HEALING OF APICECTOMY WOUNDS IN DOGS

A histological study

by

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

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PREFACE

The clinical operation of apicectomy has been practised since the 19th century, but little is known of the early changes in the tissues following this operation. There is knowledge of the later changes both from clinical and experimental sources.

The earlier work of Bauer, who reported the later histological findings after apicectomy in experimental animals, is discussed in the review of the literature in which is considered aspects of wound healing with particular reference to healing after apicectomy.

The second part of the thesis, or present investigation, is the original portion which embodies the histological findings at specified post-operative intervals following apicectomy in dogs.

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REVIEW OF LITERATURE ON ASPECTS OF WOUND HEALING, WITH PARTICULAR REFERENCE TO HEALING OF APICECTOMY WOUNDS.

INTRODUCTION

Wound healing is part of life — an everyday event. As such, it is often very much taken for granted; and, at the same time, it is a phenomenon about which relatively little is known.

Despite this ignorance, fruitful studies have been carried out on many aspects of wound healing. For example, a knowledge of factors which encourage or retard healing is obviously a desirable basic requirement for the practice of surgery; and many of these factors have been elucidated. The histological changes following wounding have also been the subject of much investigation, and there is considerable knowledge as a result of this. On the other hand, relatively little is known concerning the chemistry of wound repair, despite much research.

There has been very little investigation of the histological changes following the operation of apicectomy. An apicectomy wound is unique in that it involves healing of alveolar bone, mucoperiosteum and the resection surface of a
pulpless root filled tooth. It is, therefore, relevant to review aspects of wound healing, with particular reference to alveolar bone healing, healing of oral mucoperiosteum, healing of tooth surfaces in contact with living tissues, and tissue responses to foreign root filling materials.
CHAPTER 1

SOME ASPECTS OF WOUND HEALING

General aspects of wound healing

Wound healing is the foundation of surgery (DEVITO, 1965).

Trauma to the tissues of the body, whether caused unintentionally (as in accidents) or intentionally (as by the surgeon's incision), initiates a sequence of cellular and molecular events, the purpose of which is to restore structural continuity. An understanding of these events is fundamental to proper surgical practice (DEVITO, 1965).

Virchow's studies in cellular pathology stimulated research into the microscopic sequences of injury and healing, so that modern knowledge of these sequences was well established by the end of the 19th century (EDWARDS and DUNPHY, 1958). An extensive review of the earlier literature was published by AREY (1936).

Much of our knowledge of wound healing is the result of experimental observations upon a small number of laboratory animals. The extrapolation of much of this knowledge to man remains to be proved (EDWARDS and DUNPHY, 1958).
Vertebrates in general, and man in particular, have poor regenerative powers compared with lower forms of life. For example, salamanders will grow a new limb to replace one which is amputated ( Gibson, 1964). However, from the point of view of the surgeon, man's powers of healing are almost completely confined to terms of epithelial regeneration and fibroplasia ( Devito, 1965).

Surgical healing of an injured part is considered to have occurred when tissue continuity, approximately normal tissue strength, and an appropriate surface covering have been restored ( Grillo, 1963; Edwards and Dunphy, 1958).

The repair of a wound in a given animal is directly related to both the nature and the extent of the injury, and to the power of survival of the remaining living tissues ( Dunphy, 1960).

While the repair of damaged tissue is a remarkably specific biologic reaction, (Dunphy, 1960), it is nevertheless, difficult to describe wound healing as though it were a single process.

The time taken for a wound to heal varies with different species, with different individuals of a given species, with
different tissues and with different parts of the body. Even previous injury to the organism can modify the response to wounding. Nevertheless, despite such variable factors, it is possible to synthesise the diverse experimental data to form an outline of the process of events in wound healing, while keeping in mind the variations which may occur (DEVITO, 1965).

**Sequence of events in tissue repair**

Although much concerning the sequence of events involved in tissue repair is known generally, and in depth concerning particular aspects, it is well to remember the words of DOUGLAS (1963):

Wound healing teems with unsolved problems. One may point to our lack of knowledge of the stimulus to repair and its subsequent inhibition, the role of mucopolysaccharides and their sulphation, the origin and function of the fibroblast and the control of collagen synthesis.

Injury to the body may elicit systemic and local reactions.

**Systemic reactions to injury**

It is not the intention of this review to elaborate upon the complex processes of systemic reactions to injury, involving
such phenomena as surgical shock. May it suffice to quote the words of EDWARDS and DUNPHY (1958):

Severe regional injury results in a complex pattern of local and systemic reactions.... The precise relation between local and systemic factors in the repair of wounds remains largely speculative.

Local reactions to injury

LOCALIO et al (1943) divided the healing of a clean incised wound into three phases, as follows:

(1) the phase of traumatic inflammation,
(2) the phase of destruction, and
(3) the phase of proliferation.

To these phases, DOUGLAS (1963) adds,
(4) the phase of maturation.

CAMPANI (1960) also divided the healing of wounds into stages namely, transudatory, productive, biochemical-metabolic, and collagen stages.

(1) The phase of traumatic inflammation

The immediate local tissue response to an unspecified injury is acute inflammation. This response is both defensive and preparative to repair (EDWARDS and DUNPHY, 1958). Much has
been written concerning acute inflammation; only a few of the more salient features as related to wound healing are mentioned below.

After a deep incised wound, the edges of the wound become sealed together rapidly with a fibrin clot. Very soon there is a dilatation of capillaries of the wound margins and an increase in local capillary permeability. An outpouring of fluid occurs from the capillaries into the interstitial spaces (DOUGLAS, 1963). These phenomena are probably designed to increase the metabolic rate of the wounded tissues in preparation for repair (DOUGLAS, 1963). The exudate from the capillaries contains cellular and plasma elements (DEVITO, 1965). Within 12 hours of injury, the exudate contains polymorphonuclear leukocytes, red blood corpuscles, fibrin and macrophages (DEVITO, 1965). Local cellular injury probably releases a substance or substances which probably, at least partially, are responsible for these effects (MENKIN, 1950).

The polymorphonuclear leukocyte population increases during the first 24 hours, but they undergo fragmentation during the next 48 hours, possibly releasing chemotactic substances during fragmentation. The macrophage population increases, particularly between 24 and 72 hours (DEVITO, 1965).
(2) **The phase of destruction**

This phase is continuous and coincident with the phase of traumatic inflammation. It involves the removal of dead and dying tissue, and of material which is not contributing to repair (DOUGLAS, 1963). Leukocytes and macrophages, which have migrated into the wound, are the agents for this "cleaning-up" phase. Proteolytic enzymes are released by the leukocytes and these enzymes, for example, liquefy non-viable injured cells (HOWES et al, 1955). (HOWES et al (1955), also pointed out that ensuing processes also, such as sprouting of new capillaries and the proliferation and maturation of fibroblasts and epithelium are activated by enzymatic activity.)

The phases of traumatic inflammation and the destructive phase constitute the lag phase in wound healing. The duration of the lag phase varies from four to six days, and no repair proper proceeds during this period (DOUGLAS, 1963). The changes occurring during these early phases are "designed to increase the blood supply to the part, counteract bacterial invasion, remove necrotic tissue and blood clot, and prepare for definitive repair" (GIBSON, 1964).
(3) The phase of proliferation

This is concerned with the proliferation of connective tissue cells and of epithelial cells. These processes are described in Chapters 2 and 3 respectively.

(4) The phase of maturation

As will be mentioned in Chapter 2, fibroplasia in the healing wound reaches a peak at approximately 14 days after wounding (DOUGLAS, 1963). After this time, shrinkage and maturation of the connective tissue occur. Histologically, at this stage, the part becomes less vascular, and the proportion of the number of fibroblasts to the number of collagen fibres rapidly decreases. Coincident with the relative increase in fibre content, there is a progressive increase in the tensile strength of the wound. The process of maturation probably proceeds for a long time after clinical healing has occurred (JACKSON, 1958).

Factors which modify healing

There are systemic factors (such as nutritional and hormonal), and local factors (such as blood supply, infection, haemorrhage and the presence of foreign material), which may
influence the healing of a wound (DOUGLAS, 1963). Besides systemic or general factors which may be present to delay wound healing, it may be said that anything which decreases the optimum blood supply of the healing wound or anything in the wound which acts as an appreciable irritant will delay healing.

The biochemistry of wound healing

CHEN and POSTLETHWAIT (1964) made an extensive review of the literature concerning the biochemistry of wound healing. They concluded that:

It seems that despite the many studies reported, the sum total of knowledge concerning the metabolic aspects of wound healing is low. We do not know how a wound heals, although the histological sequence has been described repeatedly. We do not know what stimulates a wound to heal or what causes the healing process to stop when healing is complete.
CHAPTER 2

FIBROPLASIA IN HEALING WOUNDS

The restoration of continuity and strength to an injured part results from fibroplasia. Fibroplasia, together with epithelial activity, also participates in surface coverage (GRILLO, 1963). As mentioned in the previous chapter, the restoration of tissue continuity, tissue strength and surface coverage is the criterion of clinical surgical healing.

Fibroplasia in the healing wound commences as the congestion associated with the phase of traumatic inflammation subsides. Fibroblasts can be demonstrated in the wound as early as 24 hours after injury, but are most numerous after 72 hours (EDWARDS and DUNPHY, 1958).

The fibroblasts migrate into the wound and align themselves along new capillaries, which have sprouted from pre-existing ones by a process of asymmetrical mitosis (DOUGLAS, 1963). The new blood vessels appear first as solid endothelial buds within the first three days. The buds soon develop a lumen and form anastomoses with one another or the parent capillaries (GIBSON, 1964). The fibroblasts, having migrated into the wound, proliferate there (GIBSON, 1964).
The origin of the proliferating fibroblasts is still controversial. There are two schools of thought concerning this. There are those workers who propose an origin from local fibroblasts, while others favour an origin of fibroblasts in the wound from circulating cells in the blood-stream (HAIRSTONE, 1959).

ALLGÖWER (1956), and ALLGÖWER and HULLIGER (1960), advocates of the vascular origin theory, produced evidence that human mononuclear blood cells in tissue culture will produce a connective tissue network. MOEN (1935) also had found that mononuclear exudative cells in tissue culture may take on the characteristics of fibroblasts, and produce pure colonies of fibroblasts which maintain their morphological characteristics through repeated subcultures. Electron microscopic studies by ROSS and BENDITT (1961) have demonstrated sufficient similarities between macrophages and fibroblasts to conclude that fibroblasts can be derived from macrophages.

However, despite evidence for a blood-borne origin of fibroblasts, most workers favour a local source of primitive fibroblasts, either from the loose perivascular areolar tissue or from the walls of blood vessels (EDWARDS and DUNPHY, 1958). DUNPHY (1963) labelled monocytes with Indian ink, but was never able to demonstrate Indian ink within cells which appeared as
fibroblasts in the healing wound. The studies of STEARNS (1940) on the development of connective tissue in transparent chambers in the rabbit's ear, indicated that fibroblasts migrate from the periphery into the wound. MACDONALD (1959) used tritiated thymidine to identify cells in mitosis in the exudate of wounds in rats. His results pointed to a local origin of wound fibroblasts from local undifferentiated adventitial cells, which migrated into the wound, proliferated there and differentiated into fibroblasts. GRILLO'S (1963) studies on the effects of irradiation on repair also indicated that the fibroblasts of wound repair arise predominantly by proliferation of locally resident connective tissue cells, rather than from precursors derived from the vascular system.

To summarise the origin of fibroblasts, while it is possible for cells of vascular origin to produce collagen-producing cells in vitro, the significance of such cells in normal repair remains uncertain, and there is little evidence that they are the major source of fibroblasts. Most of the evidence points to a local origin of fibroblasts by an intense connective tissue response resulting in local cellular proliferation (GRILLO, 1963).
It was mentioned above how the fibroblasts, having migrated into the wound, align themselves along the new capillaries. The amount of fibroplasia in granulation tissue is related to the vascularity of the surrounding unwounded tissues (HADFIELD, 1963). GRILLO (1964) stated:

To date it has been impossible to separate the proliferative response of the capillaries from the fibroblasts which accompany them in such intimate relationship. The omnipresent fibroblast-capillary system may well be regarded as a primary reparative system in the mammal; with the exception of epithelial surfacing, this is the system which accomplishes restoration of continuity after injury.

DUNPHY (1963) has aptly referred to the fibroblast as the "ubiquitous ally for the surgeon".

The fibroblasts, having appeared in the wound, rapidly increase in number between the third and fifth days after wounding. During this early period, the wound is undergoing, by virtue of the cellular activity, an active preparation for fibrogenesis. Rather than being interpreted as a lag phase, this phase of healing should be thought of as being a productive or substrate phase according to DUNPHY and UDUPU (1955).

Following upon this phase of preparation there is the collagen phase, during which the tensile strength of the wound (previously negligible) increases progressively as collagen
fibres are laid down. This collagen phase lasts from the fifth or sixth day to about 15 days, (or until healing is complete, if the phase of maturation is considered as being a continuation of this phase).

Before considering the formation of collagen, it is pertinent to consider the ground substance of connective tissue. Ground substance is the amorphous matrix in which the cells and fibrils of connective tissue are situated. This matrix contains appreciable amounts of acid mucopolysaccharides. It exhibits metachromasia when stained with toluidine blue, and the degree of metachromasia is related to the activity of the tissue. Thus, during wound repair, metachromasia is marked (DOUGLAS, 1963). During the early fibroblastic activity of healing, metachromatic ground substance is demonstrable after the first 24 hours, reaching a peak between the third and the fifth or sixth day, declining rapidly thereafter (JACKSON, 1958; DEVITO, 1965). At one time it was believed that mast cells formed the metachromatic ground substance (RILEY, 1959), but it is now thought, on the basis of histological and histochemical determinations, that it is the product of the early fibroblast population in the wound (DEVITO, 1965). It may be that further study will show that what appear to be mast cells are
actually young fibroblasts (DUNPHY and UDUPU, 1955). The
fibroblasts are, then, responsible for at least some production
of new polysaccharides, although recent observations by EDWARDS
et al (1957) indicate that much of the polysaccharide of ground
substance is related to serum proteins, which were carried into
the wound with the inflammatory exudate.

Whatever the exact source of ground substance, it would
seem that some part of its function is to provide a nutrient
medium for the efficient synthesis of collagen (DUNPHY and
UDUPU, 1955), where the cells and fibrils of connective tissue
can live and be repaired (DOUGLAS, 1963). Nevertheless,
JACKSON (1958) stated that "there is no strong evidence that
the presence of mucopolysaccharides is essential for the
formation of collagen fibres in the healing wound". The work
of WATTS (1961) may clear the dilemma. He pointed out that
techniques of measuring mucopolysaccharides of ground substance
have not necessarily proved them to be essential precursors to
collagen formation in the wound. He applied hyaluronidase to
depolymerise the mucopolysaccharides of wounds, and found that
the collagen concentration in the wound was apparently unaffected.
However, the total amount, as opposed to concentration, of
collagen laid down was considerably reduced. WATTS (1961)
concluded, therefore, that "ground substance is an essential to collagen deposition".

Collagen deposition

STEARNs (1940) studied the development of connective tissue in transparent chambers in the rabbit's ear. Daily microscopic observations over a period of months showed that the formation of connective tissue fibres was initiated, on the average, at six days after operation. The formation of fibres was associated with the invasion of the operative area by fibroblasts from the preformed tissue. The presence of fibroblasts was found to be essential to the development of connective tissue and, moreover, the fibroblasts were seen to be intimately associated with the actual formation of connective tissue fibres. The orientation of the fibres, also, was related to the orientation of the fibroblasts. STEARNs (1940) found that connective tissue fibres could form in the transparent chambers with remarkable rapidity, a reticular fibre formation having been observed in three or four hours. During the course of 48 hours, the formation of fibres was so dense that the fibroblasts were almost obscured. Befőre forming the fibres, the fibroblasts appeared to increase in size and to become flattened
and striated and joined by cytoplasmic processes to form a syncytium. The fibroblasts appeared to participate directly in fibrillogenesis, by producing projections from their surface of vesicular masses of cytoplasm, which become separated from the fibroblasts. The cytoplasmic material disappears as the fibrils form, apparently being utilised in the production of fibrils. WASSERMANN (1954), studying fibrillogenesis in the regenerating rat tendon by electron microscopy of ultra-thin sections, found that bundles of fibrils were evident within the cytoplasm of the fibroblast, especially in the marginal region of the cell, as well as on the surface of the cell.

As mentioned previously, the ground substance provides an efficient medium for fibrogenesis. The physico-chemical conditions of ground substance provide the appropriate medium for the macromolecules of tropocollagen to bind themselves together, by cross-linkages and physico-chemical bonds, to form tropocollagen fibrils. Aggregation of tropocollagen fibrils occurs, to form procollagen fibrils. Further aggregation of the procollagen fibrils produces larger fibres of mature collagen of about 600 A.U. diameter (DEVITO, 1965).
Mature collagen is non-argyrophyllic. The early argyro-
phyllic fibrils which are formed, are distinguished by some as
being a separate fibre type, namely, reticulin. These fibrils
are, however, collagen microfibrils. Studies using electron
microscopy and x-ray diffraction have shown that reticulin and
collagen fibrils are similar in structure, although they are
different histochemically (AMLER et al, 1964).

The amount of collagen in the wound increases rapidly
between the fifth and fourteenth days, the tensile strength of
the wound increasing as the amount of collagen increases. The
initial maximum tensile strength of the wound is thus reached in
12-14 days in most species (EDWARDS and DUNPHY, 1958).

The phase of maturation of the healed connective tissue
has been mentioned in Chapter 1.
CHAPTER 3

HEALING OF EPITHELIUM OF EPIDERMAL WOUNDS

Coincident with the phase of fibroplasia (collagen phase) in a healing wound, such as a deep incisional type into the skin or oral mucoperiosteum, is the restoration of epithelial continuity. The restoration of surface continuity with the appropriate cover is one of the criteria of clinical surgical healing (GRILLO, 1963).

While the collagen phase provides most of the tensile strength to a healing wound, and granulation tissue provides a considerable degree of protection against entry of infection, coverage with epithelium provides optimal appearance, function and protection (DEVITO, 1965). The process by which the wound surface is covered by epithelium, is by amoeboid migration and mitosis of epithelial cells.

Concerning the origin of regenerating epidermis (from existing epidermis and epidermal appendages) there is general agreement, unlike the disputed origin of the fibroblasts of healing connective tissue (EDWARDS and DUNPHY, 1958). Nevertheless, there is some controversy as to whether epithelial
mitosis commences early or late, as to the relative importance of epithelial proliferation and migration in wound coverage, and concerning what cells of the epidermis are involved and whether they migrate singly or in groups (VIZIAM et al, 1964). For example, GIBSON (1964) stated that epithelial migration, and not proliferation, is the initial phenomenon concerned with the epithelialisation of epidermal wounds. He said that "cells of the exposed basal layer move amoeba-like over the raw surface. Cell migration ceases immediately the migrating edge meets another epithelial layer. Mitoses occur after cell migration". What causes wounded epithelial cells to assume amoeboid activity is unknown. WEISS (1959-60) has postulated that epithelial cells possess specific surface configurations binding them to neighbouring epithelial cells. Wounding of the epithelium causes unsatisfied lateral valencies to develop, resulting in the migration of epithelial cells towards one another, until they are surrounded by cells of their own kind.

VIZIAM et al (1964) reviewed the literature concerning some of the controversial aspects of the epithelialisation of epidermal wounds. In order to clarify some of the controversial views they made a histological study of the epithelialisation of small, shallow, incised wounds in the skin of rabbits. The
wound was inflicted by using a guarded surgical knife passing through the epidermis to the depth of the upper dermis. In some of the animals mitoses were arrested with colchicine. In others the cells which were about to divide were marked with tritiated thymidine. In addition, in order to ascertain the role of the haemopoietic system in wound repair, some of the rabbits were treated with busulfan which is a specific myeloid suppressant. There were also untreated control wounds. VIZIAM et al (1964) found that there was an initial lag period when the wound edges are retracted and the wound is filled with an exudate. The capillaries in the wound are dilated, and many intravascular and perivascular polymorphonuclear leukocytes (polymorphs) can be seen. These polymorphs, together with some lymphocytes, migrate towards the wound during the next six hours, so that after this time the wound is demarcated from the rest of the underlying dermis by a zone of polymorphs. Between 12 and 24 hours a well-defined "poly-band" has thus formed.

This poly-band provides a barrier against bacterial invasion. Superficial to the poly-band there are degenerative changes, or actual necrosis, of damaged dermal and epidermal components.
No changes, visible to the light microscope, are seen in the epidermis during the first 18 hours. (This may be compared with the statement of EDWARDS and DUNPHY (1958), that migration begins a few hours after the infliction of a small, purely epidermal, wound). However, during the next two or three hours, marked changes occurred in both treated and control animals. The thickness of the epithelial layer increases from two or three layers to six to eight layers, due to increases in the size and number of the non-keratinised cells. The thickness of the epithelium is thus rendered greatest adjacent to the wound, and diminishes with distance from the wound edge until it gradually merges with the normal epithelium. This thickening of the epidermis at and near the wound margins is due mainly to increased mitotic activity. The cellular proliferation occurs before cell migration, according to these workers, in contradistinction to the statement of GIBSON (1964) above. This conflict of statements is explained by VIZIAM et al (1964), who found that the mitoses were not evident in sections of wounds of untreated animals. On the other hand, the mitoses were evident in the animals treated with tritiated thymidine. VIZIAM et al (1964) found that migration of epithelium was not observed until 24 hours after wounding. After this time, the
epithelium moves as a wedge-shaped mass between the poly-band and the underlying intact dermis, the poly-band appearing to attract and guide the epithelium. While mitoses in the migrating epithelial cells could not be seen in sections from untreated animals, many tritiated thymidine marked cells (indicating mitosis) could be seen in the thus-treated animals. After 48 hours, the migratory epithelium is relatively thick and has a parakeratotic layer of cells, often overlying a granular layer, indicating that some differentiation of cell layers is in progress. Again, mitoses of the migratory epithelium were not observed in untreated animals, but many tritiated thymidine marked cells were seen. Where mitoses were arrested in the colchicine-treated animals, the epithelium had not progressed, at this 48 hour stage, beyond where it was at 24 hours. At 72 hours, epithelial cell migration has ceased, but proliferation continues, and the epithelium, which now covers the wound, continues to thicken in the centre of the wound. Observations of the sections of the tritiated thymidine treated rabbits indicated that mitotic activity is high in the basal layer of the wound-covering epithelium. The epithelium is fairly well differentiated, except that the stratum corneum is not present, the most superficial cells being parakeratotic. The busulfan-treated animals were unable to develop a poly-band and their
wounds remained uncovered by epithelium, because of the lack of the prerequisite intact dermal surface upon which the epithelium could migrate. The haemopoietic system would thus appear to be important for efficient epithelialisation as well as for protection against invasive bacteria.

The work of VIZIAM et al (1964), as described above, differs considerably from what has been accepted previously, before the techniques of treating with colchicine and tritiated thymidine were used. For example, reference has already been made above to the statement of GIBSON (1964) concerning whether or not migration precedes mitosis. An earlier worker, (HARTWELL, 1955) described an "extension membrane" of migrating amoeboid epithelial cells, extending from all living layers of the epithelium of the wound edges. HARTWELL (1955) also stated that "mitosis occurs secondary to cellular movement". He appreciated the importance of a "supporting base" of dermal tissue upon which the epithelial "extension membrane" could move.

Concerning the control of mitotic activity in the skin, BULLOUGH and LAURENCE (1957, 1961) in experiments on wounds in the skin of mice, found that the mitotic rate of the wounded skin increases to ten-fold that of the normal unwounded skin. They rejected the old concept of a mitogenic "wound hormone" as
a result of their work. They proposed that mitosis in undamaged skin is inhibited by the presence of local mitotic inhibitors. When a wound is sustained, the high mitotic activity which is observed is due to the local absence, or reduction in concentration, of these inhibitors. The gradient in mitotic activity (also implied by the work of VIZIAM et al (1964) referred to above) whereby the mitoses are greatest closest to the wound margins, is probably related to an inverse gradient in the concentration of the mitotic inhibitor. BULLOUGH and LAURENCE (1961) found that nearly all the mitotic cells are within one millimetre of the wound margins in purely epidermal wounds. SULLIVAN and EPSTEIN (1963) determined the mitotic response of epidermal cells following wounds on human volunteers. They found that there are transient bursts of high mitotic activity between periods of lesser mitotic response.

