EVALUATION OF ANTI-INFLAMMATORY DRUGS BY BILATERAL ORTHOPAEDIC SURGERY IN DOGS

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as University supervisors.

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ABSTRACT

EVALUATION OF ANTI-INFLAMMATORY DRUGS BY BILATERAL ORTHOPAEDIC SURGERY IN DOGS

Both traditional and modern surgery have emperically given importance to various anti-phlogistic measures, following the rationale that the inflammatory process often overshoots its objective as a defense and repair process and becomes excessive and detrimental. The anti-inflammatory drugs used in human surgery have generally been selected according to their performance in patients with rheumatoid arthritis, and essentially the same drugs have been adopted for use in veterinary surgery. Rheumatoid inflammation, however, differs markedly from an acute post-traumatic inflammatory reaction.

There is a lack of reliable models for clinical assessment of anti-inflammatory effects. Recent research in human oral surgery with a rather unique model that allows well controlled studies on how anti-inflammatory drugs may modulate a post-operative course, has for several drugs challenged the common view regarding their efficiency and suitability in controlling post-traumatic sequelae. It has remained an open question whether the findings in oral surgery also apply to surgery and traumata of other parts of the body, e.g. the extremities. Appropriate and well

controlled clinical models for such studies are lacking in human medicine.

The aim of the present work was to establish a properly controlled model for investigations on how steroidal and non-steroidal anti-inflammatory drugs (NSAID) might modulate the signs of the inflammatory reaction and the healing process following orthopaedic surgery. The experiments were designed on a placebo-controlled crossover basis, with two "identical" surgical interventions being performed on the forelimbs of each dog with an interval of 28 days, to enable a paired comparison of the post-operative courses.

In a standardized way, under general anaesthesia, the 3rd metacarpus was transected with an oscillating saw. The fracture was then stabilized with a mini dynamic compression plate before the wound was sutured. A special device was designed to allow measurements of post-operative swelling, while pain and limb function were assessed by the use of visual analogue scales. Abnormalities in the wound healing were recorded as well as clinical signs that could be indicative of adverse drug effects. Radiographs taken 2, 4, 6 and 8 weeks after the two operations were interpreted and compared for bone union, callus formation, signs of infection and foreign body acceptance. The dogs were

euthanized 8 weeks after the 2nd operation and the two 3rd metacarpi of the forelimbs excised. They were later cut in a cryo-microtome and the stained sections assessed for bone healing.

The three anti-inflammatory drugs selected for the investigations were a glucocorticoid, betamethasone; and two NSAID, phenylbutazone and indomethacin. Glucocorticoids are recognized as the most powerful anti-inflammatory drugs, but their place in surgery is disputed. Phenylbutazone was selected since it is probably still the most widely used NSAID in veterinary practice, while a main reason for including indomethacin was that it has been reported to delay or inhibit fracture healing.

In the first trial, a single pre-operative injection of 3mg betamethasone was tested against placebo in each of 8 dogs. The drug proved to significantly reduce the post-operative swelling. On the 3rd day the reduction was 43%. Less pain and limping were assessed after the glucocorticoid was injected, but the differences did not reach a level of significance. No adverse effects of the glucocorticoid on wound or fracture healing were detected. This trial included measurements of the endogenous cortisol levels. A marked decline in the serum cortisol levels followed the glucocorticoid injection. The levels remained low for about 3 days and

then returned to normal. It was concluded that the results of the study support the view that short-term glucocorticoid administration can efficiently curb an excessive post-traumatic inflammatory reaction and is essentially safe.

In the next trial 8 dogs were given 300 mg phenylbutazone by oral administration twice daily for 8 days starting on the day before surgery. Phenylbutazone did not reduce the swelling significantly as compared to placebo, although the drug gave a significant pain relief. The clinical observations indicated somewhat better wound healing after the operation when placebo was given, and that also applied to the fracture healing as evaluated by radiographs and bone sections.

In another group of 8 dogs, 25 mg indomethacin was to be administered orally twice daily for 8 days starting on the day before surgery. This medication had to be discontinued after 2 1/2 days when they had received a total dose of 125 mg indomethacin, since signs of toxicity became evident, e.g. vomiting, bloody stool and lethargy. One indomethacin-treated dog died on the 5th post-operative day. Swelling measurements showed no consistent difference, but the pain assessments were significantly lower after the operation when indomethacin was administered. No noticeable differences were observed

in wound healing, but the radiological evaluation revealed tendencies in disfavour of indomethacin.

A trial was then undertaken in another group of 8 dogs with a lower dosage of indomethacin. They each received 5 mg indomethacin twice daily for 8 days starting on the day before surgery. Even at this dosage one of the dogs developed bloody stool on the 5th post-operative day. With indomethacin there was a tendency towards less swelling, and the reduction became significant after one week. The pain assessments showed no consistent difference and there appeared to be no difference in wound and fracture healing.

