentials (Donnan potentials).   In order to obtain
membrane potentials per se, assumptions must be made about the
electrode potentials.   In the present investigation, standard
electrochemical procedures were adopted.   Calomel electrodes were
used.   It was assumed, as is usual, that the supplied calomel
electrodes balance one another exactly.   The emf of the cell thus
equals the membrane potential.

Calomel electrodes are filled with saturated KCl solution.
Diffusion of KCl from the electrodes tends to occur.   This can be
minimized by the use of low-leak electrodes or by filling the
calomel electrodes with more dilute KCl solutions than the usual
saturated ones.

Contamination of the concentration cell by diffusion from
electrodes must be kept to a minimum.   Test-immersion times were
therefore kept to a minimum.   Between readings, each calomel half
cell was shorted by immersing the tips of the KCl electrodes in
saturated KCl solutions.   The bulk solutions on either side of the
membrane were changed frequently for freshly prepared solutions.
They were always changed immediately before taking a reading.   The
immediate consequence of using calomel electrodes which leak is that
continuous readings cannot be taken without causing solution contam-
ination and therefore alteration of solution activities.   This problem

can be avoided by the use of 'direct reversible' electrodes. Ag/AgCl electrodes are at present being employed to obtain such continuous readings.

The system is very sensitive to film effect at the membrane surface though this effect is not so pronounced when monovalent electrolytes are used. Essentially the Donnan potentials are determined by solution concentrations, ionic valences and the selectivity of the membrane material. The relative ionic mobilities determine the diffusion potentials in the membrane and films. Agitation of the solutions minimizes the film effect and this was done by mechanical stirring.

Membrane cells have a high internal electrical resistance. It is necessary to use a null instrument - in this case a high sensitivity moving coil galvanometer. Instability of the measured potentials can be caused by such factors as interference from supply leads, the thermostatic control and even static changes induced by operators. Low impedence screened leads were used. A Faraday cage was not available.

(b) Temperature control. This was achieved by using a water bath. Throughout the experimental period, the temperature was maintained at $25^0C \pm 1^0C$. For an ideal cation exchange material, such a temperature difference would result in a difference of emf of only 0.2 mV which is less than the sensitivity of the apparatus used in the present investigation.

Interpretation of results

Experiment 1.   The results of experiment 1 are best discussed under
two headings:

(a)   the results obtained with cells 1-12 inclusive and

(b)   the results obtained with cells 13-25 inclusive.

(a)   Cells 1-12 were prepared from dentine caps and discs.   The
teeth were intact, functional or carious canines.   Disc preparation
was by the standard procedure.   In every case the more dilute
solution was positive with respect to the concentrated solution.
Moreover, the observed membrane potentials were always less than
expected from an ideal cation exchange resin.   It can be inferred
from the results that, under the experimental conditions, dentine was
not able to completely prevent the passage of ions of one electrical
sign.   Both cations and anions penetrated the dentine caps and
discs.   The cation penetrated more rapidly and gave its sign to the
dilute solution.

Consider the case of KCl.   The mobilities of K^+ and Cl^- ions
are almost identical.   The experimental results therefore suggest
that dentine in some way retards the Cl^- ion with respect to the K^+
which confers its positive sign on the dilute solution.   However,
as the slope for all teeth was less than for a theoretical ideal
cation exchanger, it can be concluded that Cl^- ions also penetrate
the dentine.

Lower membrane potentials were always obtained with NaCl
solutions than with KCl solutions.   This would be predicted from
factors affecting the conductance of electrolytes in aqueous

solutions and the relative velocities of the ions.   The ionic mobility of Na^+ is less than that of Cl^- and K^+.   Less positive charge is transferred to the dilute solution and is reflected in the observation that, for any given concentration gradient, the observed membrane potential with a NaCl solution was always less than with a KCl solution.

(b) Cells 13-25 were prepared from unerupted teeth, from intact, functional teeth by modified disc preparation and from carious teeth by standard disc preparation plus boiling in ethelene glycol. Table 11 lists the cell number and the details of the teeth used in this experiment.

Cells 13, 14 and 19 gave low positive values at all activity ratios.   Cells 15, 16, 17, 18, 20, 21, 22, 23, 24 and 25 gave low negative values at all activity ratios using NaCl solutions.   In each of these cases no stabilized readings were obtained with KCl solutions.

In the case of cells 13, 14 and 19, the observed results were similar in nature, but lower than the results obtained with cells 1-12 in the first part of the experiment.   In each case the dilute solution was positive with respect to the more concentrated one and KCl solutions gave higher values than NaCl solutions at all activity ratios.   It can be inferred from these results that the dentine in these cells was for some reason less perselective than the dentine in cells 1-12.