HOWES et al (1955) pointed out that enzymes are intimately concerned with the activation of wound healing, including cell proliferation. BULLOUGH and LAURENCE (1961) stated that mitosis in a wound is an aerobic process, and that it is also dependent on the presence of adequate supplies of suitable carbohydrate substrate such as glucose and fructose. NICOLAU and BALUS (1960) reported that the first changes observed in the
restoration of the epidermis were in the stratum lucidum. Proliferation of epithelium began in this layer several hours before it commenced in the Malphigian layer.

GILLMAN et al (1955) re-examined certain aspects of the histogenesis of the healing of cutaneous wounds. They concluded that the first tissue to show any response to injury was the epithelium, in that it is the first tissue to regenerate and bridge the incisional gap. Even when sutures are used to close a skin wound, the epithelium always inverts and shows active "invasive" properties, growing downwards into the incision. The downgrowth of the epithelium evokes a subepithelial connective tissue response, this response being evident four to six days after wounding. The response of the connective tissue seems to check the hyperplasia and "invasiveness" of the regenerating epithelium. DOUGLAS (1963) commented that this work, then unconfirmed, suggests an interesting reciprocity between the activity of regenerating epithelium and healing connective tissue. Where there is a surface defect, HOWES (1943) found that epithelisation of the surface begins after a latent period of three to six days. It proceeds at the rate of approximately 0.5 mm per day over newly formed granulation tissue. Migration of the epithelial cells, in this case,
precedes mitotic division. When the surface is covered, differentiation of the epithelium occurs.
CHAPTER 4

BONE HEALING IN ALVEOLAR SOCKET WOUNDS

Much of the work on healing of wounds of the jaw-bones has been concerned with the healing of the alveolar socket after tooth extraction. Inasmuch as the extraction of teeth and the operation of apicectomy involve surgery of the alveolar bone, it is pertinent to the present study to review some aspects of the healing of extraction wounds. It would seem not unreasonable to suppose that bone healing after these operations would be similar in many respects.

Bone healing following extraction of teeth

Investigations of alveolar socket healing in animals

The healing of extraction wounds in animals has been investigated by several workers, but there have been few histological studies of extraction wound healing in humans.

An outline of some of the experimental findings in dogs and in other animals follows.
Bone healing after extractions in dogs

EULER (1923a) published the first histologic and radiographic evaluation of the healing process following tooth extraction in dogs. He described the following stages:

1. Haemorrhage
2. Coagulation of the blood
3. Thrombosis of vessels of the alveolar socket wall
4. Organisation of the blood clot
5. Proliferation of epithelium over the socket entrance
6. Resorption of damaged tissue
7. Formation of new bone

EULER (1923a) observed the first evidence of new bone formation eight days after extraction. W. MEYER (1924) found that suturing wounds accelerated healing, and that foreign bodies and infection delayed healing. H. MEYER (1935), like EULER (1923a), found that the first evidence of new bone formation was at eight days. SCHRAM (1929) also observed the first formation of new bone eight days post-operatively following forceps removal of the upper first premolar in the dog. On the other hand, CLAFLIN (1936) found new bone formation as early as five days.
From the studies of the various workers it is possible to form an outline of some of the principal histological features observed during healing of the alveolar socket after the extraction of teeth in dogs, as described below. (No reference will be made in this outline to the essential and inter-related process of epithelialisation of the wound, bone-formation being the subject under consideration here.)

1. After the extraction of teeth, blood fills the alveolar socket(s), and the blood clots within a few minutes. The surrounding blood vessels are engorged. Some contraction of the blood clot occurs.

2. By two or three days post-operatively, fibroblasts have commenced to invade the blood clot, extending from the walls of the socket to the centre of the clot within the alveolar socket. Numerous newly-formed blood vessels extend into the clot also. The young fibroblasts and new blood vessels are intermingled, the columns of fibroblasts extending between the vessels. Osteoclasts make their appearance at the alveolar crest after three days.

3. New bone appears as areas of osteoid surrounded by osteoblasts at the end of five to eight days. The new bone
first forms at the fundus and side walls of the socket. At the same time, resorption of bone by osteoclastic activity is occurring over the entire alveolar socket wall and at the alveolar crest.

4. The organisation of the clot and the formation of new bone proceed. Two weeks after the extraction, some unorganised clot is still present in the centre of the socket. The organised columns of young connective tissue, extending from the alveolar socket wall, are being converted into the specialised connective tissue, bone.

5. After three weeks, the formation of new bone has progressed to the extent where the whole alveolar socket is almost filled with it. Only in the centre of the socket are there still remnants of the original clot.

While bone apposition proceeds in the socket, bone resorption of the socket walls is also taking place. Resorption of bone is also especially evident at the alveolar crest.

In addition to the deposition of bone within the socket itself, there is bone formation in surrounding regions also; for example, in the mandible, subperiosteally along the
lingual cortex, overlying the mandibular canal, and in adjacent marrow spaces (BOYNE and KRUGER, 1962).

6. After four weeks, the alveolar socket is filled with new bone, with little or no central clot remaining. Active bone resorption is no longer evident. The outline of the socket (lamina dura) is still visible. This outline will gradually disappear as remodelling of the bony architecture (by bone apposition and resorption) of the region occurs over the ensuing three months.

**Bone healing after extractions in other animals**

The process of healing of extraction wounds is essentially the same in other animals as in dogs.

HARRISON (1943) observed the formation of new bone after six days in sheep.

HUEBSCH et al. (1952) investigated the healing of mandibular first molar extraction wounds in rats, and observed the first appearance of new bone after five days.

The healing of mandibular first molar extraction wounds in hamsters was studied by LINN (1955, 1959), using the technique of
Alizarin Red S vital dye. He demonstrated the first appearance of new bone at seven days.

SIMPSON (1960) investigated the healing of tooth extraction wounds in macacus rhesus monkeys. Some of the wounds were examined histologically at a post-operative interval of three days. At this time, the socket was filled with blood clot, with leukocytic infiltration of the superficial part of the clot. Fibroblasts were beginning to invade the clot. No bone formation or bone resorption was seen at this stage. Other specimens were examined at the next post-operative interval of seven days. By this time, fibroblasts had invaded the blood clot. Bone apposition was apparent on parts of the wall of the alveolar socket and at the fundus of the socket. Resorption of bone was occurring at the alveolar crest, in some parts of the socket wall, and in a few adjacent marrow spaces. New bone had filled the socket after four weeks, the bone having gradually replaced the organised central core of fibrous tissue. Remodelling, by continuing apposition and resorption, of the new and old bone proceeded so that, at eight weeks, the socket outline was becoming indistinct. A rudimentary cortex had formed over the socket entrance by six weeks.
ALLING and KERR (1957) found that the trauma produced by burnishing the alveolar socket after tooth extractions in monkeys delayed healing.

**Investigations of human alveolar socket healing**

Few studies of the healing of alveolar sockets after the extraction of teeth in humans have been made.

MANGOS (1941) reviewed the work of earlier workers on animals, and described the first systematic histological study of the healing of human tooth extraction wounds. Some of his findings may be summarised as follows:

1. The blood clot forms in the socket(s).

2. Fibroblasts are seen to be proliferating from the walls of the socket at three days.

3. Organisation of the clot is most rapid in the upper (cervical) third of the socket.

4. New bone formation is first observed at 10 days. Bone resorption is also first evident at 10 days. There is little resorption of the alveolar crests, "merely a rounding-off of the sharp processes".
5. New bone has completely filled the socket after 15 weeks. (Radiopacity of the socket region approximates that of the surrounding bone after 15 weeks also.)

From his own study and that of earlier workers on dogs, MANGOS (1941) concluded that bone repair after extractions takes approximately three times as long in man as in dogs.

Another investigation was that of AMLER et al (1960), who obtained biopsies of the contents of human tooth extraction sockets, and studied the specimens histologically and histochemically. They found the first evidence of osteoid formation at the fundus and on the wall of the socket by the seventh day after extraction. The osteoid spicules were bordered by osteoblasts with much alkaline phosphatase in their cytoplasm. The central, more mature, part of the osteoid spicules showed a greater concentration of glycoprotein than the peripheral, younger part. The central part did not exhibit metachromasia, while the less mature peripheral part did so; but not to the extent of the surrounding ground substance. Using Von Kossa's method for demonstrating calcium, AMLER et al (1960) also demonstrated the presence of drop-like deposits of calcium salts in the peripheral portions of the osteoid spicules. The more
mature central portion of the osteoid spicules exhibited a much heavier mineralisation. As mineralisation of the matrix occurs, there may be a reconstitution of the collagen fibres therein (Loe, 1959). As the osteoid spicules develop they become confluent, trabeculae being formed. Amler et al (1960) found that the socket was at least two-thirds filled with new bone after 38 days. They pointed out that it was not possible to take more than one or two biopsies from each patient, and that individual differences in healing chronology could be a source of error.

Boyne (1966), following the experiments of Boyne and Kruger (1962) on the healing of tooth extraction wounds in dogs using tetracycline-induced fluorescence microscopy technique, studied the healing of human extraction wounds using this technique. He concluded that his observations conflicted with current concepts of extraction wound healing. He found that the first bone formed was not in the alveolar socket itself, but in the surrounding marrow vascular spaces. Bone formation in the socket was first observed along the wall of the socket, and not at the fundus, at nine to ten days after extraction. At two weeks, the new bone formation had extended from the lateral wall of the socket to the fundus. Boyne (1966) pointed out that earlier workers may not have had the advantage of using
chronologically-orientated intravital techniques to relate tissue responses accurately as to position and time. As he and Kruger (BOYNE and KRUGER, 1962) had found in dog extraction wounds, BOYNE (1966) demonstrated subperiosteal apposition of bone along the lingual cortex of human extraction wounds, though to a lesser degree than in dogs. This lingual subperiosteal bone formation and the formation of bone in the marrow vascular spaces are interpreted by BOYNE (1966) as being a compensatory healing response.

Other aspects of alveolar bone healing will be described in Chapter 11 on the healing of apicectomy wounds.

It is seen from the account above that wounds of the alveolar bone heal relatively quickly, compared with the healing rate of other bones. For example, SIMPSON (1960) referred to the work of BANCROFT (1914) who found that cavities made in the femur of dogs, of approximately the size of a tooth socket, required 190 days to become filled with new bone. This compares unfavourably with the 28 days noted by EULER (1923a) and CLAFLIN (1936) for the filling of a dog extraction wound with new bone. SIMPSON (1960), in making this comparison, considered that it is not unreasonable to suppose that saliva may have some influence on this considerable difference in rate of healing.
WADE and FLEMING (1961) studied the healing of extraction wounds in rats whose salivary glands had been ligated. They found that the healing was retarded compared with non-ligated controls.
CHAPTER 5

HEALING OF ORAL MUCOPERIOSTEAL FLAPS AND WOUNDS

The operation of apicectomy as usually performed, like many oral surgery operations, requires the raising of a mucoperiosteal flap.

DEDOLPH and CLARK (1958) have reviewed the literature concerning studies of mucoperiosteal flap reattachment. They cited SVOBODA (1947) who found that, after the raising and replacement of mucoperiosteal flaps in edentulous patients, the histological appearance of the operative region 22 days later was indistinguishable from the pre-operative appearance, apart from some difference in the bony architecture. They also referred to the work of BORDEN (1948), who made a histological study of the healing of oral mucoperiosteal flaps in dogs and humans.

DEDOLPH and CLARK (1958) themselves studied histologically the healing of gingival margin flaps in humans. They found that, 48 hours post-operatively, the gingival epithelium was separated from the tooth by an acute inflammatory exudate and blood clot. After one week, the gingival periodontal fibres were regenerating and rearranging themselves, and the
acute inflammatory cells had been replaced by chronic inflammatory cells. The gingival epithelial cells were proliferating. After three weeks, the epithelial attachment was restored, as was the attachment of fibres of the periodontal membrane. At this stage, there was little or no evidence of inflammation. The three week specimens were indistinguishable from unoperated control specimens.

SIMPSON (1959) examined histologically, at post-operative intervals from three days to eight weeks, the reattachment of mucoperiosteal flaps after surgical removal of teeth from macacus rhesus monkeys. He observed that there was little space between the flap and the bone after three days, and that this narrow space was filled with fibrinous exudate. There was a moderate inflammatory infiltration of the flap. In some of the sections, fibroblasts and capillaries could be seen growing out of foramina in the bone towards the flap. After one week, the fibrinous exudate under the flap had been replaced by young connective tissue. This connective tissue was continuous with that of the flap and with connective tissue in foramina of the alveolar bone. After two weeks, both resorption and apposition were taking place on the surface of the alveolar bone. After three weeks, the mucoperiosteal flap was regarded as being
firmly attached to the bone, although bone resorption and repair were continuing. SIMPSON (1959) observed that, microscopically, bone fragments were seen to have often been avulsed during the raising of the flap, or were left between the flap and the bone during the surgical procedure. He found that such fragments of bone were usually well tolerated by the tissues, unless they were near the wound surface, when they tended to evoke an inflammatory reaction. Indeed, healthy bone fragments sometimes acted as centres of ossification. (This does not imply that the surgeon should not be meticulous in carrying out the wound toilet, including the removal of obvious unattached bone fragments.) GLICKMAN et al (1947) found that, following tooth extraction in rats, retained root remnants and bone fragments tended to undergo dissolution or to be exfoliated, unless deeply embedded.

KOHLER and RAMPJORD (1960) observed satisfactory clinical and histological evidence of healing in all cases of gingival margin flaps in human subjects, whether the flap had been made in regions of gingivitis or in clinically normal gingivae. They found that the rate and degree of healing of the flap were the same in wounds associated with teeth exposed to normal or abnormal occlusal stresses, including teeth with no antagonists.
STAFFILENO et al (1960) studied the healing of split-thickness gingival flaps associated with permanent teeth in dogs. They found that the wound was completely sealed by epithelial proliferation in six days. The connective tissue response was secondary to the epithelial response. Included in this response was the initial mobilisation of osteoclasts at two days with ensuing bone resorption. Bone repair followed.

SIMPSON (1959), in his experiments on macacus monkeys, found that the reflection of a mucoperiosteal flap has some influence on resorption of bone under the flap. The normal bone resorption of the outer alveolar plate, which occurs following removal of teeth in monkeys, was delayed where a flap had not been raised. Normally, resorption of the lingual alveolar plate was seldom observed following the extraction of teeth in monkeys. However, where a lingual flap had been raised, resorption of the lingual plate did occur to a significant degree.

GLICKMAN et al (1963) found that, when the periosteum of gingival margin mucoperiosteal flaps was removed in dogs, there was delay in healing and a significant loss of height of alveolar bone in relation to the flaps. On the other hand, there are the findings of HATTON and SCHRAM (1929) on bone regeneration following surgical removal of teeth in dogs.
They observed that no bone formation from the periosteum of the mucoperiosteal flap was evident. Their concept of periosteum was that it acted as a limiting plane of tissue only in respect of bone formation, controlling by its position the extent of new bone formation. However, these findings can be reconciled with those of SIMPSON (1959) and GLICKMAN et al (1963). It appears that while "periosteum is not a truly osteogenetic tissue" (HATTON and SCHRAM, 1929) normally, its presence does delay or prevent resorption, when resorption would not normally occur. The periosteum forms a protective covering for the bone and its inner cambium layer is potentially osteogenic if the need arises. (In young developing bones, the inner layer of osteogenic cells is actively involved in osteogenesis. "Later in life the periosteum is thinner and less vascular, and the osteoblasts are represented by a single layer of flattened cells on its deep surface" (GRAY'S Anatomy, 1958).) HAM (1957) stated that the osteogenic cells of the deep layer of the periosteum participate in the formation of callus following fractures of bones. DALTON (1952) found that new bone formation occurs partly from the periosteum after injury to the rat maxilla.

McHUGH (1957) investigated the healing of gingival epithelium in dogs after gingivectomy, using the technique of
fluorescence microscopy. He found that the healing proceeded in four distinct phases. Up to the second post-operative day, cells of the deeper layers of epithelium migrate over the wound. Few or no mitoses are evident at this stage. From approximately two to nine days, cells of the basal layer of epithelium actively proliferate in a region some distance from the healing wound margin. From this region of proliferation, the newly-produced epithelial cells stream out towards the wound margin and beyond, migrating over the exposed dermis. In the region of proliferation, there is also an extension in the form of large rete pegs into the underlying connective tissue. From nine days and after, epithelial cells, after covering the granulation tissue with four or five layers of cells, undergo mitosis in the basal layer with formation of rete pegs. Coverage of the wound with epithelium is thus effected.

BUTCHER and KLINGSBERG (1963) investigated the healing of palatal mucosa wounds in the rat. They found that superficial mucosa wounds healed in the same sequence as skin wounds, with the epithelium moving over the underlying dermis. Young rats healed more rapidly than older ones.

GIBBINS (1964) found that, after the production of similar palatal mucosa wounds, migration of epithelial cells
was well under way by six hours after the injury.
CHAPTER 6

REPAIR BY CEMENTUM

Cementum is formed on teeth by appositional growth from the dental sac in early life, and from the periodontal membrane in later life (KERR, 1961). The deposition of cementum is a continuous process throughout life (ORBAN, 1957; ZANDER, 1958), upon which depends the integrity of the periodontal tissues.

Repair of cementum, like repair of bone, is also an appositional phenomenon. KRONFELD (1955) pointed out that the healing of a tooth root fracture is based on the same principles as the healing of a simple fracture of bone. Just as bony union will occur between the fragments of a fractured bone if they be in close and correct apposition, other factors being favourable, so also will union occur between the fragments of a favourably disposed immobilised tooth root fracture. If the root fragments are close together, other factors being favourable, they are united by cementum. If the fractured root fragments are further apart, cementum will form on the fractured surfaces, with fibrous union between the fragments. The fibres connecting the fragments are embedded in the cementum covering the fractured surfaces. BOULGER (1928)
described such a case of repair of root fractures in the apical region of two lower incisors. The pulps of these teeth had remained vital despite the root fractures.

Besides fractures completely across the roots of teeth, there may be minor cracks in the root or cemental tears.

FIGG (1928) described a tear in the cementum of an upper incisor of a monkey. A fragment of cementum was found in the periodontal membrane on the lingual side of the tooth. It was assumed by FIGG (1928) that the tooth had probably sustained a blow, the tooth moving in its socket and a piece of cementum tearing off. In FIGG'S (1928) case, part of the cementum, in the region of the tear, remained attached to the dentine of the body of the tooth, the outer part of the cementum constituting the detached fragment. It was found that new cementum had been deposited both on the fractured surface of the cementum still attached to the tooth, and on the surface of the detached splinter of cementum. A functional periodontal membrane type of connection existed, between the cementum splinter and the tooth on the one side, and between the cementum splinter and the alveolar bone on the other side. FIGG (1928) pointed out that, the fact that the cementum splinter fractured
off within the cementum layer and not at the cementodentinal junction, implied a strong connection between the cementum and the dentine.

BOLDEN and WEINMANN (1958) examined 71 human jaws at autopsy of patients from 2-77 years. They found that 11% of the teeth in these jaws had cementum fractures. Cellular cementum was found deposited on the fractured surfaces of the fragments and on the injured root surface.

THOMAS (1922) found that tooth roots, which had been retained in human jaws for varying lengths of time following attempted tooth extraction, had a covering of cementum over their fractured surfaces. THOMAS (1922) doubted whether cementum would form upon such retained roots if they did not contain pulpal tissue. He commented that, in teeth with vital pulps, "recementation of artificially exposed dentine seems an easy possibility". However, he continued, "it remains to be proven by experiment whether cementum will form upon the dentine of pulpless teeth".

ZEMSKY (1932) also reported the healing of fractured surfaces of buried retained tooth roots by a covering of new cementum, even the root canals being covered over.
SIPPY (1927) was able to demonstrate that cementum will form upon the dentine of pulpless teeth. He extirpated the pulp from a lower canine tooth of a dog and filled the root canal with gutta percha. Using an extra-oral approach, a bur injury was made through the bone over the apical third of the tooth, extending through bone, periodontal membrane, cementum, into the dentine of the root filled tooth. He found that new cementum formed over the injured dentine of this pulpless tooth in the same way that it formed over the dentine after similar injuries to teeth with vital pulps. SIPPY (1927) found there is osteoclastic activity at first in the injured region of the tooth and bone, osteoclasts being found in Howship's lacunae. Osteoblasts and cementoblasts then "array themselves in continuous rows around the periphery" of the injured region and lay down new bone and cementum respectively. LINGHORNE and O'CONNELL (1951) pointed out that there appears to be little, if any, difference between osteoblasts and cementoblasts in the reparative process.

Experiments on dogs (BEUBE, 1949; LINGHORNE and O'CONNELL, 1951) have shown that, following injury to the supporting structures of teeth, there is a reconstruction of cementum, periodontal membrane and alveolar bone. The reattachment of
soft tissues to the roots is effected by a deposition of new cementum upon the injured root surface. BEUBE and SILVERS (1934) had reported similar findings.

ZANDER (1957) observed cementoid on the curetted cementum surface of a section of a human tooth which had undergone periodontal curettage two months earlier.

It is interesting, in relation to the paper of THOMAS (1922) referred to earlier, that MORRIS (1960) found that new cementum was deposited on dentine after periodontal flap procedures; but that "this reaction was greatly modified by the status of the pulp, as demonstrated by the fact that little or no cementum was deposited on the dentine of teeth with root fillings".

HENRY and WEINMANN (1951) investigated the pattern of resorption and repair of human cementum from material obtained from autopsies. They found that trauma appeared to be the most important local factor tending to produce resorption of the roots of teeth. The apical third of the tooth was the most common site for resorption. Resorptions of the roots of permanent teeth were usually small, shallow and readily repaired by deposition of cementum.
Cementum has also been found to form over the resected surface of tooth roots. This is described in Chapter 11, on the healing of apicectomy wounds.

An excellent review of the reaction of cementum to injury and infection has been published by Coolidge (1931).
CHAPTER 7

TISSUE RESPONSES TO ENDODONTIC MATERIALS

Root canal therapy includes the insertion of material of a foreign-body nature into the correctly prepared root canal. It is generally agreed that usually the position of the apical end of the root filling should coincide with the position of the apical foramen, or with the apical end of the canal in the case of root resected teeth. The root filling should hermetically seal the root canal, especially at the level of the apical end of the root canal (RICKERT and DIXON, 1931; DOW and INGLE, 1955).

The root canal filling material usually consists of gutta percha or silver points sealed in the canal with a root canal sealer material.

As the apical end of a correctly executed root filling is expected to be, eventually at least, in contact with living tissue, (and it seems possible that in some cases it may be initially in contact with necrotic or non-viable tissue in the periapical region), it is obvious that a knowledge of reactions of the body tissues to root canal filling materials is important.
HUNTER (1957) studied histologically the effect on the healing of bone of gutta percha and silver points, and Rickert's root canal sealer. He implanted sterilised lengths of these materials into holes drilled into the tibia of guinea-pigs. He found that there was complete bony repair of the drilled holes after one month. A thin capsule of fibrous tissue surrounded the implants. After two months, there had been some resorption of the root canal sealer. The gutta percha and silver points were unchanged, as were their surrounding capsules of fibrous tissue. After six months, approximately 90% of the root canal sealer had been resorbed, whilst the gutta percha and silver points were as before. The small amount of remaining root canal sealer was permeated by a connective tissue framework which was replacing it. Phagocytosis of the sealer was evident. HUNTER (1957) concluded that gutta percha and silver points are quite compatible with adjacent living tissues.

BOULGER (1933) implanted gutta percha into connective tissue in rats and found that it was well tolerated.

COOLIDGE and KESEL (1956), in a review of healing after root canal treatment, referred to several authors (BLAYNEY, 1932; BOULGER, 1938; DAVIS, 1920; GROVE, 1916, 1921; HATTON, 1931;
KRONFELD, 1932; MOEN, 1928; COOLIDGE, 1928, 1931, 1932) who had reported favourable healing of teeth whose root canals had been filled with gutta percha. Reference to the papers of these workers reveals that the deposition of cementum within the apical part of the root canal is usually associated with incompletely root filled teeth, in which one to three millimetres of vital apical pulp tissue remains. DAVIS (1920) reported such cases of sealing of the apical foramen with hard tissue, but he stated that "no cases have yet been found wherein the foramen was closed, which gave a history that would indicate a diseased condition of the subdental tissues". Longitudinal histological sections of teeth, showing obliteration of the apical region of the root canal by cementum, can be misleading unless the entire series of sections is followed, when there may be found "a very thin canal filled with atrophic connective tissue extending to the foramen" (COOLIDGE, 1928), or unless transverse histological sections are prepared.

BOULGER (1933) inserted intramuscular implants of gutta percha into rats and found that the implants were surrounded by a fibrous tissue capsule which was free of inflammation. BOULGER (1933) also reported favourable human tissue responses to gutta percha root fillings. He described a case of a
human tooth incompletely root filled with gutta percha after
vital pulp extirpation, in which cementum was found to have
been deposited directly upon the apical end of the root filling.
The unfilled portion of the canal was free of inflammation.

KRONFELD (1955) claimed that, following root canal
filling of human teeth with gutta percha, a fibrous capsule
forms over the end of the root filling. Later on, new
cementum forms over the root surface and "sometimes also
directly on the gutta percha surface". This illustrates that
gutta percha is well tolerated by human connective tissue.
KRONFELD (1955) pointed out that, if the gutta percha root
filling extends slightly beyond the apical foramen, it is
surrounded by a dense, fibrous capsule. Occasional foreign-
body giant cells are found in this capsule. KRONFELD (1955)
claimed that there are no inflammatory cells in this capsule
if the tooth is not infected. Whilst this may be so with a
very slight extension of the root filling beyond the foramen,
COOLIDGE and KESEL (1956) advised that "it is seldom that a
perfect healing and normal periodontal tissue is found where
the filling material protrudes through the apical foramen".
LAWS (1962) investigated the use of calcium hydroxide as a possible root canal filling material. He cited the earlier work of ROHNER (1940), who had found that cementum-like tissue had sealed the apices of several of twenty teeth which had been root filled with Calxyl, a calcium hydroxide preparation. The experiments of LAWS (1962), using mixtures of calcium hydroxide in different vehicles, as intramuscular implants in rats and in human pulpotomies showed that calcium hydroxide is tolerated by the tissues. After insertion into muscle tissue of rats, the calcium hydroxide, after eliciting an initial acute inflammatory response, was gradually resorbed by macrophagic activity and replaced by granulation tissue. Similarly, using calcium hydroxide as a root filling material in humans, LAWS (1962) found that it is gradually resorbed and replaced by granulation tissue from the periodontal membrane. As the resorption of the calcium hydroxide takes place, a cementum-like tissue is deposited on the walls of the root canal.

RAPPAPORT et al (1964) studied the tissue reactions of the rat and the rabbit to various root canal sealer materials. Tissue culture toxicity and bacteriologic studies of the materials were also carried out. The materials tested were zinc oxide and eugenol, AH-26, Diaket, Proco-Sol radiopaque
silver cement, Proco-Sol non-staining root canal cement, Kerr's Sealer (Rickert's paste), Kloroperka, N2, N2-Medical, and Mynol root canal sealer. All ten of these materials were implanted into the subcutaneous connective tissue of the rat and the tissue responses were observed histologically. Four of the materials were selected for insertion into the conjunctival sac of the rabbit, and the tissue reactions studied. The workers found that zinc oxide and eugenol provoked the least irritating response initially. The material to elicit the least irritating tissue response finally was AH-26.

STEWART (1958) compared the tissue responses of the rabbit to three root canal sealing agents, namely, Kerr's sealer, Grossman's new sealer and Diaket. They were found to be equally well tolerated by soft tissues of the rabbit. These three materials were also tested in respect of their antimicrobial activity, tensile strength, permeability and clinical use. STEWART (1958) concluded that "Kerr sealer has stood the test of time, as far as clinical use is concerned", but that the other two materials tested were equally well accepted clinically and have additional more desirable properties.

A study of tissue responses to endodontic materials in rats was carried out by GUTTUSO (1963). He tested three root
canal filling materials, namely, AH-26, N2, and Riebler resin; four root canal sealers, namely, Diaket, Kerr's sealer, Proco-Sol root canal cement, and Tubli-Seal root canal sealer; and three intracanal drugs, namely, Micro-Cide A absorbent points and solution, N2-Medical, and PBSC. He found that all of these materials provoked severe tissue responses.

TORNECK (1961), after investigating the reactions of the tissues of the hamster to various agents used in the sterilisation of root canals, concluded that no completely non-irritating agent for this purpose had yet been introduced.

DIXON and RICKERT (1938) concluded that the histological reactions of the periapical tissues after root canal therapy are identical in humans and in dogs, and that this justifies the use of the dog as an animal suitable for experimental endodontic procedures.

KUKIDOME (1957) also found that "there is no basic difference in form between healing of the periapical tissues" associated with infected human teeth and infected dogs' teeth.

HILL (1932) found that dental granulomas are produced more easily in experimental dogs than in humans. He attributed this to number and arrangement of accessory pulp canals in the dog.
CHAPTER 8

ASPECTS OF PATHOLOGY OF THE PULPLESS TOOTH

A frequent end-result of infection of the dental pulp is the formation of a chronic alveolar abscess or of an apical granuloma. FREEMAN (1931) described the histology of the apical granuloma.

THOMA (1954) described the pathogenesis of an apical granuloma as follows:

Soon after infection of the dental pulp sets in a reaction takes place in the periodontal membrane at the outlet of the pulp canal or the perforation of the root. It presents an effort to wall off the infection of the tooth. Toxic bacteria, however, gradually migrate into the area and produce a chronic alveolar abscess; granulation tissue forms at the expense of the bone and proliferating fibroblasts derived from the periodontal membrane form a fibrous capsule which protects the peripheral marrow spaces from becoming involved. It localises the lesion to the immediate neighbourhood of the tooth.

This concept of the granuloma being a protective barrier for the surrounding tissues agrees with KRONFELD'S (1939) interpretation, cited by JOLLY and SULLIVAN (1956), that:

A granuloma is not an area in which bacteria live, but in which they are destroyed. The bacteria live and multiply in the infected root canal — opposite this zone of danger the body builds up a barrier of granulation tissue that destroys the
bacteria as they grow out of the root canal and prevents them from entering the periodontal tissues.

This protective function of the periapical granulation tissue is one of the bases of successful root canal therapy (WEINREB, 1960; WOLCH, 1956). It is a well-known clinical fact that, after proper preparation, sterilisation and filling of the root canal, the periapical pathology will often resolve. Whether this happens or not is usually determined by subsequent clinical and radiographic examinations.

The presence of bacteria in apical granulomas (granulomata) has often been established. JOLLY and SULLIVAN (1956) observed bacteria in 14 out of 57 granulomas examined bacteriologically. On the other hand, they pointed out that often the granuloma appears to be sterile despite heavy infection of the root canal. According to HARNDT (1926), when bacteria are found, they are usually in areas of degeneration in gaps or fissures of the granuloma. However, BOYLE (1934) described the first case of bacteria found within cells of a dental granuloma. The intracellular bacteria were gram positive bacilli and were demonstrated in large numbers within phagocytic cells in the central part of a solid granuloma.
Relationship between the bacteriologic status of the root canal and periapical repair

It is one of the accepted principles of endodontic practice that the root canal should be rendered sterile before it is filled. HEDMAN (1951) found that, when the pulp was infected, the periapical region of that tooth was very often infected also. He devised a technique for assessing whether residual infection remained after treatment of the root canal. He found that, after two successive negative cultures were obtained from the canal, there was no demonstrable residual periapical infection.

Seltzer et al (1964) referred to the study of Kitamura (1956) which has led some to doubt the validity of attempting to obtain a sterile root canal before it is filled. Seltzer et al (1964) found that, working on 64 teeth in three dogs, there was no significant difference in the repair of periapical lesions associated with teeth whose root canals had pre-root filling positive and negative cultures respectively. However, most operators would be reluctant to accept these limited results as a basis for abandoning a practice based on a well-founded surgical principle of attempting to avoid any contamination of the tissues.
Periapical areas of rarefaction

GARBER (1964) has reviewed the problem of interpretation of radiolucent periapical areas. All agree that, although there are sometimes pointers as to the diagnosis of a periapical lesion causing a periapical rarefaction in the radiograph, it is difficult to make a definitive diagnosis from the radiograph alone. It is necessary, as always, to take the history and clinical examination and progress into consideration. Biopsy of the periapical lesional tissue may be necessary to help in arriving at a final diagnosis in some cases.

JOLLY and SULLIVAN (1956) have observed microscopic cysts in granulomas no more than 2mm in diameter. They found that, in a series of 57 periapical lesions resembling granulomas radiographically but examined histologically also, up to 32% of these contained cysts or strands of proliferating epithelium. They stressed the need for adequate radiographic follow-up of all root filled teeth, because of this high incidence of cysts or proliferating epithelium. This is because cysts (and probably epithelium in granulomas), unlike granulomas, do not resorb. These workers also pointed out that, the fact that the majority of periapical lesions resolve
and that this may be ascertained by clinical and radiographic 
follow-up, renders routine apicectomy (or periapical curettage) 
unnecessary.

PENICK (1961) pointed out that the loss of periapical 
bone associated with periapical lesions may not always be 
replaced by new bone formation during repair after root canal 
treatment. Periapical repair in these cases may occur by the 
formation of connective tissue less specialised than bone, 
namely dense fibrous tissue. He cited LERICHE and POLICARD 
(1928) who considered that this type of healing was possible in 
bone lesions. GROSSMAN (1960) also mentioned the healing of 
periapical lesions by the formation of fibrous tissue.

WAIS (1958) after studying biopsies of 100 periapical 
lesions, confirmed that the majority are granulomas and not 
cysts, and that routine periapical surgery is unnecessary. 
At the same time, he suggested the limited indications for 
endodontic surgery.

Histological findings on teeth which have been root filled but 
not root resected

GROVE (1921) described the formation of cementum over the 
apical foramen of a root filled tooth. He considered that such
an apical seal by cementum was "Nature's method of making perfect root fillings following pulp removal". So that this might happen he advocated that the apical periodontal membrane should not be disturbed, especially between the entrance to the apical foramen and the cementodentinal junction in this region. HATTON (1931) also reported the sealing of the apical foramen of pulpless teeth by cementum. FISH (1948) called this cementum "calcified repair tissue". He stated that either this type of tissue or else fibrous scar tissue forms in relation to the apical end of the root filling. A more recent study by KUKIDOME (1957) described the manner in which the apical foramen is closed after root canal treatment.

The anatomy of pulp canals, especially in relation to the presence of accessory pulp canals in the apical third of the root, has been investigated by HESS (1925).

HATTON et al (1928) stated that infection within the small accessory canals of the apex of a tooth is extremely rare; and that the presence of these accessory canals does not interfere with periapical bone regeneration. HATTON (1922) reported his observations that "calcific plugs", resembling cementum, are to be found filling accessory pulp foramina and incompletely filled root canals.
KRONFELD (1955) stated that earlier workers had shown that formation of cementum over the apex of pulpless teeth occurs only in the absence of infection. COOLIDGE and KESEL (1956) have written a most informative review of the literature on repair after root canal treatment. One of the interesting cases they described shows deposition of cementum upon the wall of the apical part of a root canal which had been incompletely filled with a gutta percha root filling, after the apical part of the vital pulp had been left in situ, unextirpated. A strand of pulp tissue was still present, surrounded by the cementum deposited on the apical non-filled part of the canal wall. A very interesting finding in this case was that in one region, cementoid had been deposited directly upon part of gutta percha root filling. These authors also cited a case of KRONFELD (1932), who reported the deposition of cementum directly upon, and completely over, the apical end of a gutta percha root filling in a molar (COOLIDGE and KESEL, 1956). Other references to the sealing of the apical foramen of root filled teeth by deposition of cementum have already been referred to in Chapter 7, on tissue responses to endodontic materials.
HILL (1931) described his histological findings on an upper second premolar which had been root filled and had undergone apical curettage a little over four years earlier. He described "a thick deposition of new cementum" over "all the apical end", (although his paper does not have a figure of a section cut in a plane showing the apical end of the gutta percha root filling). The periodontal fibres in this case were described as running parallel, or occasionally obliquely, to the surface of the root in the region of the apical curettage, and not as being embedded in the root. He concluded that there is, nevertheless, a firm attachment between the new periodontal membrane and the root surface, because the periodontal fibres remained attached to the apex during the extraction of the tooth. There was no evidence of inflammatory infiltration in the periodontal membrane.

WEAVER (1947) reported repair of curetted apices of teeth with a covering of cementum. He considered that the operation of apicectomy is objectionable, because of the resultant shortening of the root, which reduced the resistance of the tooth to functional stresses. He thought that the opening up of fresh dentine tubules was another undesirable effect of apicectomy. KNECHTLE (1963) considered that the cutting of dentine tubules
during apicectomy creates a pathway between the resection surface and the root canal, and that bacterial exchange could take place along this pathway.
CHAPTER 9

BACKGROUND AND HISTORY OF THE APICECTOMY OPERATION

FARRAR (1884) reported that he had successfully practised for nine years the "radical and heroic treatment of alveolar abscess by amputation of roots of teeth". SOMMER et al (1966) claim that DESIRABODE (1843) performed the first apicectomy operation, and that MAGITOT (1866) performed a similar operation.

FARRAR (1884) pointed out that although periapical abscesses may be cured by the more conservative "syringe process", there are occasions when there are "sepulchres of sacs long since wasted away that are so large and ragged, and perhaps accompanied with necrosis, and with degenerated roots projecting into them like stalactites in a cave, that in order to (sic) a permanent cure more or less of the root must be removed". He practised the amputation of roots to an extent varying "from a small extremity about the apex to the entire root, depending on the extent of its degeneration". The amputation operation included filling the root canal to make it "securely closed and rendered harmless". The root filling material consisted of such materials as amalgam, gold foil, or a gold screw inserted before or
after the operation. FARRAR (1884) recognised the need to avoid irritation of the periapical tissues, in that he recommended the polishing of the apical end of the gold screw.

Another early exponent of the apicectomy operation was RHEIN (1890). He mentioned that the current text-books were "singularly silent about this superior procedure". From his experience of the operation RHEIN (1890) concluded that "whenever death of any portion of a tooth has taken place, the simplest cure is to amputate the necrosed portion of the root and the tissue will close firmly about the remaining healthy portion, which will suffice to support the tooth".

DIVINELLE (1880), in the Proceedings of the New York Odontological Society, described an interesting operation he performed in 1856 on the palatal root of an upper molar, this root being "entirely dead — a thorn in the flesh". He amputated and "treated" the palatal root, and filled the root canals of the two buccal roots. Whilst DIVINELLE'S (1880) operation was not an apicectomy, it casts some light on this operation because of the similarity between the two procedures, and because of the possible practice of apicectomy ("long advocated by the few" RHEIN, 1890) even before FARRAR (1884) described his use of it over the previous nine years.
SHAPIRO (1951) cited BROPHY (1880) as having described the procedure in 1880.

An unusual technique for root resection was advocated by BEACH (1902) who recommended extraction of straight-rooted teeth, amputation of the diseased part of the root, filling of the root canal and replantation of the thus-treated tooth into its socket.

MARTIN (1889) of Lyons, France, in 1881, in a communication to the Medical Congress of Algiers proposed the use of a trephane (trephine) to remove the "diseased radicular extremity". PONT (1900) claimed that MARTIN (1889) later abandoned the use of the trephine because it "gave him bad results". Since these early times, various other techniques have been used to perform the apicectomy operation, and the operation "has come to be accepted for many years as a good operative procedure" where indicated (SOMMER, 1946).

SOMMER (1946) cited OTTESEN (1942) of Oslo as having demonstrated the "open view method" of root resection at the Dental School, University of Minnesota. This is the type of operation mostly used today. After the apicectomy and retrograde amalgam root-filling, OTTESEN (1942) sutured the
mucoperiosteal flap in position for primary closure of the wound. SOMMER (1946) also referred to HARTZELL (1911) who advocated the use of the trephine so that "drainage should be maintained until the wound had healed".

SOMMER (1946) stressed the importance of eliminating sources of irritation from the root canal, and preventing their return by hermetically sealing the sterile canal with "agents of known tissue tolerance". Moreover, he emphasised that "the erroneous belief that root resection is sufficient to eliminate periapical pathology permanently is far from correct. Surgical measures alone, regardless of how skilfully performed, will not restore the periapical tissue to a normal physiologic status".

COOK (1929) had demonstrated that a poorly-filled root, when resected, may harbour bacteria which may maintain infection or permit re-infection of the periapical tissues.
CHAPTER 10

RATIONALE OF AND INDICATIONS FOR THE APICECTOMY OPERATION

Rationale of Apicectomy

The rationale of the apicectomy (root resection, root amputation) operation on a tooth was suggested by ROSS (1952). He pointed out that accessory root canals in the apical third of the root of a tooth are a 'perfect nidus for bacterial growth', the organisms living on the tissue-fluid which seeps into these canals. Leukocytes, which could combat the bacteria if they were able to gain access to them, are found localised around the openings to these accessory canals on the root surface. "By resecting the apical third of the root, the area in which bacteria can live and multiply is removed and eventually the space is filled in with new bone".

ROSS (1952) also pointed out that the cellular cementum over the apical third of human tooth roots is permeable to dyes (and, therefore, presumably to infective material); whereas the cervical two-thirds of human tooth roots is covered by acellular cementum, which is impermeable to dyes. Therefore, by performing an apicectomy, the part of the tooth which is more likely to harbour infection is removed.
STONES (1934) and ROSS (1933) have carried out permeability studies on the teeth of dogs. It was shown that, in adult dogs, the acellular cementum is completely impermeable to dyes. In young adult dogs, the apical cellular cementum is completely permeable to dyes. In older adult dogs, the apical cellular cementum is permeable in its outer layers only. The permeability of the outer part of the apical cellular cementum in dogs, and the presence of accessory root canals in the apical third of the root, could allow the harbouring of infection in the same way as ROSS (1952) suggested could occur in human teeth with infected pulps.

Concerning the thesis of ROSS (1952) that the accessory root canals may form a "perfect nidus for bacterial growth", there is, on the other hand, the opinion of HATTON et al (1928) referred to in Chapter 8, that infection of these accessory canals is extremely rare.

Besides the reasons given for performing an apicectomy in order to eliminate potential or actual nidi of infection, another reason for carrying out the operation is to ensure the removal of non-resolving periapical pathology such as radicular cysts. The problem of whether a particular periapical lesion
is an apical granuloma or cyst (or other pathology), and the planning of treatment accordingly, have been mentioned in Chapter 8.

Indications for Apicectomy

Various authors such as WAIS (1958), GROSSMAN (1960) and SOMMER et al (1966), have given indications for performing the apicectomy operation. There may be certain contraindications, such as health reasons, anatomical or clinical reasons (LUEBKE et al, 1964).

JOLLY and SULLIVAN (1956) have listed the limited indications for apicectomy, which summarise the acceptable indications as given by other workers, as follows:

1. When it is impossible to ream adequately to the apex but when cleansing and filling to the level of apicectomy is possible.

2. When a root canal is grossly overfilled with a non-resorbable paste.

3. When upon re-examination a periapical area is deemed to be enlarging.

4. When it is impossible to sterilise the root canals because of re-infection from the periapical area.

5. When a perforation has occurred in the apical third of the root or when a small fragment of a broken instrument is lodged in the apical third of the canal.
CHAPTER 11

HEALING OF APICECTOMY WOUNDS

The healing of apicectomy wounds has been observed in humans clinically, radiographically and, to some extent, histologically. There have also been experimental investigations in animals.

Observations of patients and human material

Clinical and radiographic observations in humans

The healing of apicectomy wounds in patients has been observed by many dentists on the grounds of clinical and radiographic follow-up.

In the British Dental Journal in 1923 there are notes advising that the operation of apicectomy would seem "to have a legitimate place among the operations of dentistry. One of the criticisms has been that a dead space is left and that re-infection may occur. It has been claimed that regeneration of bone can occur and that this has been proved by radiographic examination taken before and at intervals after the operation". In support of this demonstration of radiographic
evidence of new bone formation there is described a case of a patient of LACRONIQUE (1923) recorded in the Revue de Stomatologie. LACRONIQUE (1923) claimed the radiographic demonstration of new bone formation within the apicectomy wound three months post-operatively. A radiograph taken eight months after the operation showed "well-formed bone of normal density completely filling the cavity and in contact with the end of the root".

Other operators since then have recorded radiographic evidence of healing of apicectomy wounds; and it has long been an accepted and recommended procedure to follow up apicectomy (and other endodontic treatment) by periodic clinical and radiographic examinations.

SMITH (1952) recommended that a radiograph be taken twelve months after the operation to show the regeneration of alveolar bone. MOEN (1940), with experience of 250 root resections over 15 years, advocated radiographic follow-up at six-monthly intervals for two years after the resection. He reported that, in some teenage patients, there is almost complete filling-in of the resection crypt after six months.
On clinical and radiographic grounds BLUM (1932) obtained 95-98% success for apicectomy operations, whilst PHILLIPS and MAXNEN (1941) claimed 99% success of 600 cases.

SHAPIRO (1951) described a case of an upper lateral incisor which had undergone apicectomy. The tooth had a very large area of periapical (or, rather, periradicular) pathology involving about two-thirds of the root. Part of the root projecting into the lesional tissue was amputated. After removal of the periapical tissue, with no filing or smoothing of the root, the root canal was filled with gutta percha and eucap-percha. Follow-up radiographs over the years demonstrated the formation of a lamina dura mesially and distally, with a periodontal membrane space of normal width in these regions. The root was observed to have increased in length and to have become rounded, presumably by the deposition of secondary cementum. After three years, bone appeared to fill the cavity almost completely. Only a small area of apical rarefaction remained, which was assumed to represent an area of fibrous scar tissue (an assumption, which SHAPIRO (1951) pointed out, could only be proved by histological examination). Some of the apparent "rounding-off" of the root could be an illusion due to radiographic technique.
SHAPIRO (1951) claimed that "a complete rounding off of the root, an elongation of the root with secondary cementum will occur only in the absence of inflammation. If bacteria were present in the region, the regeneration of bone, cementum, and periodontal fibres could not take place". SPRINGER and BERLAD (1955) also reported good osteogenesis following root resection of a tooth with a large periapical lesion.

DAWKINS (1958a) studied the radiographic healing of root resection crypts in eight patients, using a standardised technique for taking the radiographs. He observed the beginning of mineralisation after eight weeks post-operatively, and the beginning of trabeculation of bone in eight to twenty weeks (usually about twelve weeks). There was complete filling-in by bone after 24-32 weeks.

DALITZ (1964) compared the results of DAWKINS (1958a) with the results of his own study on the radiographic evidence of bone healing after tooth extraction. He concluded that "generally it may be said that a socket becomes obliterated radiographically 20-30 weeks after the extraction of a tooth". Therefore, it may be concluded that the rate of healing of bone after tooth extraction and apicectomy respectively is
approximately the same; but, as DALITZ (1964) pointed out, there may be considerable variations in the rate of alveolar bone healing.

FISHBEIN (1943) made a clinical and radiographic study of the use of synthetic bone paste in root resection crypts. Control patients had root resections without the insertion of bone paste. FISHBEIN (1943) concluded that bone formation in the resection crypt started sooner in those treated with bone paste than in the controls. He found that age was not a factor in the healing of apicectomy wounds. The apparent radiographic size of the crypt did not affect the time of commencement of mineralisation of the crypt. (BOYNE and LYON (1960) found that larger alveolar defects especially, exhibited more advanced osteogenesis following implantation of heterogenous and homogenous osseous implants after apical curettage.)

Histological evidence of healing of apicectomy wounds in humans

It appears that there have been few, if any, histological investigations of the healing of human apicectomy wounds. What histological evidence there is of the healing of human apicectomy wounds appears to have been obtained solely from
histological observations of extracted teeth which had undergone apicectomy at some earlier time.