In the case of the remaining cells 15-25 (19 excepted), no stable membrane potentials were observed using KCl. With NaCl, the observed potentials were negative in value and approached the

theoretical liquid junction potential for two NaCl solutions of
activity ratio. It can be inferred from these results that the
major barriers to diffusion have been removed in the preparation of
the discs, and that the salt solutions are diffusing along open
physiological channels.

It should be noted that true stabilized potentials were not
obtained with any of the cells. Even with NaCl solutions, the
obtained potentials developed within 12 hours and fell off within 24
hours. Liquid junction potentials would be expected to be virtually
spontaneous and to diminish quickly with time. The ideal liquid
junction potentials have been calculated for each activity ratio and
are shown in the appropriate tables and graphs.

The experiments do not indicate the mechanisms within the
dentine which cause the retardation of anions with respect to cations
as suggested in the first part of the experiment. This could be
accomplished by fixed negatively charged ionic groups in excess of
fixed positively charged ionic groups within dentine. Caps and
discs prepared from functional, intact and carious teeth act as non-
ideal cation exchange membranes. Non-functional, unerupted teeth,
however, show varying degrees of increased permittivity. In three
cases, near liquid junction potentials arose suggestive of the
existence of patent dentinal tubules extending from the amelo-dentinal
junction to the pulp. The boiling in ethelene glycol of discs
prepared by the standard method resulted in near liquid junction
potentials similar in value to the results obtained with three of
the unerupted teeth. This suggests that the barrier to diffusion is
organic in nature and is removed by boiling in ethelene glycol. It

is further suggested that this organic barrier to diffusion is laid down between the time of eruption and the time the tooth becomes functional. Finally, discs prepared by grinding at least 1mm of dentine from the pulpal aspect also lose their permselectivity. The barrier to diffusion would appear to be located in that part of the dentine about 1mm from the pulp.

Further inferences concerning the nature of the barriers within dentine cannot be made from the results of the present investigations. The following papers, however, are of particular interest. Brannstrom & Garberoglio (1972), using scanning electron microscopy, concluded that the odontoblast processes in young premolars did not extend beyond 0.7mm from the pulp and mostly terminated 0.4mm from the pulp. Tsatsas & Frank (1972) studied 20 intact caries free human premolars and molars without attrition. The dentine consisted of dead tracts. The authors concluded that these arose from the progressive hyaline transformation of odontoblast processes and surrounding organic structures. The dead tracts were presumed to be filled with extracellular fluid.

The description of dentinal tubules as given by Brannstrom & Garberoglio (1972) and Tsatsas & Frank (1972) differs from the traditional text book description. In terms of permeability barriers, it is fundamentally similar to the classical description of the dentinal response to mild irritation or caries (Furrer, 1922; Beust, 1931; Fish, 1932; Bradford, 1960; Miller & Massler, 1962; Barber & Massler, 1964). Fig. 24 illustrates the three basic situations which seem to exist.

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Fig. 24.   Schematic representation of the relationship between dentinal tubules and the odontoblast process.

1. Carious Attack.

Enamel.

Normal Dentine.

Carious Cavity.

Dead Tracts.

Reparative Dentine.

(after Fish, 1932).

Hyaline layer, non-tubular. (permselective)

2. Intact Functional Tooth.

Enamel.

Amelo-Dentinal Junction.

Dead Tract.

Hyaline or Organic Plug.

(after Garberoglio & Brannstrom, 1976).

Odontoblast Process occupying the Pulpal 1/4 of the Tubule.

3. Unerupted Developing Tooth.

Enamel.

Amelo-Dentinal Junction.

Dentine.