KRONFELD (1928) described his histological findings on a lower second premolar, on which a root amputation had been performed approximately a year earlier. On examining sections of the tooth, he found that new cementum had formed on the resection surface, but that this deposit of new cementum was upon the old cementum only. That is, no new cementum was found on the resected surface of dentine. It was assumed that the reason for this was that the dentine was infected, and that this cross-section of exposed infected dentine could promote further periapical infection.

Reference was made in Chapter 6 to the doubts of THOMAS (1922) concerning whether cementum would form on dentine which is not "vital", and he remarked that he had been "hopeless when expectation of cementum deposit upon the bared dentine of resected roots is mentioned", although he admitted that examination of a sufficient number of resected teeth could disprove this. THOMAS (1922) concluded, as mentioned previously, that "it remains to be proven by experiment whether cementum will form upon the dentine of pulpless teeth".
That cementum could form upon the "bared dentine" of pulpless teeth was proved by the experiments of SIPPY (1927) on dogs, referred to in Chapter 6. COOLIDGE and KESEL (1956) referred to a report by BLAYNEY and WACH (1924) of partial healing of resected roots.

COOLIDGE (1930) described the formation of new cementum over the dentine surface exposed by apicectomy in a human. His case of "complete repair" was that of a chronically infected upper right first premolar which had had a root filling inserted in 1914, with apicectomy 33 days after root filling. The tooth was extracted 14 years later, and examined histologically. (There was evidence of healing radiographically.) Histological examination (figure 1) of the extracted premolar revealed that new cementum had covered the entire root resection surface, extending up to (but not over) the border of the gutta percha root filling. The cementum had formed over the exposed surface of dentine without prior resorption of dentine; in some places; on other parts of the resection surface, resorption of dentine had occurred first, the resorbed area then being covered by cementum. Opposite the apical end of the gutta percha root filling (the only part of the resected apex not covered by cementum), there was a
Fig. 1. New cementum, C', deposited on root resection surface (COOLIDGE, 1930).
dense band of fibrous connective tissue, forming a capsule over the exposed end of the gutta percha. This capsule was free of inflammatory cells. There were also no inflammatory cells to be found in the tissue surrounding other parts of the resection surface. COOLIDGE (1930) concluded that, provided the root had been properly treated and filled before apicectomy, this operation makes it possible to eliminate a chronic periapical infection. As mentioned earlier, it is possible to eliminate chronic periapical infection by proper root treatment, without resort to apicectomy, which should be performed only when indicated (JOLLY and SULLIVAN, 1956).

Since the report of COOLIDGE (1930), others have reported similar findings (BLUM, 1932). AISENBERG (1931) described his histological findings on a tooth resected 4 years earlier. New cementum had formed over part of the resected dentine surface, the partial covering of cementum extending from the periphery of the resection surface towards, but not up to, the border of the root filling. Periodontal fibres were seen to be embedded in the new cementum over the resection surface. Opposite the gutta percha root filling, a band of fibrous tissue had formed as described by COOLIDGE (1930). Round-cell infiltration of the band (capsule) was
evident in the specimen examined by Aisenberg (1931).

Moen (1940) reported on six teeth which had undergone apicectomy. The teeth were from patients aged 24-65. He found it interesting that there was no round cell infiltration of the apical periodontal membrane in any case. A significant finding was that "in no case did we find the cementum building over the apical foramen. It seemed to build up to the foramen, but did not bridge it".

Histological examination of root resected teeth was also reported by Herbert (1941, 1943). He reported (1943) on two teeth, an upper lateral incisor and an upper central incisor. The lateral incisor was examined histologically approximately seven years after root resection. Sections cut near the periphery of the resection surface showed the deposition of new cementum over the old cementum and over the whole of the dentine resection surface. Resorption of dentine, before deposition of cementum, was seen in one part of the section. Sections cut in a plane closer to the centre of the tooth (that is, closer to the root filling) showed little deposition of cementum over dentine. Opposite the root filling of "osteoc and thymol", (upon which no deposition of cementum had occurred), there was some round-cell infiltra-
tion. Sections of the other tooth, the central incisor, showed deposition of new cementum over most of the cut surface of the root, with healthy connective tissue (new periodontal fibres) attached to it. Apposition of cementum occurred over the dentine both with and without prior resorption of dentine. As AISNEBERG (1931) had found, HERBERT (1943) found fibrous tissue with a slight amount of round-cell infiltration opposite the osteo and thymol root filling. HERBERT (1943) commented that "such inflammatory reaction as remains in connection with these teeth is strictly limited to the tissues immediately adjacent to the apical end of the root canal filling and is not found in association with the cut surface of the dentine, which shows evidence of repair over most of its surface". He suggested, as ROSS (1952) implied, that as long as the porous apical third of an infected tooth is removed, any danger of future infection is more likely to come from an imperfect, hermetic seal of the root canal than from retained infected dentine.

JOLLY and SULLIVAN (1956) have pointed out that, from their observations, any bacteria present in the dentinal tubules (which is uncommon) are in close proximity to the root canal; so that enlargement of the canal by thorough
reaming and filing of the canal would be likely to remove these bacteria. If this is so, and if the removed apical third of the root contains the other possible sites for harbouring of bacteria, then the tooth itself is unlikely to be a source of infection following proper root treatment and root resection.

HERBERT (1937) described how he collected material under sterile conditions from the periapical region of teeth which had undergone root resection at an earlier time and were judged to be healed. The material was collected from 12 cases and examined bacteriologically. Only two of these cases produced any bacterial growth and these were air-borne contaminants.

**Observations on the healing of apicectomy wounds in experimental animals**

The first histological study of the healing of apicectomy wounds appears to have been made by BAUER (1922), who published his findings on root resections performed on cats. His observations were of material obtained after six apicectomies in three cats. The root resected teeth had their pulps extirpated, and the pulp canals were enlarged with acid and filled with gutta percha points. Two of the wounds were left open, while the other four were sutured. The animals were
sacrificed at intervals of one to six months post-operatively, and the specimens of resected teeth and surrounding tissues examined histologically.

BAUER'S (1922) paper has figures, which are drawings of histological sections. They are reproduced in figures 2 and 3. BAUER'S (1922) figure 1 is a drawing of a section from a three month post-operative specimen. The section has been cut in a longitudinal plane which passes through a region between the periphery of the root and the filled root canal. What BAUER (1922) called bone-cement ("knockenzement"), that is cementum, covers the entire resection in this region, "like a cast over-layer". BAUER (1922) stressed that this new cementum does not "melt" in with the dentine, which BAUER (1922) said had been erroneously suggested by another worker in connection with the formation of cementum over fractured retained root remnants.

BAUER'S (1922) figure 1 also shows some pieces of dentine "entirely wrapped up in bone-cement".

His figure 2 and figure 3 (an enlargement of part of his figure 2) are longitudinal sections from a similar region. A space (Sp) is apparent in his figure 2 which appears to be within the layer of new cementum over the resected root end.
W. Bauer. Histologische Befunde an Zähnen nach Wurzelspitzenamputation

Fig. 1.

Fig. 2. Drawings from histological observations after root resections in cats (BAUER, 1922).

Fig. 3.
Fig. 3. Drawings from histological observations after root resections in cats (BAUER, 1922).
This space is an artefact. Several detached dentine fragments (DG) are covered with cementum, and are woven together by cementum also.

BAUER'S (1922) figure 4, with its partial enlargement in figure 5, are longitudinal sections passing through the apical end of the gutta percha root filling. These are drawings of a section of a two month specimen. New cementum is seen to have formed upon the resected root surface in this region, to the extent of almost covering the resection surface. On the "left" side of the pulp canal, the new cementum extends over about three-quarters of the distance between the periphery of the root and the filled root canal. It is interesting to observe that there is an area of increased density opposite the apical end of the space where the gutta percha root filling (lost in histological preparation) was. The present author interprets this (in the absence of reference to it by BAUER (1922) in his paper) to be an accumulation of inflammatory cells or a fibrous capsule on the basis of comparison with his own work and COOLIDGE'S (1930) report. In these figures, on the right side, there is to be seen a fragment of detached dentine, surrounded by new cementum, and attached to the root of the tooth by a bridge of cementum also. To the right of
this relatively large piece of cementum-covered dentine is a fragment of old cementum, which is covered like the dentine fragment(s) with new cementum. The split in the dentine of the root, on the right side of the drawing, and the region where the large dentine fragment was originally connected, are also covered by new cementum.

BAUER (1922) concluded from his histological observations upon the six root resections on three cats, that at first there is a bony ankylosis between the surrounding alveolar bone and the "bone-cement" covering the resection surface. He described this union as being a fixed bridge ("fixe Brucke"). He postulated, on the basis of his interpretations of findings up to six months post-operatively, that ankylosis between the alveolar bone and the resected root end "dissolves" under the influence of functional irritation. He wrote

(The ingrown dentine splinters are resorbed, the bony connection between the root stump and the alveolar bone disappears, and there remains a root stump covered with a nearly uniformly-wide layer of bone-cement. This root stump is separated from the alveolar bone by a layer of connective tissue which slowly differentiates into a normal periodontal membrane.)

However, BAUER'S (1922) own figures of two and three month specimens show that there is no ankylosis in general between the root and the alveolar bone. While BAUER (1922) implied that other sections (of specimens later than three months), not shown in his paper, may have shown the absence of dentine splinters, the present author cannot agree with his implication, on the basis of information given in his paper, that there has been entire resorption of dentine splinters merely because they were not seen in these later sections. In other words, the absence of these dentine splinters in later specimens does not necessarily mean that they were present at an earlier time post-operatively and resorbed later. It may be that, during the operations on the cats from which the later specimens were obtained, there was a more thorough debridement of the operative region so that there were fewer, if any, dentine splinters retained in the wound. The same may be said of the implied extension of his argument for the supposed presence (for which no evidence is presented) of a bony ankylosis between the root end and the alveolar bone, and
its supposed disappearance later due to resorption.

Altogether, the evidence presented by BAUER (1922) for a connecting bridge of bone between the surrounding alveolar bone and the resected root is entirely unconvincing. However, it is agreed that a periodontal membrane might form between the resected root end and the alveolar bone. But the theory that a bridge of bone existed prior to the formation of such a periodontal membrane is unacceptable. BAUER (1922) stressed the importance of "functional influence" on the development of this periodontal membrane. This seems reasonable in itself, but there is no evidence to support this theory that a supposed bony ankylosis is "lost after root resection due to functional irritation". It would seem more acceptable to postulate that a layer of non-differentiated, non-calcified, connective tissue forms after the operation between the resected root end and the new bone which forms in the resection crypt; and that this connective tissue differentiates, probably under functional influence, to form a periodontal membrane. The functional connection between the alveolar bone and the resection surface might be through the attachment of Sharpey's fibres inserted into the bone and cementum respectively (GUSTAFSON and PERSSON, 1960; ORBAN,
1957). The presence of an intermediate plexus in the periodontal membrane, wherein new functional links are formed, has been described by ORBAN (1957) and by SICHER (1959). On the other hand, SELVIG (1965) made a study of the attachment of periodontal fibres to human cementum and alveolar bone by means of optical microscopy, microangiography and electron microscopy. He considered that there could be a rebuilding of the collagen fibres of the periodontal membrane without presupposing the presence of a distinctive, histologically recognisable intermediate plexus.

Although BAUER'S (1922) concept of a rigid calcified connection between the root end and the alveolar bone, which undergoes resorption due to function, is untenable, there would no doubt be a remodelling of the newly-formed alveolar bone; and this remodelling would be influenced by function.

Concerning the apposition of cementum over the resected root surface, BAUER (1922) reported that, in his cases, there had been very little resorption of the resection surface prior to the deposition of cementum. He acknowledged the possibility of cementum apposition with or without resorption, however, as reported by others in connection with the apposition of cementum on root fractures. He related the very small amount
of prior resorption in his study to the ideal conditions under which his experimental operations were "correctly performed" in the absence of infection. In one of his cats, when the wound was left unsutured, there was evidence of epithelial proliferation across the resected surface with cyst formation. In this animal there was very little formation of new bone.

Concerning points of technique, BAUER (1922) advocated that the root canal be filled, preferably with ivory points, before the apicectomy operation. He recommended the use of a fine fissure bur, not a chisel, for the root resection. He pointed out that splinters of tooth substance and bone retained in the wound would retard the formation of new bone, and advised their removal by irrigation with saline. Following the debridement of the wound, the margins of the flaps should be carefully sutured in correct apposition.

BAUER (1925) also published in 1925 a paper on the influence of function on the healing of 10 apicectomy wounds in two cats and three dogs in which he reiterated much of the philosophy of his 1922 paper, and this later paper did not significantly add to his earlier findings or alter his interpretation of them. He briefly dismissed the criticism by EULER (1923b) of his 1922 paper, referred to below, on the
grounds that most of EULER'S (1923b) root resection wounds on
dogs did not heal normally, and because he considered that the
term of the latter's study did not extend over a long enough
period. BAUER (1925) claimed that the apical end of the root
canal of a resected tooth filled with an ivory point was
covered with cementum.

EULER'S (1923b) study was published soon after BAUER'S
(1922) paper. As mentioned above, the experimental subjects
in his study of healing of root resection wounds were dogs.
Seven canine teeth were operated upon. Five of these root
resextions were performed by an extra-oral approach, two by an
intra-oral approach. In one of the operations, the pulp of
the resected tooth was not extirpated. In this particular
operation, after the root resection via an extra-oral approach
had been carried out with a chisel, the resected apex was left
in the tissues. After 12 weeks the animal was killed, and
the operated region histologically examined. The resected
apex, which had been left in the tissues, was covered with a
layer of bone. The pulp of the tooth had undergone meta-
plasia, having been converted into bone. Cementum was found
lining the wall of the pulp canal. The resection surface was
also covered with a thin layer of new cementum. Here and
there, the section exhibited lifting of the layer of cementum 
on the resection surface, this probably being an artefact. 
New bone had filled the resection crypt. Between the new 
bone filling the bony cavity and the resection surface there 
was a layer of connective tissue which was hardly distinguish-
able morphologically from a periodontal membrane.

EULER'S (1923b) other root resections on dogs all 
entailed the insertion of a root filling. The main theme of 
his paper was the influence of different root filling materials 
upon the healing of root resection wounds. He considered that 
current controversies concerning whether the root canal should 
be filled via the canal or using a retrograde method (depending 
on whether the canal was considered "aseptic" or "septic" 
respectively) are not the main factors which influence the 
success or otherwise of the operation. Rather, he stressed 
that a more important consideration was the reaction of the 
tissues to the various root filling materials. He thought 
that too little attention had been given to this. He 
therefore experimented with gutta percha, phosphate cement, 
Walkhoff's iodoform paste, and gold amalgam as root filling 
materials in root resected teeth. A total of only six 
resections was carried out. The operated animals were
sacrificed from $5\frac{1}{2}$ to 12 weeks post-operatively. Four of the resections were carried out from an extra-oral approach, two from an intra-oral approach. In no case was there formation of cement over the resection surface. On the whole, the healing was poor. Cyst formation occurred in the tooth filled with gutta percha which was from a 12 week specimen. Even after this time, only a narrow strip of osteoid had formed around the periphery of the bony cavity. The cyst had arisen from epithelium which proliferated across the root surface from the skin epithelium. EULER (1923b) admitted that the presence of the epithelium, and probably not the gutta percha root filling, was the cause of the poor osteogenesis. Two of the other operated areas developed abscesses with sinus formation. No definite general conclusions can therefore be made from this study of EULER (1923b) when they are read in conjunction with the work of BAUER (1922), because of the poor healing, the short term of the experimental study and the limited amount of experimental material. However, one very interesting aspect of the study was the nature of the healing of the wound, where the pulp had not been removed.

Perhaps because of the uniformly poor results of the healing of his dogs' resection wounds when there was a root
filling present (the only successful one being, as mentioned above, when the pulp was retained) EULER (1923b) was critical of the work of BAUER (1922). He argued that drawings in BAUER'S (1922) paper did not correspond with the textual description of them. This criticism appears to the present author to be unjustified in general. However, some of EULER'S (1923b) criticism of BAUER'S (1922) paper could only be completely evaluated after observation of the original histological sections of BAUER (1922).
SUMMARY AND CONCLUSIONS OF REVIEW OF LITERATURE

1. The operation of apicectomy (root resection) of a tooth is sometimes indicated.

2. Correctly executed root resection operations in humans are often successful, judged on clinical and radiographic criteria.

3. There is radiographic evidence of new bone formation in root resection crypts in humans.

4. Radiographic studies in humans indicate that the rate of new bone formation in root resection crypts approximates that in alveolar sockets after tooth extraction.

5. There is histological evidence that the healing of bone (and mucosa) after tooth extraction in humans is slower than in dogs.

6. No histological evidence has been published concerning either the earliest time of formation of new bone in root resection crypts, or the earliest time of deposition of cementum on root resection surfaces, in humans or in animals.
7. New bone formation in a root resection crypt has filled the crypt by eight weeks post-operatively in a cat. Deposition of new cementum on the root resection surface has occurred in the same specimen. Earlier specimens showing new bone and cementum formation have not been described, but the extent of new bone formation and cementum deposition in this animal after apicectomy indicate that the formation of these tissues commenced well before eight weeks post-operatively.

8. Although knowledge of the chronology of new bone formation and cementum deposition after apicectomy is uncertain, there is ample evidence that these processes do occur in humans and in the experimental animals studied, namely, cats and dogs.

9. Cementum deposition on the resected surface of a gutta percha root filling in a root resected tooth has never been demonstrated histologically either in humans or in animals.

10. There is evidence that the apical foramen of non-resected teeth with gutta-percha root fillings may be sealed by deposition of cementum, especially if the
root canal is not quite completely filled and if the pulp were vital prior to this incomplete extirpation. Cementum deposition does not occur on gutta percha root fillings which protrude beyond the apical foramen.

11. Gutta percha has been shown to be relatively well tolerated by human and animal tissues.

12. Many root sealing agents are irritating to the tissues of experimental animals.
THE PRESENT INVESTIGATION OF HEALING OF APICECTOMY WOUNDS IN DOGS

INTRODUCTION

From the review of the literature, it is clear that the operation of apicectomy, when indicated and properly carried out, serves a useful purpose in conserving many teeth which would otherwise be unsavable.

It is also evident, from the information derived from clinical and experimental sources, that there is knowledge of the histological state of the resected tooth and surrounding tissues in the later, but not in the earlier, stages of healing after apicectomy. For example, it is known that osteogenesis may occur within the resection crypt, and that the resection surface of the tooth may be covered partially or completely by new cementum. However, this knowledge is incomplete in that there is little direct evidence concerning the histological changes occurring in the wound between the time of operation and the onset of osteogenesis and cementogenesis.

It is the purpose of the present investigation to determine what histological changes, observable by light microscopy, take place during the healing of apicectomy wounds in dogs.
SUMMARY

A histological study of healing of apicectomy wounds has been carried out. Post-mortem specimens were obtained after the following post-operative survival periods of:

2 hours, 8 hours; 24 hours; 48 hours,
7 days, 2 weeks, 4 weeks, 8 weeks,
18 weeks, 26 weeks, 52 weeks.

It has been confirmed that osteogenesis occurs within the root resection (apicectomy) crypt, commencing before seven days post-operatively. New bone fills the resection crypt by four weeks. The manner and chronology of osteogenesis following apicectomy in dogs resemble that seen in alveolar sockets after tooth extraction in dogs.

Cementogenesis occurs on the resected root surface, certainly by four weeks post-operatively, with suggestion of early cementogenesis after two weeks. The time of earliest cementogenesis upon root resection surfaces has not been previously reported.

The early phases of healing of apicectomy wounds, not previously reported, are described. These conform to the pattern of wound healing in general, and in particular
resemble the changes seen in the alveolar socket after tooth extraction. The significance of the histological findings is discussed.
CHAPTER 12

MATERIALS AND METHODS

Selection of experimental animal

The dog was selected as a suitable experimental animal for the investigation. It is readily and cheaply obtainable, easily handled and has suitable teeth and supporting structures.

The dental formula of the adult dog is:

I 3/3  C 1/1  P 4/4  M 2/3 x 2 = 20/22 = 42

(SCOTT and SYMONS, 1961)

Descriptions of the anatomy of the dentition of the dog have been made by various authors (TOMES, 1923; WIDDOWSON, 1946; SCOTT and SYMONS, 1961). These will not be repeated here, but it is pertinent to describe the dog's dentition as related to selection of a suitable experimental tooth.

Selection of experimental tooth

An experimental tooth was sought having the following characteristics — single-rooted; with a suitably shaped and sized pulp canal, with favourable anatomical relationships.
and with good access for operating.

The pulp canals of the incisor teeth were not considered to be ideal, because they are not round in transverse section, being compressed mesio-distally, as is evident from a comparison of figures 4 and 5. The labio-palatal inclination of the upper incisor teeth, especially the first and second incisors, is such that the apices are in a rather inaccessible position under the anterior aspect of the floor of the nose. There are also low, broad muscle attachments in the upper incisor region which predispose to excessive bleeding, with resultant unsatisfactory visibility despite the use of good suction, when incisions are made in this region.

The canine teeth were considered to be unsuitable for the investigation, because the pulp canals are extremely large.

The second, third and fourth premolar teeth and the molar teeth have more than one root and some of them do not allow suitable operative access.

After eliminating the incisor teeth, canine teeth, second, third and fourth premolar teeth and the molar teeth, there remained the upper and lower first premolars to be
The root of the lower canine tooth curves distally and is sometimes in close proximity to the root of the lower first premolar.

It was considered that the upper first premolar would be a suitable experimental tooth.

**Anatomy of the upper first premolar of the dog in relation to this investigation**

The crown of the upper first premolar tooth of the dog is peg-like in form. The buccal surface inclines slightly palatally. From the operative point of view, the buccal surface provides a good approach to the pulp canal.

The mesio-distal inclination of the tooth is such that the apex lies more distally than the tip of the cusp.

When the teeth of the dog are in occlusion, the upper first premolar is not in contact with any lower teeth.

There is a space between the upper canine tooth and the upper first premolar, and usually a space between the latter tooth and the second premolar. In dogs with shorter muzzles the first and second upper premolar teeth are closer together.
(However, during apicectomy operations on the dogs used in this investigation, there was no difficulty in avoiding injury to adjacent tooth roots, as they were not exposed during the operation on the upper first premolar.)

There are no low muscle attachments in the region of the upper first premolar. The buccal alveolar plate over this tooth forms a flat table of bone. The apex of the tooth is approximately 2 millimetres under the surface of the buccal alveolar plate, as illustrated in figure 6. The relationship of the first premolar to the nasal cavity is shown in figures 6 and 7. The apex of this tooth may be in close proximity to the nasal cavity, even to the extent that there may be at times no intervening bone. This is shown in figure 9 where there is only soft connective tissue between the root apex and the epithelium of the nasal mucous membrane. (This close relationship of the apex of the tooth to the nasal cavity was considered to be an operative disadvantage. A small exposure of the nasal cavity was made in one of the operated dogs, dog B.)

The main pulp canal of the upper first premolar is straight and round in transverse section. At a distance of approximately one millimetre from the apex, there is a branching of the main pulp canal, forming many accessory apical pulp canals (figure 8). There is thus a delta-like
arrangement of accessory pulp canals at the apex (figure 9). The diameter of these accessory apical canals is such that it is not possible to insert a root canal reamer beyond a point where the main canal ramifies. As mentioned above, this is a point approximately one millimetre from the apex. This apical ramification of the main pulp canal is not peculiar to the upper first premolar, but is a feature in other dogs' teeth (figure 10).

Schedule of apicectomy operations and post-operative survival periods

A schedule of operation times and survival periods was planned, to enable specimens of the operated regions to be obtained at specified intervals after apicectomy operations. These specimens were to be examined histologically in order to determine what changes had taken place in the tissues after the elapsed post-operative intervals, and thereby ascertain the chronology of healing of apicectomy wounds in dogs.

It was not possible to obtain a sufficient number of dogs of the same pedigree and age. Therefore, healthy mongrel adult dogs of different ages and sexes were used as experimental animals. A total of 23 post-apicectomy specimens was obtained from 17 dogs. (Some dogs had apicectomy
**Fig. 4a.** Mesio-distal radiograph (printed directly for greater clarity) of three upper first premolars of dogs. The mesio-distal width of the pulp canals approximates the bucco-palatal dimension as shown in figure 5a of the same teeth.