Predentine.
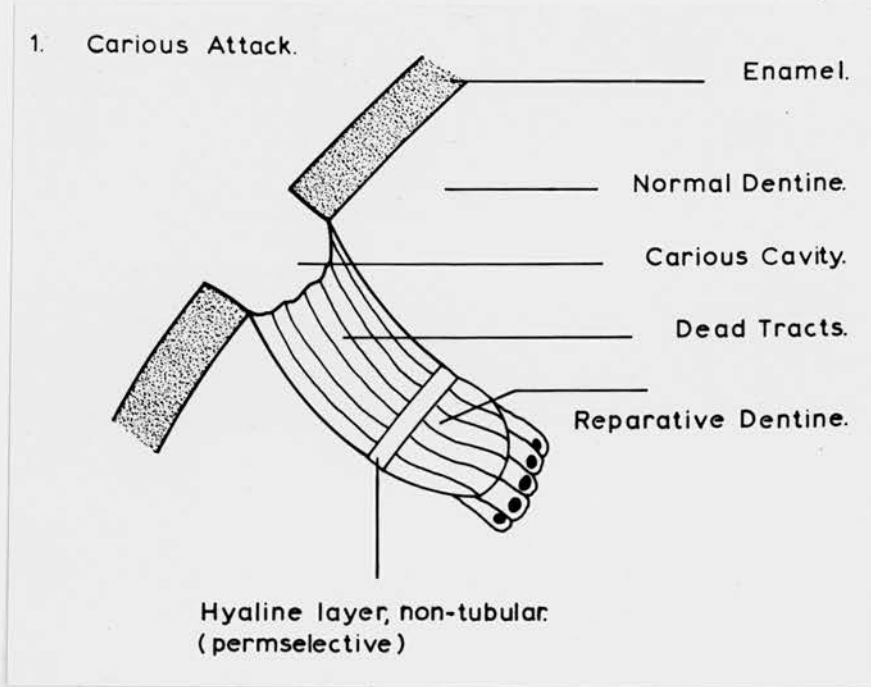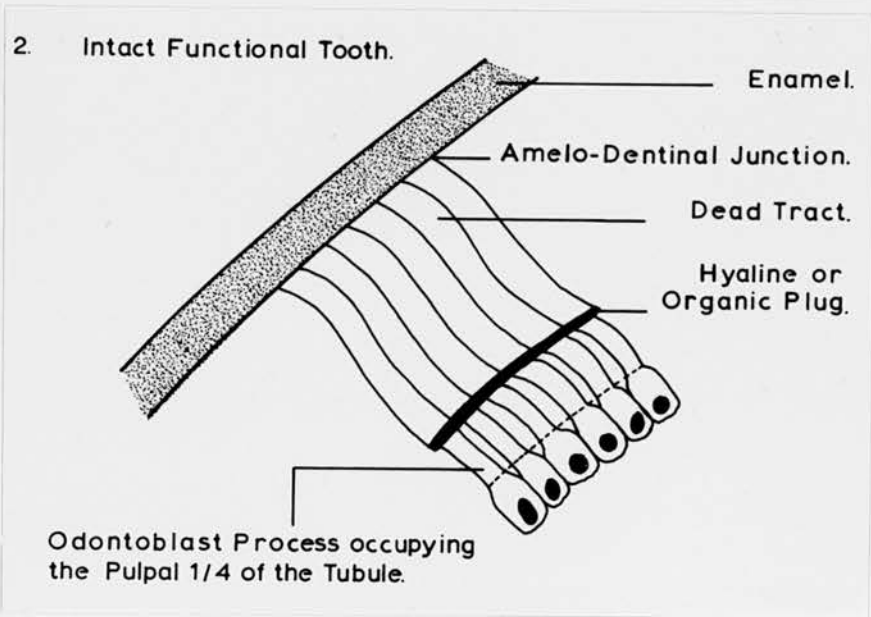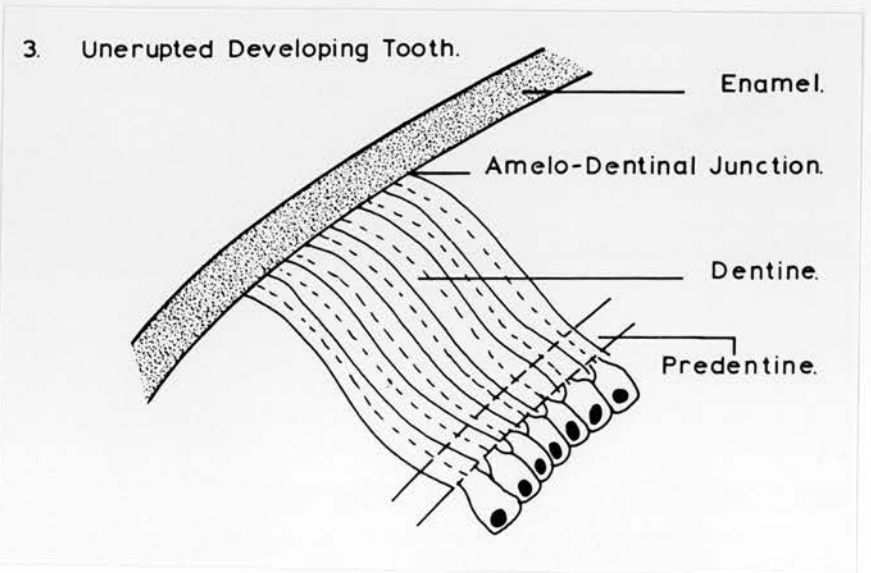
(after Gaunt et al., 1971).