**Fig. 4b.** Mesio-distal radiograph of an upper incisor of a dog. The mesio-distal width of the pulp canal is less than the labio-palatal dimension as shown in figure 5b of the same tooth. This is especially so towards the apex of the root.
Fig. 5a. Bucco-palatal radiograph of three upper first premolars of dogs. The bucco-palatal width of the pulp canals approximates the mesio-distal dimension as shown in figure 4a.

Fig. 5b. Labio-palatal radiograph of an upper incisor of a dog. The labio-palatal width of the pulp canal is greater than the mesio-distal dimension as shown in figure 4b.
Fig. 6. Bucco-palatal radiograph of three upper first premolar teeth of dogs. The thickness of buccal alveolar bone over the apices of the roots is shown, as is the close relationship of the apices to the nasal cavity. The apex of the root of the shortest premolar here (with periodontal membrane space retouched) appears to project slightly into the nasal cavity.
Fig. 7. Radiograph of the upper first, second and third premolar region of a dog, showing the close relationship of the apex of the root of the first premolar (pm 1) to the nasal cavity (nas). On the right is seen part of the canine tooth (can) with its very large pulp.
Fig. 8. Photomicrograph x 25, transverse section of apex of an upper first premolar of a dog. Apical accessory pulp canals are indicated by arrows.
Fig. 9. Photomicrograph x 25, longitudinal section of apical half of the root of an upper first premolar of a dog. The main pulp canal (pc) divides into a delta-like arrangement of apical accessory pulp canals, arrowed. In this tooth, in the plane of section, there is no bone between the apex of the root and the nasal cavity (nas).
Fig. 10. Radiographs (printed directly for greater clarity) of three upper first premolar teeth on the left, and an upper incisor tooth on the right, of dogs. A number 1 sized reamer has been inserted into the pulp canal of each tooth as far as possible.
operations on one upper first premolar; others had operations on both upper first premolars, at different times for each.)

The specimens, consisting of the operated tooth and surrounding hard and soft tissues, were removed from the dogs immediately after their death.

The post-operative survival periods, number of specimens of each survival period, and the dogs from which each specimen was obtained, were as follows:

<table>
<thead>
<tr>
<th>Time</th>
<th>Specimens</th>
<th>Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours</td>
<td>two specimens</td>
<td>H and O</td>
</tr>
<tr>
<td>8 hours</td>
<td>two specimens</td>
<td>Q and R</td>
</tr>
<tr>
<td>24 hours</td>
<td>two specimens</td>
<td>N and O</td>
</tr>
<tr>
<td>48 hours</td>
<td>two specimens</td>
<td>M and P</td>
</tr>
<tr>
<td>7 days</td>
<td>two specimens</td>
<td>J and L</td>
</tr>
<tr>
<td>2 weeks</td>
<td>two specimens</td>
<td>G and K</td>
</tr>
<tr>
<td>4 weeks</td>
<td>two specimens</td>
<td>J and K</td>
</tr>
<tr>
<td>8 weeks</td>
<td>two specimens</td>
<td>F and I</td>
</tr>
<tr>
<td>18 weeks</td>
<td>two specimens</td>
<td>C and S</td>
</tr>
<tr>
<td>26 weeks</td>
<td>three specimens</td>
<td>A, B and H</td>
</tr>
<tr>
<td>52 weeks</td>
<td>two specimens</td>
<td>A and B</td>
</tr>
</tbody>
</table>
Anaesthesia

The apicectomy operations on the dogs were carried out under general anaesthesia. This was obtained by an intravenous injection into a foreleg vein of pentobarbitone sodium solution ("Sagatal" — May and Baker Ltd.), containing 60 mg. of pentobarbitone sodium per millilitre. The anaesthetic dose administered was 1 ml. per 2 Kg. body weight. The weights of the experimental dogs varied from 8.5 Kg. to 17.5 Kg. Usually a single intravenous injection was sufficient to maintain a satisfactory plane of anaesthesia throughout the operation. During two (out of 23) operations an additional small intravenous dose of pentobarbitone sodium was given.

In addition to the general anaesthetic, before the incision for the apicectomy operation was made, a total of approximately 0.5 ml. of 3% prilocaine local anaesthetic with 1:300,000 adrenaline ("Citanest 30" — Astra Pharmaceuticals, Australia, Pty. Ltd.) was injected by infiltration into the buccal and palatal aspects of the operative region. This local anaesthetic with vasoconstrictor was administered primarily to assist haemostasis during the operation. The palatal mucosa of the operation region was not injured during operation, but it was thought that palatal vessels could con-
tribute to bleeding from the resection crypt bony wound; therefore, a palatal injection was given in addition to a buccal injection in order to assist haemostasis.

The dog's tongue was positioned to protrude well out of the side of the mouth on the opposite side to the operation in order to preserve a good airway. Sterile gauze tampons in the mouth, proper positioning of the head and good suction prevented aspiration of blood, saline irrigation solution and any other material.

Asepsis

All instruments and materials used for the root filling and apicectomy (including saline irrigation solution, paper points, gutta percha points and glass mixing slab) were sterilised except for the root canal sealer ("Tubli-Seal" — Kerr Manufacturing Company) which was used directly from the two tubes (base and accelerator) as supplied by the manufacturer.

Before the pulp canal was opened for root canal treatment, the operated tooth and surrounding tissues were swabbed with chlorhexidine and cetrimide antiseptic ("Hibitol" — Pharmacy Département, The Queen Elizabeth Hospital, Woodville, S.A.).
A "no-touch" technique, without sterile gloves and gown, was adopted for the root filling procedure. Disposable head-cap and face mask were worn by the operator.

When the root-filling procedure was completed, the trolley containing used instruments and materials was put aside. Fresh sterile instruments and materials were used for the apicectomy operation. Prior to the apicectomy, the operative region and surrounding tissues were swabbed again with chlorhexidine and cetrime. The dog was draped with sterile sheets and towels, allowing only the operative region and antiseptic-swabbed surrounding tissues, and the anterior nares, to be exposed. Fresh gauze tampons were carefully arranged in the mouth.

For the apicectomy operation, the operator wore sterile gloves and gown after "scrubbing up".

(No antibiotics were administered to the dogs at any stage of the project.)

**Operative procedure**

The operative procedure for the root-filling and apicectomy was as follows. (Some of the stages of the
operation are shown in figs. 1-24.)

1. Access to the pulp canal was readily and directly obtained by drilling through the buccal aspect of the crown with a no. 2 round bur. A pin-point of blood was seen when the pulp chamber had been opened into. The opening into the pulp chamber was widened to about \( \frac{3}{4} \text{ mm.} \) diameter, and the access cavity in the crown of the tooth was gradually widened coronally from this point, so that the diameter of the outline form of the cavity was approximately two millimetres. This provided sufficient space for instrumentation with reamers.

2. A number 1 sized reamer was inserted into the pulp canal and gently directed apically as far as it would go. (As mentioned earlier, there is little danger of pushing the reamer beyond the apex in dogs' teeth, because of the ramification of the main canal into many minute apical accessory canals — figs. 8, 9, 10.) The reamer was invariably prevented from going any further apically than the point of ramification of the main pulp canal. This point is approximately 1 mm. from the apex of the tooth. The depth to which the number 1 reamer was inserted was measured, and subsequent reaming with wider reamers was carried out to the same depth and not beyond. The root canal was reamed to number 10 sized reamer
diameter. The root canal was irrigated with normal saline between the stages of reaming. No filing of the root canal was considered necessary, as the reamers produced a cleanly cut, uniformly round canal.

3. After the canal had been reamed, it was thoroughly irrigated with normal saline introduced into the canal through a 25 gauge hypodermic needle. After irrigation, the root canal appeared extremely clean to the naked eye, being free of pulp tissue and dentine shavings.

4. After irrigation with saline, the root canal was dried with paper points.

5. A pink no 10 gutta percha point was tried in the prepared root canal. The gutta percha point was generally a little loose. This was corrected by cutting off two or three millimetres of the narrow end of the tapered point. The required amount was cut off until a fairly snug fit of the point within the root canal was obtained. A suitable length of the closely fitting gutta percha point was cut to make allowance for the amalgam filling which would be inserted later to seal the access cavity.
6. Equal 1 cm. lengths of Kerr's "Tubli-Seal" root canal sealer base and accelerator were mixed according to the manufacturer's directions on a glass mixing slab. A no. 8 sized root canal reamer was used to introduce some of the mixed root canal sealer into the prepared canal, rotating the reamer anticlockwise in order to facilitate the coating of the wall of the root canal with sealer. A small amount of the root sealer was applied to the selected length of gutta percha point before its insertion into the root canal. The gutta percha point was pressed home into its correct position with a plastic instrument. The excess root canal sealer which extruded coronally was removed with a small spoon excavator. (The approximate composition of mixed "Tubli-Seal" root canal sealer according to the manufacturers is given in the Appendix.)

7. When the Kerr's "Tubli-Seal" root canal sealer was set, an amalgam restoration was inserted into the access cavity after suitable cavity preparation. The amalgam filling was considered to be a suitably durable material for sealing off the root filling from the oral environment.

8. After a change to fresh sterile instruments and materials, and after suitable preparation of the operative
field, and with the operator wearing sterile gloves and gown, a total of 0.5 ml. 3% prilocaine with 1:300,000 adrenaline ("Citane 30" — Astra Pharmaceuticals, Australia, Pty. Ltd.) was infiltrated into the soft tissues buccally and palatally to the upper first premolar. This injection was mainly to assist haemostasis. (The degree of haemostasis obtained, combined with adequate suction and good lighting, provided the operator with satisfactory visibility during the operation.)

9. An incision was made with a number 15 scalpel blade through the buccal alveolar mucoperiosteum along the predetermined incision line. The horizontal part of the incision line was a straight incision approximately 3.5 to 4.5 mm. from the buccal gingival margin. The length of this horizontal part of the incision was approximately 0.8 cm. From each end of the horizontal part of the incision line, oblique incisions extended towards, but not up to, the buccal sulcus for a distance of about 0.8 cm. (Care was taken especially to avoid extending the distal oblique arm of the incision too far, as the pulsations of a buccal artery were usually to be observed in this second premolar region.) The incision used provided a mucoperiosteal flap with a broad base having a good blood supply. The margins of the
flap, when repositioned with sutures at the completion of the operation, lay upon a firm foundation of alveolar bone, clear of the resection crypt. The incision also allowed an adequate blood supply to be maintained to the mucosa-gingival to the incision line. (An alternative incision could have been a gingival margin incision for the horizontal part of the incision.)

10. The mucoperiosteal flap was raised with a periosteal elevator beginning the elevation at the mesial oblique part of the incision line. The flap was readily raised as the periosteum had been cleanly divided during the incision. A flat surface of buccal alveolar bone was exposed. Continuous suction was required to maintain a relatively bloodless field throughout the operation.

11. The region of the apex of the tooth was determined, keeping in mind that the apex is slightly distal to the crown, and by referring to the length of the tooth as predetermined during the root filling procedure.

12. Using a number 5 round bur in a handpiece revolving at 12,000 r.p.m., under a continuous generous flow of normal saline, a cavity was cut in the buccal alveolar bone over the apex of the tooth. The diameter of the surface outline form
of the cavity, which was circular or approximately so, was approximately 4 millimetres. The depth of the cavity in the bone was increased by further drilling until the apex of the tooth was exposed. The cavity was further increased in depth until the root apex was considered to have been almost half drilled through, as shown by the appearance of the apical end of the gutta percha point just coming into view. (The continuous irrigation with saline, during all cutting of bone and tooth substance, combined with adequate suction, enabled shavings of bone, tooth substance and root filling material to be removed from the wound as soon as they were formed.)

13. A number 700 tapering fissure bur was used to resect the apical third of the root, using a slightly sawing mesio-distal motion. Care was taken to drill just through the tooth and not beyond, if possible, in order to avoid opening into the nasal cavity. The resected apical portion was invariably still firmly attached. It was released by drilling around its mesial, distal and sometimes apical aspects with the number 700 tapering fissure bur. The same bur was used to widen the gap between the resection surfaces of the main root and apical root fragment. After these manoeuvres, the remaining apical fragment was elevated into this gap and out of the wound,
using a small spoon excavator as an elevator. The lamina dura related to most of the resected apex on its palatal, and sometimes apical aspects was not usually disturbed by drilling. Smoothing of those parts of the bony wall of the resection crypt which had been previously drilled was carried out if necessary, leaving a rather hemispherical resection crypt. The resected root end was also rendered smooth if necessary using a number 5 round bur. This left a very slightly concave root resection surface. During the smoothing of the root end, particular care was taken to ensure that the gutta percha root filling was cleanly and smoothly divided. Any minute tags caused by the dragging effect of the resection bur on the relatively soft gutta percha material were removed with the round bur. Usually only the round resected surface of the gutta percha was seen, but occasionally a very thin ring of the root canal sealer was visible around the resected gutta percha surface.

14. A careful debridement of the wound was carried out, removing any remaining visible loose particles of bone, tooth substance and root filling material by thorough irrigation with normal saline and careful suction with a fine sucker point. Care was taken that no particles were under the mucoperiosteal flap.
15. After the wound toilet, the wound was allowed to fill with blood, which readily occurred in a few seconds.

16. The mucoperiosteal flap was sutured into the correct position with 4/0 chromic catgut sutures. Only two sutures were required, one at each end of the horizontal part of the incision.

The operated teeth were not mobile in most cases; in some cases, however, there was slight mobility after the apicectomy.

17. The dog was returned to its cage immediately after the operation, the duration of which was 40 to 55 minutes.

Post-operative progress

The dogs made uneventful recoveries from the operation, regaining consciousness usually two to three hours after the operation. The animal house attendants reported a short excitement phase in some animals during the period of recovery from anaesthesia.

A soft diet was given to the dogs for a few days. Dogs were seen (unless they were shorter term survival dogs and already sacrificed before then) the day after the operation.
Fig. 11. Obtaining access to the pulp canal

Fig. 12. Reamer in pulp canal of upper first premolar
Fig. 13. Drying the prepared root canal with a paper point

Fig. 14. Inserting gutta percha point into root canal
Fig. 15. Dog 'towelled up' with sterile covers prior to apicectomy operation

Fig. 16. Injecting local anaesthetic solution containing vasoconstrictor
Fig. 17. Making the incision

Fig. 18. The incision through mucoperiosteum
Fig. 19. Raising the mucoperiosteal flap

Fig. 20. The resection crypt
Fig. 21. Irrigating the wound with normal saline during wound toilet

Fig. 22. Allowing the wound to fill with blood before suturing
Fig. 23. Suturing the mucoperiosteal flap

Fig. 24. Sutures in position at end of operation
They invariably appeared to be in no distress or pain, looked well, were up and about as much as non-operated dogs and were not off their food. In other words, there appeared to be no general ill-effects as the result of the operation.

Dogs were examined from time to time. The healing of the mucosa seemed to be remarkably rapid. For example, after only two days post-operatively, the incision line appeared to have closed on the surface. After one week, the incision line was firmly united. Another feature was the rapidity with which the chromic catgut disappeared from the wound; usually no sutures were to be seen on the surface after one week, or occasionally only a small strand or two. There were no clinical signs of inflammation of the soft tissues after one week. The operated teeth at this time were usually firm or only very slightly mobile. In one dog, F (8 weeks and 18 weeks), it was found that at the time of sacrifice in the region of the 18-week operation, the tooth was no longer present. The 8-week contralateral upper first premolar was still in situ and not mobile. Another 18-week specimen was obtained from dog S.
Sacrifice of animals and obtaining post-mortem specimens

The dogs were killed at predetermined post-operative intervals, as scheduled, in order to obtain specimens of the operated teeth and surrounding hard and soft tissues. As mentioned earlier, specimens were obtained after survival periods as follows:

2 hours, 8 hours, 24 hours, 48 hours,
7 days, 2 weeks, 4 weeks, 8 weeks,
18 weeks, 26 weeks, 52 weeks.

The dogs were killed by a lethal dose of intravenous pentobarbitone sodium ("Sagatal" — May and Baker, Ltd.) into a foreleg vein. The lethal dose is twice the anaesthetic dose, being 1 ml. per 1 Kg. body weight. The intravenous injection caused painless death in a few seconds.

Immediately after death, the operated tooth or teeth and surrounding tissues were excised. For post-operative specimens one week or longer, the surrounding tissues were removed, with enclosed tooth, to an extent of at least three millimetres clearance from all parts of the tooth and resection crypt. For specimens two days or less, the excision cut in the surrounding hard and soft tissues was made beyond the
operation incision line; this was to avoid any disruption of
the incision line during excision of the specimen.

The technique for excision of specimens was as follows. The mucoperiosteum, in the predetermined line of excision, was
incised with a scalpel. A fine, spiral-bladed dental
laboratory fret-saw was used to cut through bone and any
adjacent teeth in the excision line. After very quickly
washing off under tap water any blood or saw shavings from the
specimen, it was immediately placed in 10% buffered formalin
for fixation.

After fixation, the specimens were radiographed bucco-
palatally and mesio-distally prior to decalcification.

Details of further treatment of the specimen during
histological processing, and the procedures of section cutting
and staining are given in the Appendix.

The histological sections of specimens were examined
under a light microscope and observations recorded.
CHAPTER 13

RESULTS

Examination of the many histological sections of post-apicectomy specimens from animals of different post-operative survival periods was made, and the observations were recorded as follows. The headings refer to the post-operative intervals and the histological findings of the corresponding specimens.

2 HOURS post-operative

The epithelium in the region of the incision line is inverted and corresponding layers are not in apposition (figs. 25 and 26). Apart from this inaccurate apposition of the incised epithelial layer, the epithelium of the two sides of the incision line is frequently not in contact, being separated by a narrow gap which is filled with blood clot containing moderate numbers of polymorphonuclear leukocytes. These acute inflammatory cells are also found in the fibrin which is present in the V-shaped depression on the surface of the incision line (figs. 26 and 27). Vacuoles are seen in the prickle-cell layer of the epithelium on the buccal and
palatal sides in the region where the local anaesthetic was injected.

The connective tissue of the mucoperiosteal flap is also separated, in the region of the incision line, from the unelevated incised surface of the gingival side of the incision by a thin layer of blood clot containing polymorphonuclear leukocytes. This thin layer of blood clot is continuous with that between the epithelium and that filling the resection crypt (fig. 25).

A mild infiltration of polymorphonuclear leukocytes is also present in the tissue spaces of the mucoperiosteal flap, which exhibits typical changes associated with the early stages of acute inflammation, such as the presence of congested, dilated blood vessels with margination (pavementing) of polymorphonuclear leukocytes, and emigration of these leukocytes between the swollen endothelial cells of the walls of the capillaries and small venules into the surrounding tissues. As well as the walls of the small blood vessels being swollen, they are also disrupted in places (fig. 28). Diapedesis of red blood corpuscles into the tissue spaces, and inflammatory exudate in these spaces are also apparent.
There is a narrow space between the periosteum of the mucoperiosteal flap and the surface of the unoperated alveolar bone surrounding the bony wound of the resection crypt. This space contains blood clot moderately infiltrated with polymorphonuclear leukocytes.

The resection crypt is occupied by blood clot, much of which has been lost during histological preparation of the section, causing an artefact space (fig. 29). Unlike the blood clot between the incision surfaces, and that between the mucoperiosteal flap and the unoperated alveolar bone, the blood clot within the resection crypt contains no more leukocytes than are to be seen in a fresh blood smear. The composition of the blood clot, in terms of relative numbers of constituent blood cells, is uniform throughout the blood clot in the resection crypt.

No changes are seen either on the bony walls of the resection crypt, or in the adjacent marrow spaces.
Fig. 25. Photomicrograph x 25,
dog 0, 2 hours.

inv  inversion of epithelium in incision line
bc  blood clot between incised surfaces
rc  resection crypt
Fig. 26. Photomicrograph x 100, dog O, 2 hours.

bc blood clot between incised surfaces is infiltrated by polymorphonuclear leukocytes
Fig. 27. Photomicrograph x 400, dog 0, 2 hours.

epi epithelium of each side of incision line, the surface of which is filled with fibrin clot containing many polymorphonuclear leukocytes, one of which is indicated by an arrow
Fig. 28. Photomicrograph x 400, dog 0, 2 hours.

rbc red blood corpuscles in dilated venule, in which there is margination of polymorphonuclear leukocytes as shown by arrows

p polymorphonuclear leukocytes which have emigrated into the tissue spaces from the blood vessel
Fig. 29. Photomicrograph x 25, dog 0, 2 hours.

gp  gutta percha root filling
bc  blood clot in resection crypt
art artefact space caused by loss of blood clot from resection crypt during histological preparation
8 HOURS post-operative

The epithelium in the region of the incision line exhibits the same features as described for the two-hour specimens. Inversion of the epithelium is pronounced.

A striking and disturbing feature is seen in the epithelium of the regions where the local anaesthetic had been injected buccally and palatally before the apicectomy operation. In these regions there is necrosis of epithelium (fig. 30) on the buccal side, and hydrophic changes are seen in the palatal epithelium. Polymorphonuclear leukocytes are present between the dead epithelial cells. Vesicles are seen in the necrotic layer of the epithelium in places. In some regions the entire epithelial layer is necrotic, whilst in most places there is a thin layer of living cells of the basal layer of epithelium.

The connective tissue of the mucoperiosteal flap is now moderately infiltrated with polymorphonuclear leukocytes which are present between fibrocytes and collagen fibres of the connective tissue, and between the few voluntary muscle fibres which are present in the buccal sulcus. In these tissue spaces the polymorphonuclear leukocytes are concentrated
around the small blood vessels which are engorged with red blood corpuscles and contain many polymorphonuclear leukocytes, with margination of these leukocytes. Red blood corpuscles are lightly scattered through the tissue spaces of the flap.

The space between the mucoperiosteal flap and the unoperated alveolar bone, which contains blood clot, is now densely infiltrated with polymorphonuclear leukocytes, as is the blood clot between the incised surfaces of mucoperiosteum.

The blood clot in the resection crypt, relatively free of inflammatory cells in the two-hour specimens, has now become moderately infiltrated with polymorphonuclear leukocytes. Moreover, there are focal concentrations of these cells. A noteworthy and constant feature is the presence of a concentration of polymorphonuclear leukocytes in relation to the gutta percha root filling material (fig. 31), forming a band of leukocytes in this area. Much of the gutta percha was lost from the specimens during processing (chloroform) of specimens and during the procedures associated with staining. This left an artefact space where the gutta percha was lost. References to gutta percha in the following descriptions of findings and in the photomicrographs will be taken to include
the space which represents gutta percha lost during histological preparation.

There is no tendency at this stage for concentrations of polymorphonuclear leukocytes to be evident either in relation to the cut root resection surface or in relation to the cut surface of the bony wall of the resection crypt.

No changes are seen in the cut surface of the alveolar bone in the resection crypt. However, the adjacent marrow spaces now contain appreciable numbers of polymorphonuclear leukocytes.
Fig. 30. Photomicrograph x 400, dog Q, 8 hours.

nec epi  necrotic epithelium which is infiltrated by polymorphonuclear leukocytes, arrowed

bl  living cells of the basal layer of epithelium
Fig. 31. Photomicrograph x 400, dog Q, 8 hours.

gp gutta percha root filling

p concentration of polymorphonuclear leukocytes adjacent to gutta percha root filling

bc blood clot in resection crypt
24 HOURS post-operative

In the region of the incision line, the epithelium has proliferated downwards (fig. 32), with projection of rete pegs into the corium in the region of the incised surfaces. Mitoses are seen (fig. 33). The proliferation downwards was observed in both 24 hour specimens. In one of these the epithelium has united tenuously across the incision line (fig. 34). Hydrophic changes are present in some parts of the prickle cell layer. There are still polymorphonuclear leukocytes on the surface of the incision line.

In the regions buccally and palatally where the local anaesthetic was injected, in one of the 24-hour specimens, the superficial layers of epithelium have sloughed off, but the lost epithelium is being replaced by proliferation of cells of the basal layer which has sent down rete pegs into the corium.