The results of experiment 2, together with the findings of Brannstrom & Garberoglio (1972) and Tsatsas & Frank (1972), suggest that there is a physiological closing of dentinal tubules by an organic barrier before the onset of caries and that this barrier is located in the zone 1mm from the pulp (Garberoglio & Brannstrom, 1976).

Experiment 2. The results suggest that the observed membrane potential is a function of the pH of the solutions. This further suggests that dentine acts as an ion-exchange membrane, the electro-chemical properties of which are determined by the ionic environment (see discussion of Theory).

The iso-electric point of an ampholyte is defined as the pH at which the positive and negative ions within the substance have equal concentrations. Zero values for membrane potentials will occur when the fraction of current carried by the cations equals the fraction carried by the anions. When a solution of NaCl is used, as in the present experiments, in order that Na^+ ions transport half the current, it is necessary that the concentration of Na^+ ions in dentine is higher than the concentration of Cl^- ions (assuming that the mobility of the ions is the same as in free solution). This will occur when the pH of the dentine is such that there still exists a net negative charge. The true iso-electric point of dentine will be lower than the value given by the regression equation intersecting the horizontal scale.

The regression equation intersects the X axis at pH 4.7. The approximate true value of the iso-electric point of dentine is less than 4.7.

Experiment 3.   The results of this experiment give an approximate
value of 0.138 equivalents/litre tissue water for the fixed net
negative charge within dentine at 25°C when unbuffered NaCl is used
to construct concentration cells across dentine discs.

Previous experimental evidence suggests that dentine acts as a
non-ideal cation exchange resin (see results of experiments 1 and
2).   The interpretation of these results has led to the suggestion
that an organic fraction within dentine contributes the major part
of these cation exchange properties.

A value for the total negative charge contributed by the dentine
glycoaminoglycans was arrived at by making the following approxima-
tions: chondroitin 6-sulphate is the major glycoaminoglycan in
dentine (Eastoe, 1967) and provides two negative groups per
repeating disaccharide unit.   The calculated value of the fixed
negative charge from chemical analysis data is 0.176 equivalents/
litre tissue water.   In arriving at this value, it is assumed that
all the ionogenic groups are dissociated.   It should be remembered
that the dissociation of the ionogenic groups is a variable character-
istic and dependent on the pH within dentine and therefore on the
experimental conditions.   The weak acid groups (carboxyl groups)
would only be expected to be fully ionised at high pH values.

The similarity in value of the net charge obtained by the two
methods suggests that the carboxyl and sulphate groups of dentine
glycoaminoglycans were almost completely ionised under the experi-
mental conditions.   Moreoever, the results imply that the negative
groups of dentine glycoaminoglycans are not linked electrovalently
with other ground substance constituents.   This is in agreement with
the views of Herring (1968).

Experiment 4.   The mean pH at the base of an active carious cavity
was found to be 3.9 ± 0.077 ($\overline{X}$ ± SEM).   This compares with the
approximate value of 4.2 for the iso-electric point of dentine as
calculated in experiment 2.   The pH of a carious lesion is such
that the dentine subjacent to the lesion is at or near its iso-
electric point.

Amphoteric ion-exchangers with weak-acid and weak-base groups
depend strongly on the pH within the exchanger.   At or near the
iso-electric point, both the acid or base groups are essentially
non-ionized and Donnan exclusion is insignificant.   A membrane
consisting of amphoteric molecules is not selective at the iso-
electric point.

The inflammatory changes which commonly occur in relation to
deep caries arise in the first instance prior to the invasion of the
pulp by bacteria.   Such non-infective pathological changes are
characterized by macrophages, lymphocytes and plasma cells suggestive
of a delayed hypersensitivity reaction.   Metabolites or endotoxins
produced by micro-organisms within the carious lesion could initiate
such reactions.   The diffusion of these substances from the lesion
into the pulp can be expected to occur with maximum ease at the iso-
electric point of dentine which has been shown to be near the pH of
the base of the carious lesion.

Conclusions from the investigation as a whole

1. Dentine behaves as a non-ideal ion-exchanger with an excess of
    fixed negative charges at pH values greater than 4.7.

2. Most of the ion-exchange characteristics are conferred on the
    dentine by alkaline ethelene-glycol soluble components.

3. The major permeability barrier lies approximately 1mm from the
    pulpo-dentinal junction in erupted functional teeth.

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