The connective tissue of the mucoperiosteal flap still contains polymorphonuclear leukocytes, but they are reduced in number. A few macrophages are seen among the polymorphonuclear leukocytes in the incision line. The small blood vessels in the flap are now much less engorged with red blood corpuscles than in the earlier specimens. There are fewer polymorpho-
nuclear leukocytes and red blood corpuscles in the space between the flap and the unoperated alveolar bone at this stage. A few young fibroblasts are present in the fibrin clot between the incision surfaces in the corium.

The blood clot in the resection crypt is moderately infiltrated with polymorphonuclear leukocytes. A heavy concentration of polymorphonuclear leukocytes is present in contact with the gutta percha root filling material (fig. 35). Such concentrations of acute inflammatory cells are not seen in relation to the root resection surface or to the operated bone. No fibroblasts are evident in the resection crypt.

No changes (such as empty lacunae) are seen in the bone forming part of the wall of the resection crypt, except for the presence of moderate numbers of polymorphonuclear leukocytes in the adjacent marrow spaces.
Fig. 32. Photomicrograph x 40, dog 0, 24 hours.

inv inversion of epithelium in incision line
bc blood clot between incised surfaces
Fig. 33. Photomicrograph x 400,
dog 0, 24 hours,

m epithelial mitosis in basal layer
near incision region

inc approximate line of incision

un epi union of epithelium across incision line
Fig. 34. Photomicrograph x 250, dog 0, 24 hours.

un epi union of epithelium across incision line
hyd hydrophic changes in prickle cell layer
p polymorphonuclear leukocytes in fibrin clot on surface of incision line
Fig. 35. Photomicrograph x 40, dog 0, 24 hours.

gp gutta percha root filling

p concentration of polymorphonuclear leukocytes adjacent to gutta percha root filling

bc blood clot in resection crypt
48 HOURS post-operative

The epithelium in the incision region has now united well, forming a thickened wedge-shaped band, widest in the incision line and gradually diminishing with distance from the incision (figs. 36 and 37). The basal cell layer and the prickle cell layer are well-differentiated. There is parakeratosis of the surface epithelium. There is evidence of slight hydrophic changes in the deeper parts of the invaginated epithelium. The epithelium in the region of local anaesthetic injection buccally and palatally is being increased in thickness by proliferation of epithelial cells of the basal layer in the form of rete pegs.

The connective tissue of the flap still has a slight infiltration of the tissue spaces with polymorphonuclear leukocytes. The blood vessels in the flap are no longer congested. The fibrin clot between the incised corium surfaces is beginning to undergo organisation, as evidenced by the presence of young fibroblasts. A few lymphocytes and plasma cells are present in the organising clot in this region, but the predominant cells are polymorphonuclear leukocytes. Polymorphonuclear leukocytes are present in moderate numbers
in the blood clot between the periosteum of the flap and the unoperated alveolar bone. The blood clot here is also commencing to become organised by young connective tissue.

The blood clot in the resection crypt (fig. 38) is just beginning to be organised, with the appearance of a few fibroblasts at the periphery of the resection crypt. There is still a moderate infiltration of the clot by polymorphonuclear leukocytes, and there is a concentration of polymorphonuclear leukocytes adjacent to the gutta percha root filling material (fig. 39). Many of these leukocytes are in a state of degeneration as shown by fragmentation of nuclei. A concentration of polymorphonuclear leukocytes is present in the portion of the clot under the part of the flap overlying the resection crypt.

No changes are seen in the operated alveolar bone apart from the presence of appreciable numbers of polymorphonuclear leukocytes in adjacent marrow spaces.
Fig. 36. Photomicrograph x 25, dog P, 48 hours.

epi united epithelium in the incision region forms a wedge-shaped band

bc blood clot between incised surfaces of corium is commencing to be organised
Fig. 37. Composite photomicrograph x 100, dog P, 48 hours.

rp rete peg in area adjacent to incision region. The epithelium is thicker in the incision region.

bc blood clot between incised surfaces of corium is commencing to be organised
Fig. 38. Photomicrograph x 25, dog P, 48 hours.

gp   gutta percha root filling

bc   blood clot in resection crypt is infiltrated by polymorphonuclear leukocytes
Fig. 39. Photomicrograph x 400, dog P, 48 hours.

gp gutta percha root filling with polymorphonuclear leukocytes concentrated adjacent to it
7 DAYS post-operative

The epithelium in the incision line is thickened. Hydrophic changes are seen in the prickle cell layer. Parakeratosis is present (figs. 40 and 41).

In the connective tissue of the mucoperiosteal flap only an occasional polymorph is present. Young fibroblasts, and a few macrophages and plasma cells are seen in tissue spaces within the flap. Red blood corpuscles are no longer present in the tissue spaces.

There is now no blood clot in the region of the incision line, the clot having been replaced by young connective tissue (fig. 41).

The mucoperiosteal flap has become reattached to the unoperated alveolar bone by young connective tissue which has replaced the subperiosteal blood clot. There is a suggestion of early new bone formation in some areas between the periosteum and the unoperated bone, the new bone appearing on the surface of the old bone. No osteoclasts are seen under the flap. There are now no polymorphonuclear leukocytes in this region.
In the resection crypt invasion of the blood clot by proliferation of young fibroblasts from the periphery of the crypt is well under way. The blood clot has been approximately one quarter replaced by this invasion of young connective tissue (figs. 42 and 51). Infiltration of the clot by polymorphonuclear leukocytes is no longer a feature. A few macrophages, some containing blood pigment and some containing polymorphonuclear leukocytes, are seen. In one specimen (figure 43) there are a few lymphocytes in the blood clot adjacent to the gutta percha root filling material, with only an occasional polymorphonuclear leukocyte present. There is a fragment of gutta percha in the resection crypt in the same specimen (figs. 42, 49 and 50). Young connective tissue surrounds this gutta percha material. The fragment of gutta percha appears to be well tolerated in this dog, because the surrounding young connective tissue is free of inflammatory cells.

Apart from the complete replacement of the blood clot in the peripheral part of the resection crypt by young connective tissue, the remaining central blood clot is permeated by granulation tissue with young fibroblasts and proliferating capillaries present among the red blood
corpuscles (fig. 45). Mitoses of fibroblasts permeating the central blood clot are seen (fig. 46). The manner in which the young fibroblastic connective tissue tends to stream in centripetally, invading the central blood clot, is shown in figs. 47 and 48.

The young connective tissue, at the periphery of the resection crypt, is itself being replaced by new bone in places. In this description, and that to follow, the term "new bone" will be taken to include young uncalcified osteoid tissue, older osteoid in various stages of calcification and older calcified, even remodelled, "new" bone. The term "osteogenesis" will indicate both the presence, and the formation, of new bone. Less differentiated osteogenic cells present in the old bone can readily differentiate into osteoblasts and both these types of cells possess an osteogenic potential (JOLLY, 1953). Both osteogenic cells and osteoblasts can divide by mitosis. In this description and that to follow, the term "osteoblasts" will be defined in a broader sense to include the less differentiated osteogenic cells.

Formation of new bone is evident in figs. 49, 50, 52 and 53, of a seven-day specimen. It is evident from the extent
of osteogenesis seen in figs. 49 and 50, that this osteogenesis had commenced some little time before seven days. No specimens, between two and seven days post-operatively, were available from which to attempt to ascertain the very first evidence of osteogenesis.

No osteoclasts are seen in the region of the operation wound. There are some osteoclasts present at the buccal alveolar crest, well removed from the apicectomy site.
Fig. 40. Photomicrograph x 25, dog J, 7 days.

cor corium in incision region where organisation of blood clot has taken place
Fig. 41. Photomicrograph x 100, dog J, 7 days.

hyd hydrophic changes in prickle cell layer

cor young connective tissue in corium in incision region
Fig. 42. Photomicrograph x 25, dog J, 7 days.

$gp$ fragment of detached gutta percha root filling material

$bc$ blood clot is being invaded and organised by young connective tissue as indicated by small arrows
Fig. 43. Photomicrograph x 400, dog J, 7 days.

gp gutta percha root filling
l lymphocyte
p polymorphonuclear leukocyte
fb young fibroblast
Fig. 44. Photomicrograph x 400,
dog J, 7 days.

gp fragment of detached gutta percha root
filling material

yct young connective tissue surrounding the
gutta percha material is free of
inflammation
Fig. 45. Photomicrograph x 250, dog J, 7 days.

fb  young fibroblasts of granulation tissue
cap  capillaries of granulation tissue
Fig. 46. Photomicrograph x 400, dog J, 7 days.

fb m possible fibroblast mitosis. A more definite fibroblast mitosis may be seen in figure 48.

bc blood clot in resection crypt
Fig. 47. Photomicrograph x 100,
dog J, 7 days.
(Enlargement of part of this figure
is shown in figure 48)

bc  blood clot

ob  old bone forming part of the walls of the resection crypt

fb  young fibroblasts streaming in from the periphery of the crypt to invade and organise the blood clot
Fig. 48. Photomicrograph x 250, dog J, 7 days.

bc  blood clot being invaded by young connective tissue

fb m  fibroblast mitosis
Fig. 49. Photomicrograph x 40,
dog J, 7 days.
(Enlargement of part of this figure
is shown in figure 50)

ob old bone forming part of wall of resection
crypt

nb new bone is forming in peripheral part of
resection crypt

bc blood clot in resection crypt is being
replaced by young connective tissue

gp fragment of detached gutta percha root
filling material is surrounded by young
connective tissue
Fig. 50. Photomicrograph x 100, dog J, 7 days.

ob old bone

nb new bone, replacing young connective tissue

gp fragment of detached gutta percha root filling material

yct young connective tissue surrounding gutta percha is free of inflammation
Fig. 51. Photomicrograph x 25, dog J, 7 days. (Enlargement of outlined area is shown in figure 52)

bc blood clot in resection crypt is being invaded by young connective tissue as indicated by arrows

yct young connective tissue which has replaced blood clot in peripheral parts of the resection crypt
Fig. 52. Photomicrograph x 100, dog J, 7 days. (Enlargement of outlined area is shown in figure 53)

ob old bone of wall of resection crypt
nb new bone formed in resection crypt
bc blood clot being replaced by young connective tissue
Fig. 53. Composite photomicrograph x 250, dog J, 7 days.
2 WEEKS post-operative

The epithelium in the region of the incision is almost indistinguishable from normal. In some of the sections, a very slight thickening of the epithelium and slight depression on the surface mark where the incision had been made.

In one of the specimens there was a section of chromic catgut material surrounded by epithelium which had proliferated and invaginated around the suture material (fig. 54). The connective tissue around this epithelium is heavily infiltrated with polymorphonuclear leukocytes and macrophages, some of the latter containing polymorphonuclear leukocytes.

Only about a quarter or less of the original blood clot remains in the resection crypt, the clot having been largely invaded and replaced by young connective tissue. The remaining blood clot forms a central mass, permeated by granulation tissue which is replacing it (fig. 55). New bone formation is well advanced in the resection crypt. In one of the specimens, this new bone is found mainly on the side of the resection crypt removed from the resected tooth surface (fig. 55). On the other side of the resection crypt, between the central blood clot and the resection surface, is fibrous
connective tissue containing a moderate number of lymphocytes and plasma cells. Macrophages containing blood pigment are seen in this region also. A concentration of polymorphonuclear leukocytes is present in relation to the gutta percha root filling material. In the granulation tissue between the bars of new bone, red blood corpuscles tend to be grouped around the small blood vessels (fig. 56).

In the other two-week specimen new bone has formed close to the resection surface, separated from it by fibrous connective tissue. In relation to the gutta percha root filling material there is a band of fibrous tissue infiltrated with round cells (figs. 59 and 60). In sections cut near the periphery of the root, new bone has filled the resection crypt. Only a very occasional osteoclast is seen on the surface, and in the marrow spaces of, the old bone. No osteoclasts are seen in relation to the new bone.

In some sections of one of the specimens (figs. 57 and 58), there is an appearance suggestive of early cementoid over parts of the periphery of the resection surface, upon both the dentine and old cementum after prior resorption. There is an alignment of cells on the surface of this matrix material.
These cells are more elongated than typical cementoblasts. However, some cells appear to be enclosed in the matrix like cementocytes.
Fig. 54. Photomicrograph x 40, dog G, 2 weeks.

catg chromic catgut suture material

epi epithelium surrounding catgut

p polymorphonuclear leukocytes in the connective tissue surrounding the proliferated epithelium
Fig. 55. Photomicrograph x 40, dog G, 2 weeks.

gp  gutta percha root filling

p  concentration of polymorphonuclear leukocytes adjacent to gutta percha root filling

ct  connective tissue

bc  blood clot

nb  new bone
Fig. 56. Photomicrograph x 250, dog G, 2 weeks.

bv  blood vessel
rbc red blood corpuscles around blood vessel
yct young connective tissue
nb  new bone
os b osteoblast
os cyt osteocyte
Fig. 57. Photomicrograph x 100,
dog G, 2 weeks.
(Enlargement of outlined area
is shown in figure 58)

nc  probable new cementum deposited on
     resection surface

nb  new bone in resection crypt

bc  central blood clot which is being
     organised
Fig. 58. Photomicrograph x 400, dog G, 2 weeks.

oc old cementum of root resected tooth

d dentine

nc probable new cementum deposited on resection surface, after prior resorption of the latter

cb cementoblast

ct fibrous connective tissue
Fig. 59. Photomicrograph x 25, dog K, 2 weeks. (Enlargement of outlined area is shown in figure 60)

**gp** gutta percha root filling

**art** artefact space into which some of the gutta percha has extruded during histological preparation

**nb** new bone forming close to the resection surface
Fig. 60. Composite photomicrograph x 250, dog K, 2 weeks.
4 WEEKS post-operative

The epithelium and connective tissue of the flap are indistinguishable from normal in all parts, including the incision region.

No blood clot is present in the resection crypt. Bone formed in the resection crypt is fairly mature. It is being remodelled by a process of resorption and apposition. Resorption of bone formed in the resection crypt is especially active. Many osteoclasts are to be found (figs. 63, 64 and 65), lying in Howship's lacunae.

On one four-week specimen there is a heavy concentration of cells opposite the gutta percha root filling material (fig. 68). Those cells closest to the gutta percha are mostly polymorphonuclear leukocytes whilst those further out are mainly lymphocytes and plasma cells.

In the other four-week specimen, there is a band of fibrous connective tissue in relation to the gutta percha root filling material. In this case the fibrous tissue is free of inflammatory cells.
Cellular cementum has been deposited on some parts of the peripheral part of the resection surface, both upon the cut surface of the old cementum and upon the cut dentine surface, and this is quite thick in some areas, (figs. 61, 62, 63, 64, 66 and 67). In some areas, resorption of the old cementum is taking place. In one region, resorption of old cementum and apposition of new cementum are taking place side by side (figs. 63 and 64).

In this description and that to follow, new cementum will be taken to include uncalcified new cementoid and calcified new cementum, and will refer to cellular cementum as opposed to acellular cementum.
Fig. 61. Photomicrograph x 25, dog J, 4 weeks.
(Enlargement of outlined area is shown in figure 62)

oc  old cementum

d  dentine

nc  new cementum deposited on resection surface
Fig. 62. Photomicrograph x 100,
dog J, 4 weeks.

oc old cementum

d dentine

nc new cementum deposited on resection surface

cb cementoblasts on surface of new cementum

cb connective tissue between resection surface
and new bone

nb new bone
Fig. 63. Photomicrograph x 100,
dog J, 4 weeks.
(Enlargement of part of this figure
is shown in figure 64)

oc old cementum
d dentine
nc new cementum deposited on old cementum and dentine
cf fibrous connective tissue
os cl osteoclasts lying in Howship's lacunae
rsp resorbed area of old cementum
Fig. 64. Photomicrograph x 250, dog J, 4 weeks.

oc old cementum
nc new cementum deposited on resection surface
rsp resorption of old cementum at top of figure and resorption of bone at bottom of figure
os cl osteoclast
el empty lacunae in part of bone which is undergoing resorption during remodelling
Fig. 65. Photomicrograph x 250,
dog J, 4 weeks.

**gp**  gutta percha root filling

**ct**  fibrous connective tissue

**os cl**  osteoclast in Howship's lacuna
Fig. 66. Photomicrograph x 25, dog K, 4 weeks.

oc  old cementum

d  dentine

res  resection surface

nc  new cementum
Fig. 67. Photomicrograph x 100, dog K, 4 weeks.

res  resection surface
nc   new cementum
art  artefact spaces
ct   fibrous connective tissue
Fig. 68. Photomicrograph x 25, dog K, 4 weeks.

gp  gutta percha root filling

ic  inflammatory cells, mostly polymorpho-
nuclear leukocytes

nc  new cementum

nb  new bone
8 WEEKS post-operative

The bone almost filling the resection crypt is quite mature, looking like the old bone. However, it is still being remodelled, as shown by the presence of osteoclasts on the surface opposite the resected root end and in some of the marrow spaces (fig. 70 and 71). Resting lines are seen in the bone. The "new" bone in the resection crypt is fairly dense compared with the old bone further out from the resection area, which has large fatty marrow spaces.

A thin layer of new cementum has formed over almost all of the resection surface in one of the specimens. In this specimen again, there is a marked concentration of cells opposite the gutta percha root filling material. In this specimen they are mostly polymorphonuclear leukocytes (figs. 69 and 70).

In the other eight-week specimen there is only a thin layer of new cementum over parts of the periphery of the root mainly on the palatal aspect (figs. 72 and 73). Osteoclasts are present on the resection surface on the buccal side in the same section (figs. 72 and 74). In this specimen, as opposed to the findings in the other eight-week specimen,
there are no acute or chronic inflammatory cells in the fibrous capsule which has formed in relation to the gutta percha root filling material.

In both specimens the intercellular fibres of the fibrous connective tissue between the root resection surface and the bone tend to run parallel to the resection surface (figs. 70 and 72).
Fig. 69. Photomicrograph x 25,  
dog I, 8 weeks,  
(Enlargement of part of this figure  
is shown in figure 70)

gp gutta percha root filling

ic inflammatory cells adjacent to gutta percha  
root filling

nc new cementum deposited on resection surface

ct fibrous connective tissue
Fig. 70. Composite photomicrograph x 100, dog I, 8 weeks.
Fig. 71. Photomicrograph x 100, dog I, 8 weeks.

os cl osteoclasts in marrow spaces of new bone which is being remodelled
new cementum on resection surface
(Enlargement of similar area in adjacent section in series is shown in figure 73)

osteoclast on area of resection surface undergoing resorption
(Enlargement of similar area in adjacent section is shown in figure 74)

Fig. 72. Composite photomicrograph x 100, dog F, 8 weeks.
Fig. 73. Photomicrograph x 400, dog F, 8 weeks.

d dentine of resected tooth
nc new cementum deposited on resection surface
cb cementoblast on surface of new cementum
Fig. 74. Photomicrograph x 400, dog F, 8 weeks.

os cl  osteoclasts on resection surface

oc  old cementum

d  dentine
18 WEEKS post-operative

The epithelium in one of these specimens is still thickened in the region where the incision had been made. The fibrous connective tissue of the buccal mucoperiosteum is normal in the operation site.

The bone filling the resection crypt now resembles the old bone in that it is cancellous with large marrow spaces. There are still a few osteoclasts in the marrow spaces. A thick layer of cellular cementum covers the root resection surface over almost all of its surface, the covering just not quite reaching the gutta percha root filling (figs. 75, 76 and 77). It is apparent, from the presence of Howship's lacunae, that resorption of the resected surface had occurred before the deposition of new cementum took place.

In relation to the gutta percha root filling which is extruded, there is a loose fibrous capsule which is oedematous and infiltrated by round cells (fig. 78). In the oedematous connective tissue near the root filling, macrophages are to be seen, containing particles of foreign material, probably root canal sealer or gutta percha root filling material (fig. 79).
The fibrous connective tissue further away from the root filling material is still loosely arranged, but chronic inflammatory cells (lymphocytes and plasma cells) are only lightly spread among the tissues. Between the resected root surface and the alveolar bone, the connective tissue fibres tend to run parallel to these two surfaces (fig. 77). In some sections the fibrous connective tissue, in relation to the root resection surface, lifts away during histological preparation, producing an artefact space. When this happens, the cementoblasts on the surface of the "new" cementum tend to remain adherent to the new cementum.
Fig. 75. Photomicrograph x 25, dog C, 18 weeks.

oc  old cementum

d  dentine

nc  new cementum deposited on resection surface

ct  fibrous connective tissue

nb  mature new bone
Fig. 76. Photomicrograph x 25, dog C, 18 weeks.
(Enlargement of outlined area is shown in figure 77)

gp gutta percha root filling
oc old cementum
d dentine
nc new cementum deposited on resection surface
ic inflammatory cells concentrated adjacent to gutta percha root filling
nb mature new bone
Fig. 77. Composite photomicrograph x 100, dog C, 18 weeks.
Fig. 78. Photomicrograph x 400, dog C, 18 weeks.

gp  gutta percha root filling

ic  concentration of chronic inflammatory cells adjacent to gutta percha root filling

ct  oedematous connective tissue infiltrated by chronic inflammatory cells
Fig. 79. Photomicrograph x 400, dog C, 18 weeks.

mac macrophages containing foreign material, possibly root canal sealer or gutta percha root filling material

ct oedematous connective tissue

ic chronic inflammatory cell, plasma cell.
26 WEEKS post-operative

In both specimens cementum has formed over almost all of the resection surface, resorption having preceded cementum apposition. In one specimen, loose oedematous fibrous connective tissue, infiltrated with lymphocytes and plasma cells, lies opposite the gutta percha root filling. In the other specimen, there is a fibrous capsule in relation to the gutta percha root filling material. The fibrous tissue in this specimen is free of inflammatory cells. The fibrous connective tissue further out from the root filling is less dense. In both specimens the fibres of the connective tissue tend to run parallel to the resection surface.

In one of the specimens, part of the old cementum of the apex of the root is retained. The cut surface has been covered with a layer of new cementum (fig. 80). In the same specimen, two fragments of dentine and old acellular cementum have become detached from the buccal aspect of the root and are lying in the periodontal membrane nearby, almost level with the resection surface. The detached fragments are covered and joined by a layer of new cementum (fig. 82). New cementum is also covering the part of the tooth from
which the detachment of the fragments occurred. Coronal to this region where the detachment of tooth structure and subsequent repair by new cementum has occurred, the manner in which the fibres of the periodontal membrane extend into the tooth root almost at right angles, is in contrast to the parallel orientation of the apical fibrous tissue in relation to the resection surface. The bone in the resection region resembles normal bone. Osteoclasts are not present.
Fig. 80. Photomicrograph x 25, dog B, 26 weeks.

nc new cementum

oc old cementum of retained apical root fragment

cf fibrous connective tissue surrounding retained apex is free of inflammation
Fig. 81. Photomicrograph x 100,
dog B, 26 weeks.

**gp** gutta percha root filling

**ct** fibrous connective tissue adjacent to gutta percha root filling is free of inflammation and its cells and fibres tend to be aligned parallel to the resection surface
Fig. 82. Composite photomicrograph x 100, dog B, 26 weeks.
52 WEEKS post-operative

In one of the specimens, the cancellous bone in the region of the former resection crypt is mature (fig. 83). Cementum covers almost all the root surface except for a very small area immediately adjacent to the end of the gutta percha root filling. The fibrous tissue in relation to the apical end of the gutta percha root filling is loose and oedematous and heavily infiltrated with lymphocytes and plasma cells, with an occasional polymorphonuclear leukocyte. In this loose, oedematous connective tissue are to be found macrophages containing particles of what is probably root canal sealer or gutta percha root filling material (fig. 84).

In the other specimen, an apical granuloma has formed with dense infiltration with lymphocytes and plasma cells throughout the resection crypt region, but with especially heavy concentrations of these round cells opposite the gutta percha root filling (figs. 85, 86 and 87). A few bars of bone have formed in the peripheral part of the resection crypt.

A thick layer of new cementum has covered about two thirds of the resection surface, the inner third of the
resection surface around the root filled canal not being covered. Incremental lines are seen in the layer of cellular cementum over the resection surface.
Fig. 83. Photomicrograph x 25, dog B, 52 weeks.

nc new cementum deposited on resection surface

nb mature new bone
Fig. 84. Photomicrograph x 400, dog B, 52 weeks.

mac  macrophages containing foreign material, possibly root canal sealer or gutta percha root filling material

ct  oedematous connective tissue
Fig. 85. Photomicrograph x 25, dog A, 52 weeks.

nc new cementum deposited on resection surface
ic concentration of chronic inflammatory cells
Fig. 86. Photomicrograph x 25, dog A, 52 weeks.

sp  gutta percha root filling

ap gran  apical granuloma
Radiographic evidence of healing of apicectomy wounds in dogs

Post-mortem specimens of resected teeth and surrounding tissues were radiographed as described in the Appendix.

A strictly standardised radiographic technique, as advocated by DAWKINS (1958b) was not used. No radiographs were taken during the life of the dogs. Therefore, there was no radiographic evidence of the different stages of healing in any one dog. (A technique for intra-oral radiography of living dogs has been described by KOCH (1938).)

For the reasons given above, no radiographs of specimens are included here because of their limited value. However, the radiographic evidence of healing correlated with the histological findings in individual dogs. For example, all specimens, with one exception, eight weeks post-operative and later, showed radiographic evidence of filling of the resection crypt with new bone. The exception mentioned was one of the 52-week specimens, in which a radiolucency was present in the region of the resection crypt. This radiolucency corresponded to the apical granuloma found in this specimen.
CHAPTER 14

DISCUSSION

Appraisal of the results obtained in this investigation reveals that the general pattern of healing of apicectomy wounds in dogs conforms to that of wound healing in general.

Phases of healing

The healing of apicectomy wounds in dogs may be divided into four phases, namely, the phase of traumatic inflammation, the phase of phagocytosis (phase of destruction — LOCALIO et al, 1943), the phase of proliferation, and the phase of maturation, as considered by LOCALIO et al (1943) and DOUGLAS (1963).

The phase of traumatic inflammation

As early as two hours post-operatively, classical changes characteristic of acute inflammation were seen in the apicectomy wound. These changes were seen in the muco-periosteal flap and indicated by the presence of congested, dilated capillaries and venules with swollen endothelial walls, and the presence of many polymorphonuclear leukocytes in the lumen of these blood vessels. There was margination
of leukocytes, emigration of some of these into the tissue spaces, and diapedesis of red blood corpuscles. — all these features being typical of an acute inflammatory response to injury (MENKIN, 1950; MUIR, 1958).

This acute response was at its maximum after eight hours in the mucoperiosteal flap. Resolution had commenced by 24 hours. For example, after 24 hours, appreciably fewer polymorphonuclear leukocytes were present in the tissue spaces of the flap than were seen at eight hours, and there was less engorgement of the small blood vessels with red blood corpuscles and acute inflammatory cells.

The blood supply to different parts of the wound appeared to have some relationship to these changes. This was well illustrated in the delayed appearance at eight hours (compared with two hours in the mucoperiosteal flap) of increased numbers of polymorphonuclear leukocytes in the clot within the resection crypt. In this part of the wound, the blood clot, especially towards its central part, is relatively well removed from blood vessels, compared with the blood clot in the incision line. It is obvious that it therefore takes longer for emigration of polymorphonuclear leukocytes to take
place into the blood clot within the resection crypt than into the blood clot between the incision surfaces or in the subperiosteal clot.

It was not determined whether a general leukocytosis was evoked as a result of the injury inflicted by the apicectomy operation. It is known that different forms of "stress" such as trauma and haemorrhage may elicit a leukocytosis (MACFARLANE, 1954). Whether a leukocytosis contributed to the number of polymorphonuclear leukocytes seen in the blood clot in different parts of the wound was not determined.

A very interesting finding was the manner in which polymorphonuclear leukocytes were found, after eight hours, to have accumulated in a concentrated band in relation to the apical end of the gutta-percha root filling material. This material (which is not pure gutta-percha, generally containing additives inserted by the manufacturer according to SKINNER, 1947) appeared to exert a definite positive chemotactic attraction for the polymorphonuclear leukocytes.

A disturbing feature found in the two-hour to 24-hour specimens was the presence of degenerative and necrotic changes in some parts of the epithelium. These changes were
confined to the region near the buccal sulcus and on the palate where local anaesthetic with vasoconstrictor had been locally injected by infiltration. The exact cause of these changes was not determined, but they may be related to faulty technique such as perhaps inadvertent deposition of some of the anaesthetic solution within the epithelial layer instead of into the corium in spite of care taken to avoid this. This could account for some of the small vesicles seen within the epithelium. The changes might also be due to the composition of the sterile anaesthetic solution used, and unfavourable tissue reactions in the dog to this solution. It also seems possible that the injection of even a small amount of local anaesthetic containing a low concentration (1:300,000) of adrenaline could produce local changes sufficient to interfere with the nutrition of the epithelial cells. However, it is not possible to form definite conclusions as to whether or not injection of local anaesthetic with vasoconstrictor was the cause of the changes in the epithelium, in the absence of controlled research concerning this.

As well as changes within the epithelium, the inflammatory changes in the connective tissue of the mucoperiosteal flap could be due to the injection of local anaesthetic.
However this appears to be unlikely because the connective tissue on the palatal side, also injected with local anaesthetic solution, did not exhibit similar acute inflammatory changes, at two hours, as were present in the buccal mucoperiosteal flap.

The phase of phagocytosis

This phase follows closely on, and is partly coincident with, the phase of acute traumatic inflammation. These changes are concerned with the "clearing-up" process (Muir, 1958) in the wound. Polymorphonuclear leukocytes are concerned with the ingestion of fibrin of the blood clot. In the acute defensive reaction, some of the polymorphonuclear leukocytes become non-viable or actually die and fragment. The damaged and dead leukocytes, and material not required by the wound for repair, are ingested by macrophages (Muir, 1958).

In this investigation, macrophages were seen in the fibrin clot between the incision surfaces after 24 hours.

In the resection crypt, after 48 hours, many fragmented polymorphonuclear leukocytes were seen especially in relation to the gutta percha root filling material. After seven days, macrophages were present in the resection crypt, some con-
taining enclosed degenerate and fragmented polymorphonuclear leukocytes, and others containing blood pigment. Macrophages containing blood pigment were especially in evidence in one of the two-week specimens. In the same two-week specimen, macrophages containing non-viable and dead polymorphonuclear leukocytes were seen, especially in the periphery of a concentration of polymorphonuclear leukocytes around the epithelium surrounding a fragment of chromic catgut suturing material. The manner in which the epithelium enveloped the catgut resembles that in which epithelium invaginates in order to surround and expel superficial retained root remnants and bone fragments (GLICKMAN et al, 1947; SMITH, 1958).

SIMPSON (1959) reported the replacement of the fibrinous exudate under mucoperiosteal flaps in macacus rhesus monkeys by young connective tissue seven days after operation. This was verified in the present investigation. This removal of fibrin comes under the phase of phagocytosis while its replacement by young connective tissue is part of the next stage, the phase of proliferation.
The phase of proliferation

Under this heading will be considered the proliferation of epithelium, and fibroplasia as seen in the healing of apicectomy wounds in dogs.

After two hours, the incised epithelial surfaces are inverted. GILLMAN et al (1955) noted that, concerning the epithelialisation of epidermal wounds, the "epithelium always inverts and then actively grows into the incision, even when evertting sutures are used for closure". This was borne out in the present investigation. Inversion of the epithelium in the incision line was even more pronounced after eight hours. By 24 hours, proliferation of the inverted epithelium had occurred. In one of the specimens this had progressed to actual union of the epithelium from each side of the incision line across the incisional gap, although this was only present in some parts of the incision line and the band of union was only one or two cells thick. Hydrophic changes were seen in the prickle cell layer after 24 hours and again after 48 hours. By 48 hours, the epithelial surfaces were well united by a thick wedge-shaped band of epithelium. This wedge-shaped band was still evident after seven days. Hydrophic changes
in the prickle cell layer were considerable in one of the seven-
day specimens. After two weeks the epithelium in the incision
line was of normal thickness. Only a slight surface depression
marked the site of the incision. It was impossible to
distinguish histologically the site of the incision after four
weeks.

The epithelium in the incision line was quickly dif-
ferentiated into parakeratotic, prickle cell and basal cell
layers by 48 hours. This is in accord with the findings of
VIZIAM et al (1964) who found that differentiation of the
epithelial cell layers had occurred 48 hours after shallow
incised wounds in the skin of rabbits.

In this investigation the relative importance of
epithelial migration and epithelial proliferation in the
coverage of the wound was not determined. As VIZIAM et al
(1964) pointed out, it would be necessary or desirable to use
colchicine and tritiated thymidine or similar techniques in
order to elucidate this.

The fact that the epithelium is thickest in the incision
region after 48 hours would seem to imply agreement with
BULLOUGH and LAURENCE (1957, 1961) who found that most of the
epithelial mitoses were within one millimetre of the incision line after epidermal wounds in mice, and that mitotic activity was greatest in the region of the incision, gradually reducing in intensity with distance from the incision line. VIZIAM et al (1964) also found that epithelium is thickest in the region of the incision and that the thickness of the epithelial layer diminishes with distance from the wound margins until it gradually merges with normal epithelium.

The first evidence of fibroplasia in this investigation was after 24 hours, when a few fibroblasts were present at the margins of the fibrin clot between the incised surfaces of the corium in the incision line. Fibroblasts were more in evidence after 48 hours in the same region and in the blood clot between the periosteum of the flap and the unoperated alveolar bone. The first appearance of fibroblasts in the blood clot in the resection crypt was after 48 hours, in some parts of the periphery of the crypt. EDWARDS and DUNPHY (1958) reported that fibroblasts can be demonstrated in the wound as early as 24 hours and are most numerous after 72 hours. DEVITO (1965) stated that there is a rapid increase in the fibroblast population in the wound between 72 hours and 5 days.
These references to the time of appearance of fibroblasts are borne out by the present findings.

After 7 days, fibroplasia had progressed considerably in the apicectomy wound. The blood clot in the incision line and under the flap had been invaded and replaced (that is, organised) by young connective tissue. In the resection crypt also, organisation of the blood clot was very well under way, the clot being approximately one-quarter replaced. Fibroplastic and endothelial proliferation from the periphery of the resection crypt proceeded, the granulation tissue invading the central blood clot which was thus gradually reduced in size, until it was three-quarters or more replaced after two weeks. The manner in which the blood clot in the resection crypt is invaded by the young vascular connective tissue is similar to that seen in the replacement of the blood clot in alveolar socket wounds after tooth extraction in dogs, other animals and humans (EULER, 1923a; SCHRAM, 1929; CLAFLIN, 1936; HUEBSCH et al, 1952; SIMPSON, 1960; MANGOS, 1941). The time of appearance of fibroblasts in the resection crypt (two days) and the time taken for complete organisation of the entire blood clot (between two and four weeks) in the resection crypt, were the same as those in the alveolar socket after
extraction of teeth in dogs (EULER, 1923a; SCHRAM, 1929; CLAFLIN, 1936).

There seems to be a correlation between time of appearance of fibroblasts and time taken for organisation of the blood clot on the one hand, and the blood supply in different parts of the wound on the other. For example, fibroblasts made their appearance in the incision line after 24 hours, but were not seen in the resection crypt until 48 hours, possibly because all parts of the clot in the incision line were closer to a blood supply. Only the peripheral parts of the blood clot in the resection crypt are relatively close to a pre-existing blood supply from vessels in the bony wall of the resection crypt and from vessels in the periosteum of the part of the flap overlying the resection crypt. Again, the blood clot in the incision line, and that in the space between the flap and the unoperated bone, lie in a sheet between the incision surfaces and in the narrow subperiosteal space respectively. Because of the proximity of blood supply, the clot in these regions was readily organised after seven days, compared with the time taken (between two and four weeks) for complete organisation of the mass of blood clot in the resection crypt.
The formation of new bone within the resection crypt is part of the phase of proliferation. Osteogenesis was first observed in the resection crypt at seven days. The new bone was replacing the young connective tissue which had itself replaced the blood clot. Osteogenesis in the wound will be discussed later.

The phase of maturation

Young connective tissue was present between the incised surfaces of the corium after seven days. In the same region, after two weeks, there had been considerable formation by fibroblasts of mature collagen fibres of almost normal thickness. The arrangement and thickness of the collagen fibres in the incision region was indistinguishable from normal after four weeks. The fibrocytes of the connective tissue here were also indistinguishable from the fibrocytes elsewhere.

Osteogenesis in the experimental apicectomy wound

The first evidence of new bone formation in this investigation was on the bony walls of the resection crypt at seven days post-operatively. Osteogenesis was sufficiently advanced at this stage to conclude that it had commenced
before seven days. No specimens were available between two and seven days, from which to ascertain when the very first formation of new bone occurred. The utilisation of intermediate intervals and histochemical techniques would have allowed such an estimation to be made.

At seven days there was a suggestion of early new bone matrix in some places on the surface of the unoperated bone, subperiosteally in the region adjacent to, but not over, the resection crypt. However, in all specimens the periosteum of the part of the flap overlying the resection crypt appeared to act as a limiting plane of tissue only and not to be actively concerned in osteogenesis. This agrees with the findings of HATTON and SCHRAM (1929) after surgical removal of teeth in dogs.

The face that osteogenesis from the periosteum was not observed does not exclude the possibility that it could occur given the required stimulus, as periosteum is potentially osteogenic. Periosteum is, in fact, actually osteogenic during the healing of fractured bones (HAM, 1957).

Osteogenesis proceeded rapidly in the resection crypt after seven days so that by two weeks much of the young con-
nective tissue in the crypt had been replaced by new bone. At this stage, there was still some blood clot remaining in the central part of the crypt.

After four weeks, the resection crypt was filled with new bone in all parts. The new bone by this time was fairly mature, remodelling having commenced.

The process of remodelling of the new bone continued by a process of apposition and resorption. Osteoclasts were much in evidence in areas of bone resorption. Remodelling of bone is influenced by functional demands. In this context, it is recalled that the upper first premolar of the dog is not in contact with lower teeth when the teeth are in occlusion. Therefore, the functional demands upon the upper first premolar would probably be different from those upon teeth which are in occlusion. However, according to SCOTT and SYMONS (1961), these teeth are "used for carrying objects such as bones, sticks, etc."; there is therefore some function demanded of these teeth, and probably remodelling is determined to some extent by the degree and nature of this function.

The process of remodelling of bone formed in the resection crypt continued. Even after 18 weeks, there were
still a few osteoclasts present in this bone. The bone resembled normal cancellous alveolar bone at this stage, with a cortical layer buccally and palatally. The thick trabeculae of bone seen at eight weeks opposite the resection surface were not seen at this time. The bone in the resection region was quite normal by 26 weeks.

The response of the bone to surgery was quite favourable in that there was little evidence of necrosis of bone (as would be shown by the presence of empty lacunae) as a result of drilling into bone. This absence of bone necrosis is attributed to efficient cutting of bone with a sharp bur under continuous irrigation with normal saline coolant and to the avoidance of unnecessary trauma whilst removing the resected root apex. THOMPSON (1958) found that aseptic thermal necrosis of bone resulted from drilling into bone without using coolant. COSTICH et al (1964) found that bone repair in dogs proceeded more rapidly following the production of defects in bone by using ultra-speed bone cutting and coolant than by using conventional drilling speeds with coolant. Where the bone cutting was performed using conventional drilling speeds without coolant, the healing was delayed. Although actual necrosis was not in evidence, there was an acute inflammatory
response in the bony walls of the resection crypt, as shown by
the presence of polymorphonuclear leukocytes in the adjacent
marrow spaces after eight hours. There were still appreciable
numbers of these leukocytes in the adjacent marrow spaces
after 48 hours. At the next post-operative interval, seven
days, increased numbers of polymorphonuclear leukocytes were
no longer in evidence in the marrow spaces.

**Cementogenesis in the experimental apicectomy wound**

Observations of some of the sections of one of the two-
week specimens revealed the formation of material upon sections
of the periphery of the root resection surface. The material
formed was suggestive of cementoid. On the surface of the
cementoid material there was an alignment of cells. Some of
these cells were somewhat elongated and not cuboidal as is
typical of cementoblasts. Some of the cells were enclosed
within the matrix, in a manner similar to that in which
cementocytes are enclosed in cementoid. The author is of the
opinion that the material found on parts of the periphery of
the resection surface in this two-week specimen may be actually
cementoid. In support of this is the paper of HELD (1951)
who stated, after examining the first layers of cementum formed
on the roots of young human teeth that:
It is clearly seen that there is a progressive transition from the periodontal connective tissue towards the cementum.

The fibroblast sometimes presents a more or less contracted shape, sometimes it develops stellate shape, forming a network by anastomoses of its extensions. Vacuoles appear which seem to incorporate themselves progressively into the growing cementum. ....we have never had the opportunity of clearly following the transition from the fibroblastic state to the cementoblastic state; the only thing we are able to observe is the enclavement of few fibroblasts in the outer cementum.

ORBAN (1957), discussing normal cementogenesis, stated that cuboidal cells, the cementoblasts, originate by differentiation of the loose connective tissue in contact with the root surface.

In this investigation the first evidence of new cementum formation on the resection surface was invariably towards the peripheral part of the resection surface. The manner in which connective tissue fibres tended to curve around the peripheral part of the resected root was frequently observed in the specimens studied in this investigation (fig. 57 and 58). This arrangement of connective tissue fibres bears some resemblance to the cushioned hammock ligament seen in the apical peripheral region of developing tooth roots. The first site of cementogenesis, at the periphery
of the resection surface, may be related to the site of the closest cementoblasts, which is immediately coronal to the periphery of the resection surface on the surface of the old cementum covering the lateral aspects of the tooth.

Definite evidence of new cementum was observed in four week apicectomy specimens. The deposit of new cementum was quite thick in places on the resection surface. It was evident that resorption of old cementum and dentine often preceded the apposition of new cementum.

By eight weeks, almost the whole of the resection surface was covered by a layer of new cementum in one of the specimens, although the layer of new cementum was thin on most parts of the resection surface.

In the 18-week specimens, there was a thick layer of new cellular cementum over the resection surface. Again, the new cementum did not quite reach the region of the root-filled canal. It may be that the foreign body in the root canal exerted some inhibition to cementogenesis.

Incremental lines of Salter were seen in most of the later specimens exhibiting deposition of new cementum,
indicating periodic formation and resting periods during cementogenesis (ORBAN, 1957).

It was interesting to observe the formation of a thick layer of cementum upon the resection surface (except near the root filling where there was a very heavy concentration of round cells) in one of the 52-week specimens which had developed an apical granuloma. This is not surprising when reference is made to THOMA (1954) who stated that, in the apical region of teeth with apical granulomas, "deposit of cementum may occur only at the border line of infected and normal periodontal membrane, where the mildly irritating effect of the inflammation stimulates cementoblastic activity. .....

There is no deposit of cementum in the region where the inflammatory tissue is in contact with the root." TAGGER (1964) induced periapical lesions in rats, and observed that continuous apposition of cellular cementum proceeded in the apical region of the teeth even in the presence of gross periapical lesions.

Arrangement of the apical connective tissue

The process of cementogenesis during the life of a tooth is intimately associated with the biology of the fibres
of the periodontal membrane. The arrangement of the various groups of principal periodontal fibres has been described by ORBAN (1957), who stated that fibres of the apical group are "irregularly arranged and radiate from the apical region of the root to the surrounding bone".

This kind of arrangement was not found in the orientation of the apical connective tissue fibres following apicectomy. In most specimens, the fibres were observed to run approximately parallel to the resection surface and to the surface of new alveolar bone facing the resection surface. The spaces in which Sharpey's fibres were embedded were clearly seen in the old cementum on the lateral aspects of the tooth, but they were not evident in the new cementum formed on the resection surface. The fact that the upper first premolar of the dog is not in contact with an antagonist in the mandible could mean that there is little functional stress in the apical region and that this lack of functional stress could be reflected in the parallel orientation of the apical connective tissue. On the other hand, this parallel arrangement could exert a cushioning effect when stresses are applied along the longitudinal axis of the tooth. It seems probable that such stresses could be in force while dogs are holding objects in the premolar region.
As well as cementogenesis occurring on the root resection surface it also took place in relation to detached fragments of dentine and old cement seen in one of the specimens. The manner in which the fragments and the injured tooth surface were repaired by deposition of new cementum was as described by FIGG (1928).

Responses to endodontic materials

The materials used to fill the root canal were gutta percha points, and Kerr's "Tubli-seal" root canal sealer. Any "Tubli-seal" exposed in the resection region was in the form of a very thin ring around the resected surface of the apical end of the gutta percha root filling. No concentrations of cells were noted near the peripheral portions of the root filling, where the influence of any exposed "Tubli-seal" could be expected to be found. Any concentrations of inflammatory cells were usually related to the whole of the resected surface of the apical end of the root filling and therefore, presumably, to the resected surface of the gutta percha point.

The tissue response to the gutta percha root filling material was extremely variable. It varied from encap-
sulation of the end of the root filling by fibrous tissue free of inflammatory cells, as described by COOLIDGE (1930), through encapsulation by fibrous tissue infiltrated by round cells (AUSENBERG, 1931; HERBERT, 1943) to heavy concentrations of polymorphonuclear leukocytes in relation to the gutta percha root filling material. It may be that some of the inflammatory changes were related to additives (SKINNER, 1947) present in the gutta percha points. The exterior of the gutta percha points would certainly have been sterile, the points having been immersed in instrument sterilising solution for many hours or even days, prior to their use. Whether the interior of the gutta percha points could possibly be contaminated after such treatment was not determined. However, it would seem to be unlikely.

In one specimen a fragment of gutta percha root filling material was present loose in the resection crypt. After one week it was surrounded by young connective tissue free of inflammation.

Whatever the final tissue reaction to the gutta percha material was, the initial reaction to its presence was invariably the formation of a band of polymorphonuclear leukocytes in relation to it. This was seen as soon as
eight hours post-operatively.

The inflammatory changes evoked by the gutta percha material might be due, especially in the early stages, to mechanical effects of a relatively rough gutta percha resection surface. A feature noted was the apparent absence of foreign body giant cells in relation to the foreign body in the root canal. However, macrophages were observed in some of the specimens, containing fragments of foreign material which were probably particles of either root canal sealer or gutta percha root filling material.

Some further aspects of pathology of experimentally resected teeth

In the present investigation, apicectomy was carried out on teeth which had vital, and presumably sterile normal pulps. Clinical apicectomies on human patients are performed when indicated on teeth with pulpal, and in most cases, periapical, involvement.

It is also important to realise that the actual operation wound did not exactly simulate a clinical apicectomy wound, in that a clinical periapical lesion, especially if it be a very chronic apical granuloma or radicular cyst, is
usually surrounded by a layer of cortical bone, like the lamina dura of a tooth socket; whereas, the experimental wound produced in this investigation had a bony wall consisting of wounded cancellous bone with bur-invaded marrow spaces. The only part of the bony wall of the experimental wound, with a layer of compact bone similar to that which might form the bony wall of a periapical lesion, was that part of the lamina dura which was not disturbed by drilling. This was usually in those parts related to the palatal, and sometimes apical, aspects of the resected apex.

The fact that a pre-existing periapical lesion is present in the clinical apicectomy could feasibly alter the pattern of healing. Moreover, the resection surface of the clinically resected tooth could afford a less receptive surface for the deposition of new cementum, than the resection surface of a tooth, which had a vital sterile pulp immediately prior to the operation.

Comparison of experimental results with findings in humans

Evidence of healing of apicectomy wounds in humans is based mainly on post-operative radiographic and clinical findings. DAWKINS (1958a) found that it takes 24-32 weeks
for radiographic evidence of complete filling-in with bone of the root resection crypt. The radiographic technique used in the present investigation was not standardised, but it is clear that only 8-18 weeks were required in dogs for radiographic evidence of complete filling of the resection crypt with bone.

There appear to be no reports in the literature concerning when new cementum is first formed on human teeth after apicectomy. Reference has been made in the review of the literature to histological findings in human teeth extracted, usually some years, after apicectomy (COOLIDGE, 1930; AISENBERG, 1931; MOEN, 1940; HERBERT, 1941, 1943). These workers found that a deposit of new cementum, partial or complete, may be formed on the resection surface. The present investigation confirmed that new cementum may form on the resection surface after apicectomy in the dog also. MOEN (1940) pointed out, and this was confirmed here, that "in no case did we find cementum building over the apical (end of the root filling)".
Comparison of present experimental findings with previous experimental investigations of healing of apicectomy wounds

As described under "cementogenesis", a deposit of new cementum was found on the resection surface certainly as early as four weeks. The work of BAUER (1922, 1925) has been discussed at length in the review of the literature. He studied histologically the healing of apicectomy wound in cats and obtained post-operative specimens from one to six months. He did not report his findings in the one month specimen. His two month specimen showed considerable deposition of new cementum ("bone-cement") over almost all the root resection surface. A dense region at the apical end of the gutta percha root filling is seen in one of his drawings. This may represent a fibrous capsule or accumulation of inflammatory cells. BAUER (1922) makes no reference to this region of apical density in his paper, however.

The present investigation confirmed the repair of detached dentine and old cementum fragments and injured tooth surfaces, as described by BAUER (1922).

No evidence was found in the present study of the formation of a bony ankylosis (BAUER, 1922) between the resection surface and the new bone. This has been dis-
cussed in the review of the literature, in which it was pointed out that there was no evidence produced by BAUER (1922) himself for such an ankylosis.

In the present study, it was found that apposition of new cementum was often preceded by resorption of the resection surfaces, whereas BAUER (1922) found that there had been very little resorption prior to deposition of cementum. The work of EULER (1923b) has been discussed in the review of the literature and does not need to be enlarged upon here.
CONCLUSIONS

1. The early phases of healing of apicectomy wounds in dogs as observed histologically, conform to those exhibited in wound healing generally.

2. The early tissue changes, fibroplasia and osteogenesis, proceed in the root resection (apicectomy) crypt in a similar manner to these processes as observed in the alveolar socket after tooth extraction.

3. Osteogenesis in the root resection crypt commences before seven days post-operatively.

4. Cementogenesis occurs on the root resection surface after-apicectomies on dogs, confirming earlier reports on human and experimental material following apicectomy.

5. Cementogenesis on the root resection surface commences possibly as early as two weeks, and certainly before four weeks, post-operatively.

6. Epithelial coverage of the incision region is complete by 48 hours post-operatively.
7. Gutta-percha root filling material sometimes evokes an inflammatory response, but is well tolerated by the tissues of some dogs.
APPENDIX

Composition of "Tubli-Seal" root canal sealer

The approximate composition of mixed "Tubli-Seal" according to the manufacturers, Kerr Manufacturing Company, is:

<table>
<thead>
<tr>
<th>Component</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>Zinc Oxide</td>
<td>59.0%</td>
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<tr>
<td>Bismuth Trioxide</td>
<td>7.5%</td>
</tr>
<tr>
<td>Thymol Iodide</td>
<td>5.0%</td>
</tr>
<tr>
<td>Oleo Resins</td>
<td>18.5%</td>
</tr>
<tr>
<td>Oil and Waxes</td>
<td>10.0%</td>
</tr>
</tbody>
</table>

Fixation of specimens

The specimen was left in an adequate volume of the 10% buffered formalin for at least 48 hours to ensure thorough fixation. (The specimens, after decalcification, went into formalin again in the tissue processor.)

Radiographs of post-mortem specimens

Before decalcification, each specimen was radiographed bucco-palatally and mesio-distally. The radiographic films used were Kodak Ultra-speed Dental x-ray films, (Kodak code number DF 45). An exposure of 0.1 seconds with a cone-object distance of 3", using a Siemens portable 50 kV, 7 ma, x-ray machine was made.
Decalcification of specimens

After fixation, the specimens were decalcified in formic formate after carefully removing the amalgam filling in the tooth with a tungsten carbide bur driven by a water-cooled air-rotor. Decalcification of specimens took from 12-20 days, as determined by radiographic control, using a 7" cone-object distance, with a Siemens portable 50 kV, 7 ma, x-ray machine and Kodak Ultra-Speed Dental x-ray films. (The formic formate decalcifying fluid used was made by dissolving 140 G. of anhydrous sodium formate in 800 ml. of 100°/o formic acid, and then making up the volume to 2000 ml. with distilled water.)

Neutralisation of decalcifying fluid

After decalcification, the specimens were placed in 5°/o sodium sulphate solution for several hours in order to neutralise the acid decalcifying fluid.

Processing of specimens

The fixed, decalcified specimen was given to the histology laboratory technicians for processing, which was carried out in the "Elliott" Tissue Processor (Elliott, Liverpool, Ltd.). The specimen was processed as follows:
<table>
<thead>
<tr>
<th>Beaker</th>
<th>Solution</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st beaker</td>
<td>10% buffered formalin</td>
<td>4 hours</td>
</tr>
<tr>
<td>2nd beaker</td>
<td>70% alcohol</td>
<td>1/2 hour</td>
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<tr>
<td>3rd beaker</td>
<td>80% alcohol</td>
<td>1/2 hour</td>
</tr>
<tr>
<td>4th beaker</td>
<td>95% alcohol I</td>
<td>1 hour</td>
</tr>
<tr>
<td>5th beaker</td>
<td>95% alcohol II</td>
<td>1 hour</td>
</tr>
<tr>
<td>6th beaker</td>
<td>absolute alcohol I</td>
<td>1 hour</td>
</tr>
<tr>
<td>7th beaker</td>
<td>absolute alcohol II</td>
<td>1 hour</td>
</tr>
<tr>
<td>8th beaker</td>
<td>absolute alcohol III</td>
<td>1 hour</td>
</tr>
<tr>
<td>9th beaker</td>
<td>chloroform I</td>
<td>1 1/2 hours</td>
</tr>
<tr>
<td>10th beaker</td>
<td>chloroform II</td>
<td>1 1/2 hours</td>
</tr>
<tr>
<td>11th beaker</td>
<td>Paraffin Wax I</td>
<td>1 1/2 hours</td>
</tr>
<tr>
<td>12th beaker</td>
<td>Paraffin Wax II</td>
<td>1 1/2 hours</td>
</tr>
</tbody>
</table>

To ensure thorough impregnation of the specimen with paraffin wax, the specimen was then transferred into paraffin wax III in a "Labco" vacuum embedder (Sunvic Controls Ltd., London) at 60-65 degrees Centigrade for half an hour. The paraffin wax used was of superior quality ("Bioloid" paraffin embedding compound M.P. 53-55°C, Will Scientific Inc. U.S.A.).

The author was assured by the histological technician (The Queen Elizabeth Hospital, Woodville) that results obtained by using this wax were as satisfactory for hard tissues as those obtained by using double-embedding techniques.
trial specimen containing bone and tooth-structure was
doubly-embedded and histological sections cut and stained.
The quality of these stained sections was approximately the
same as that of sections obtained after single embedding with
paraffin wax.

After impregnation with paraffin wax, the specimen
was blocked in paraffin wax.

**Section cutting**

Except for one of the earlier-obtained specimens
(Dog C, 18 weeks) and some trial specimens, which were cut at
6 microns, all sections were cut at a thickness of 8 microns.
The sections were cut by the author under the supervision of
a histological technician. The microtome used was the
"Cambridge" rocking microtome (Cambridge Instrument Co. Ltd.,
London and Cambridge).

All sections were cut bucco-palatally (bucco-
lingually) commencing from the mesial aspect of the specimen.
Step sections were cut through the specimen, giving sections in
planes mesial to the tooth, through the tooth longitudinally,
and distal to the tooth. Up to 75 sections of each tooth
were cut.
The sections were floated onto the surface of a thermostatically controlled water-bath and any creases removed by gentle teasing with the tip of a moistened camel-hair brush.

When the sections were flattened out, they were transferred onto labelled, albuminised glass slides. Excess water was allowed to drain off the slides by placing them upright. After a minute or so, the slides were placed into an incubator to dry out overnight.

Staining and mounting of specimens

The sections were stained with Lillie-Mayer's alum haematoxylin and with eosin (1°/o eosin in 2°/o calcium chloride) following a routine technique as follows (CULLING, 1963):

1. Removal of paraffin wax from section with xylol.
2. Hydration of section through graded alcohols.
3. Staining with haematoxylin and eosin.
4. Dehydration of section through graded alcohols.
5. Clearing of section with xylol.
6. Mounting the section under a coverslip with P.I.X. mounting medium.
REFERENCES

AISENBERG, M.S. 1931.
Root resection after four years: report of a case.

ALLGOWER, M. 1956.
Cellular basis of wound repair.

—— and HULLIGER, L. 1960.
Origin of fibroblasts from mononuclear blood cells:
a study on in vitro formation of the collagen precursor,
hydroxyproline, in buffy coat cultures.
Surgery, 47:603-610.

ALLING, C.C. and KERR, D.A. 1957.
Trauma as a factor causing delayed repair of dental
extraction sites.

Histological and histochemical investigation of human
alveolar socket healing in undisturbed extraction wounds.

Reticular and collagen fibre characteristics in human
bone healing.

AREY, L.B. 1936.
Wound healing.

BANCROFT, F.W. 1914.
Process of bone repair following trauma.

BAUER, W. 1922.
Histologische Befunde an Zähnen nach Wurzelspitzenam-
putation.
1925.

BEACH, H.H. 1902.
in Discussion, Nat. Dent. Ass.
Dent. Cosmos, 44:385-386.

BEUBE, F.E. 1949.
Factors in the repair of alveolar bone and cementum.

--- and SILVERS, H.F. 1934.
Influence of devitalised heterogenous bone powder on regeneration of alveolar and maxillary bone of dogs.

BLAYNEY, J.R. 1932.
Fundamentals governing pulp-canal therapy.
Dent. Cosmos, 74:635.

--- and WACH, E.C. 1924.
A study of tissue repair around a resected root end.
Dent. Forum, 1:58.

BLUM, T. 1932.
Additional notes on root amputation, including a study of 38 new cases.

Incidence of fractures of cementum in human dentitions.

BORDEN, S.M. 1948.
Histological study of healing following detachment of tissue as is commonly carried out in the vertical incision for the surgical removal of teeth.

BOULGER, E.P. 1928.
Histologic study of a specimen of fractured roots.
1933.
The foreign body reaction of rat tissue and human tissue to gutta percha,

BOYLE, P.E. 1934.
Intracellular bacteria in a dental granuloma.

BOYNE, P.J. 1966.
Osseous repair of the post-extraction alveolus in man.

Effects of osseous implant materials on regeneration of alveolar cortex.

Fluorescence microscopy of alveolar bone repair.

BROPHY, T.W. 1880.
Caries of the superior maxilla.

BULLOUGH, W.S. and LAURENCE, E.B. 1957.
A technique for the study of small epidermal wounds.

The control of mitotic activity in the skin.
Wound healing: A symposium.

Age, gonadectomy and wound healing in the palatal mucosa of the rat.

A comparison of healing rate of bone after the production of defects by various rotary instruments.
CAMPANI, M. 1960.
Research on cutaneous wound healing.
Dent. Abstr., 5:115-116, (from Panminervi Medica,
1:120-121, 1959).

The biochemistry of wound healing.

CLAFLIN, R.S. 1936.
Healing of disturbed and undisturbed extraction wounds.

COOK, T.J. 1929.
Dental granuloma ten years after apicectomy.

COOLIDGE, E.D. 1928.
Pulp pathology and treatment problems.

——— 1930.
Root resection as a cure for chronic periapical infection:
A histologic report of a case showing complete repair.

——— 1931.
The reaction of cementum in the presence of injury and
infection.

——— 1932.
Pathology, diagnosis and treatment of the pulp and
preparation of root canals for filling.

——— and KESEL, R.G. 1956.
A textbook of endodontontology.

A study of the effects of high-speed rotary instruments
on bone repair in dogs.
Handbook of histopathological techniques.

A radiographic study of the rate at which human
extraction wounds heal.

DALTON, W.J. 1952.
A study of the healing process following operative
interference with the continuity of the rat maxilla.

DAVIS, W.C. 1920.
Histopathology of the cementum as related to pulp canal
surgery.

DAWKINS, J. 1958a.
An apparatus for obtaining serial roentgenograms.

—— 1958b.
An investigation into bone healing following apicectomy.

A histological study of mucoperiosteal flap healing.

DÉSIRABODE, A.M. 1843.
Nouveaux éléments complets de la science et de l'art du
dentiste.
Paris, Labe.

DEVITO, R.V. 1965.
Healing of wounds.

DIVINELLE, W.H. 1880.
in Proc. N.Y. Odont. Soc.
Histological verification of results of root canal 
therapy in experimental animals. 

Wound healing and management. 
A monograph for surgeons. 

DOW, P.R. and INGLE, J.I. 1955. 
Isotopic determination of root canal failure. 

DUNPHY, J.E. 1960. 
On the nature and care of wounds. 

--- 1963. 
The fibroblast — ubiquitous ally for the surgeon. 

--- and UDUPU, K.N. 1955. 
Chemical and histochemical sequences in the normal 
healing of wounds. 

EDWARDS, L.C., PERNOKAS, L.N. and DUNPHY, J.E. 1957. 
The use of a plastic sponge to sample regenerating 
tissue in healing wounds. 

--- and DUNPHY, J.E. 1958. 
Wound healing. I. Injury and normal repair. 

EULER, H. 1923a. 
Die Heilung von Extrakttionswunden: eine tierexperimentelle 
studie. 

--- 1923b. 
Experimentelle studien über den Heilverlauf nach 
Wurzelspitzenresektionen und über den Einfluss verschiedener 
Wurzelfüllungsmaterialen auf den Heilverlauf. 
FARRAR, J.N. 1884.
Radical and heroic treatment of alveolar abscess by amputation of roots of teeth.
Dent. Cosmos, 26:79-81, 135-139.

FIGG, W.A. 1928.
A tear in cementum.

FISH, E.W. 1948.
Surgical pathology of the mouth.

FISHBEIN, J.G. 1943.
A chemical and radiographic study of the use of synthetic bone paste in root resection crypts.

FREEMAN, N. 1931.
Histopathological investigation of the dental granuloma.

GARBER, F.N. 1964.
Roentgenolucent periapical areas.

GIBBINS, J. 1964.
Further studies with the electron microscope of the early phases of epithelial migration during wound healing in the oral mucosa of the rat.

GIBSON, T. 1964.
Modern trends in plastic surgery.

A re-examination of certain aspects of the histogenesis of the healing of cutaneous wounds.
A preliminary report.
The healing of extraction wounds in the presence of 
retained root remnants and bone fragments.  
33:263-283.

SMULOW, J.B., O'BRIEN, T. and TANNEN, R. 1963.  
Healing of the periodontium following mucogingival surgery. 

GRAY, H. 1958.  
Anatomy.  
Revised Johnston, T.B., Davies, D.V. and Davies F.  
32nd Ed. London, New York, Toronto, Longmans, Green,  
1604p.

Origin of fibroblasts in wound healing: An autoradiographic  
study of inhibition of cellular proliferation by local  
X-irradiation.  

1964.  
Derivation of fibroblasts in the healing wound.  

Endodontic practice.  

GROVE, C.J. 1916.  
Some important causes of periapical infections.  

1921.  
Nature's method of making perfect root fillings following  
pulp removal, with a brief consideration of the  
development of secondary cementum.  

The relationship between the direction of Sharpey's  
fibres and the deposition of cementum.  
1957).
GUTTUSO, J. 1963. 
Histopathologic study of rat connective tissue responses to endodontic materials. 

HADFIELD, G. 1963. 
The tissue of origin of the fibroblasts of granulation tissue. 

HAIRSTONE, M.A. 1959. 
Structural cytology of the healing wound. I. The fibroblast. 

HAM, A.W. 1957. 
Histology. 

HARNDT, E. 1926. 
cit. by BOYLE, E. re Histō-bacteriological study of 50 dental granulomata. 

HARRISON, J.A. 1943. 
Healing of routine and severely traumatised exodontic wounds. 
The Bur, 43:107.

HARTWELL, S.W. 1955. 
The mechanisms of healing in human wounds. 

HARTZELL, T.B. 1911. 
Root tip amputation. 

HATTON, E.H. 1922. 
Histopathology of apical region of teeth with partly filled root canals. 
Dent. Summary, 42:138-143.
1931.
Histologic studies of living tissue reactions associated with pulpless teeth that may be taken as evidence of a satisfactory or physiologic healing.

Histologic findings in teeth with treated and filled root canals.

and SCHRAM, W.R. 1929.
Bone regeneration following extraction of teeth in dogs.

HEDMAN, W.J. 1951.
An investigation into residual periapical infection after pulp canal therapy.

HELD, A-J. 1951.
Cementogenesis and the normal and pathologic structure of cementum.

HENRY, J.L. and WEINMANN, J.P. 1951.
The pattern of resorption and repair of human cementum.

HERBERT, W.E. 1937.
Results of root resection.
Dent. Record, 51:250.

1941.
Cases treated by root resection.

1943.
Histological examination of two teeth treated by root resection.
HESS, W. 1925.
The anatomy of the root canals of the teeth of the permanent dentition.
3rd Ed. John Bale, Sons and Danielsson Ltd. 199p.

HILL, T.J. 1931.
Regeneration of periodontal membrane after root currettment.

—— 1932.
Experimental dental granulomas in dogs.

HOWES, E.L. 1943.
The rate and nature of epithelisation in wounds with loss of substance.

—— ARMITAGE, C.M. and MANDL, I. 1955.
Enzymes in the healing wound.

The healing process following molar extraction.
I. Normal male rats (Long-Evans strain).

HUNTER, H.A. 1957.
The effect of gutta percha, silver points and Rickert's root sealer on bone healing.

JACKSON, D.S. 1958.
Some biochemical aspects of fibrogenesis and wound healing.

JOLLY, M. 1953.
The formation of bone.

—— and SULLIVAN, H.R. 1956.
A basic approach to endodontic approach.


——— 1932. The present status of the pulpless tooth. The Bur, 32:16.


KUKIDOME, H. 1957.
Histopathological study on healing of periapical tissues after infected root canal treatment in humans.

LACRONIQUE, Dr. 1923.
ocit. in ed. Notes on osseous restoration following apical resection.

Calcium hydroxide as a possible root filling material.

LERICHE, R. and POLICARD, A. 1928.
The normal and pathologic physiology of bone.
St. Louis, Mosby. 236p.

LINGHORNE, W.J. and O'CONNELL, D.C. 1951.
Studies in the regeneration and attachment of supporting structures of the teeth.
II. Regeneration of alveolar process.

LINN, R.H. 1955.
Wound healing in the jaws of hamsters with Alizarin Red S vital dye.

—— 1959.
Alizarin Red S dye in the study of wound healing in the jaws of hamsters.

Wound healing: experimental and statistical study.

LÖE, H. 1959.
Bone tissue formation: A morphological and histochemical study.
Indications and contraindications for endodontic surgery. 

MACDONALD, R.A. 1959. 
Origin of fibroblasts in experimental healing wounds: 
autoradiographic studies using tritiated thymidine. 

MACFARLANE, R.G. 1954. 
The reactions of the blood to injury. 
in Lectures on general pathology. 

McHUGH, W.D. 1957. 
Fluorescence microscopy of healing gingival epithelium 
in dogs. 

MAGITOT, E. 1866. 
Remarks upon local anaesthesia produced by Dr. 
Richardson's ether spray in tooth extraction. 
Dent. Rev. (New Series), 3:115-120.

MANGOS, J.F. 1941. 
The healing of extraction wounds: An experimental study 
based on microscopic and radiographic investigation. 

MARTIN, C. 1889. 
Trepansing of the radicular extremities in the dental 
alveolar periosteum. 

MENKIN, V. 1950. 
Newer concepts of inflammation. 

MEYER, H. 1935. 
Heilungsvorgänge in der Alveole nach normaler 
Zahneextraktion. 
MEYER, W. 1924.
Die Heilung von Extraktionswunden unter abnormen
Verhältnissen.

MOEN, J.K. 1935.
The development of pure cultures of fibroblasts from
single mononuclear cells.

MOEN, O.H. 1928.
Tissue changes in treated teeth of known history.

— 1940.
Verification of results of root resection by photomicro-
graphs.

Studies on periodontal healing.
Dent. Abstr., 5:285 (from Rev. A. Odont. Argentina,

MUIR, R. 1958.
Text-book of pathology.

A study of the mechanisms involved in the restoration of
the epidermis in experimental linear wounds.

ORBAN, B.J. 1957.
Oral histology and embryology.

OTTESEN, I. 1942.
Root resection.

Periapical repair by dense fibrous connective tissue
following conservative endodontic therapy.
PHILLIPS, W.A. and MAXMEN, H.A. 1941.
A practical root resection technique for young permanent anterior teeth.
Dent. Digest, 47:60-64.

PONT, Dr. 1900.
in Discussion at 3rd International Dental Congress.

Toxicity of endodontic materials.

RHEIN, M.L. 1890.
Dent. Cosmos, 32:904.

RICKERT, O.G. and DIXON, C.M. 1931.
The Controlling of Root Surgery.
8th International Dental Congress, Tr. Sect. IIIa.

The mast cells.

ROHNER, A. 1940.
Caixyl als Wurzelfüllungs-material nach Pulpaexstirpation.

Wound healing and collagen formation.

ROSS, W.S. 1933.
Permeability of cementum and reaction to irritation.

——— 1952.
Apicectomy.

SCHRAM, W.R. 1929.
A histologic study of repair in the maxillary bones following surgery.
Introduction to dental anatomy.

A histologic evaluation of periapical repair following positive and negative root canal cultures.

SELVIG, K.A. 1965.
The fine structure of human cementum.

SHAPIRO, M. 1951.
Apicectomy: report and discussion of a case.

SICHER, H. 1959.
Changing concepts of the supporting dental structures.

The healing of extraction wounds.

— 1959.
The reattachment of mucoperiosteal flaps in surgical extraction wounds in macacus rhesus monkeys.

— 1960.
Experimental investigation into the healing of extraction wounds in macacus rhesus monkeys.

SIPPY, B.O. 1927.
Regeneration of tissues following experimental injury of the tooth roots.

The science of dental materials.
SMITH, H.W. 1952.
Alveolar bone regeneration.

The role of epithelium in the healing of experimental extraction wounds.

SOMMER, R.F. 1946.
Essentials for successful root resection.
32:76-100.

Clinical endodontics.

Healing following radical root resection.

Histogenesis of healing of a split-thickness flap in dogs.

STEARNS, M.L. 1940.
Studies on the development of connective tissue in transparent chambers in the rabbit's ear.

A comparative study of three root canal sealing agents.

STONES, H.H. 1934.
The permeability of cementum.

SULLIVAN, D.J. and EPSTEIN, W.L. 1963.
Mitotic activity in wounded human epidermis.
Clinical study of repair of bone after alveolectomy.

TAGGAR, M. 1964.
Behaviour of cementum of rat molars in experimental periapical lesions.

THOMA, K.H. 1954.
Oral Pathology.

THOMAS, N.G. 1922.
Studies in protective cementum development.

Effect of drilling into bone.

TOMES, C.S. 1923.
A manual of dental anatomy, human and comparative.
Ed. Tims, H.W.M. and Henry, C.B.

TORNECK, C.V. 1961.
Reaction of hamster tissue to drugs used in sterilisation of the root canal.

Epithelialisation of small wounds.

Salivary gland ligation and extraction wound healing.

Significance of findings following biopsy and histologic study of 100 periapical lesions.
WASSERMANN, F. 1954.
Fibrillogenesis in the regenerating rat tendon with special reference to growth and composition of the collagenous fibril.

Collagen in wound healing.
Wound healing: A symposium.

WEAVER, S.M. 1947.
Root canal treatment with visual evidence of histologic repair.

The conservative treatment of extensive areas of periapical pathology.

WEISS, P. 1959/60.
The biological foundations of wound repair.

WIDDOWSON, T.W. 1946.
Special or dental anatomy and physiology and dental histology, human and comparative.

WOLCH, I. 1956.
Are apicectomies necessary.

ZANDER, H.A. 1957.
Mechanism of healing of periodontal tissues.

——— 1958.
Continuous cementum apposition.

ZEMSKY, J.L. 1932.
Histological study of roentgenographically-negative buried roots.