The difficulties encountered in selecting an appropriate dosage of indomethacin provide a striking example of how differences in pharmacokinetics may explain differences in drug response both within as well as between species. It was difficult to obtain consistent and reliable assessments of pain and limping even if the dogs served as their own controls. These results should therefore be cautiously interpreted.

The present studies provide evidence that the drug effects on post-operative swelling observed in oral surgery, also apply to acute traumatic swellings in other parts of the body, since the recordings with limb volumetry showed a remarkably good correlation with

corresponding results obtained in oral surgery. This conclusion was recently also reached by another researcher in tests on paracetamol using this model with limb surgery. In addition to significantly reducing the swelling, paracetamol also proved to efficiently reduce pain without any signs of adverse effects.

Indomethacin does not appear to be recommendable in dogs, while phenylbutazone presents a relatively wide safety margin. The anti-phlogistic potential was, however, not impressive for any of the two NSAID. A short-term glucocorticoid administration or paracetamol seem to be better choices for curbing the sequelae of an acute post-traumatic inflammatory reaction.

CHAPTER ONE

INTRODUCTION

Inflammation is a response of living tissue to injury, characterized by heat, redness, swelling, pain and loss of function. It is part of the normal defense and repair mechanisms, and healing cannot occur without inflammation. The process may in many instances, however, overshoot its objective producing unnecessary suffering, tissue damage and scar formation. Both primitive and modern surgery have empherically given great importance to various anti-inflammatory measures (Allgöwer and Perren, 1967). The aim of such measures is to prevent the adverse consequences of the inflammatory process without affecting its beneficial effects.

Anti-inflammatory drugs used in human surgery and traumatology seem mostly to have been selected according to their performance in patients with rheumatoid arthritis (Lökken and Skjelbred, 1981), and essentially the same drugs have been adopted for use in veterinary surgery. However, rheumatoid inflammation differs markedly from an acute post-traumatic inflammation, (Ryan and Majno, 1977) and anti-inflammatory drug effects are not necessarily the same in these different conditions.

Recent research has demonstrated a remarkable lack of correlation between the actions of substances

in vitro and their actions in *in vivo* models of acute inflammation. Whenever possible, mechanistic studies should therefore start from *in vivo* evidence (Vinegar and Truax, 1982).

There is a lack of reliable models for clinical assessment of anti-inflammatory effects. A rather unique model for evaluation of anti-inflammatory effects in human surgery, is based on patients in need of prophylactic surgical removal of "identical" bilaterally impacted molar teeth. In these patients essentially the same operation can be performed on two separate occasions, and they may serve as their own controls in cross-over studies (Lökken *et al.*, 1975). Studies in this model have revealed that drugs which efficiently reduce rheumatoid swelling, e.g. acetylsalicylic acid, oxyphenbutazone and ibuprofen, may have little or no effect on post-operative swelling. On the other hand, paracetamol which often has been claimed to be without anti-inflammatory activity, reduced swelling by about 30%. A single injection of a glucocorticoid proved even more efficient. It reduced swelling by about 50% and there was a striking pain relief. Thirty five out of 36 patients preferred the post-operative course when they received the glucocorticoid injection (Lökken and Skjelbred, 1981; Skjelbred and Lökken, 1982a, 1982b).

Although the findings with glucocorticoids in oral surgery are promising, further controlled studies are needed to evaluate their safety and their efficacy in alleviating suffering and excessive inflammatory responses after various types of surgical interventions and accidental trauma. It has also to be taken into account, that findings in oral surgery may not necessarily apply to surgery and trauma in other parts of the body, e.g. of the extremities. Human patients requiring limb surgery, who could be used to establish a so well controlled model as that with bilateral oral surgery, are not available.

The aim of the present work was to establish a well controlled model for tests on how steroidal and non-steroidal anti-inflammatory drugs may modulate the course and healing process after standardized soft tissue/bone injuries on the forelimbs in dogs. It was to be investigated whether the effects on the extremities of dogs would be similar to those obtained with oral surgery in humans. The use of experimental animals should allow a more detailed study of the healing process since they can be euthanized.

The experiments were designed on a placebo-controlled crossover basis, with the two forelimbs being operated with an interval of 4 weeks. The surgical intervention under general anaesthesia involved

a vertical skin incision over the 3rd metacarpus, transection of the bone with an oscillating saw, thereafter stabilization of the fracture with a 6-hole mini dynamic compression plate, followed by suture of the skin. Efforts were made to follow exactly the same procedure at both occasions. Several objective and subjective assessments were recorded for a paired comparison of the post-operative courses. The swelling was measured with foot volumetry, while visual analogue scales served to assess pain and limping. Clinically observable side effects which could be related to the medication were recorded, as well as infections and abnormalities in the wound healing. Blood samples were collected for haematological examination and for tests on the liver and kidney functions. Radiographs of the operation site were taken immediately after surgery and then biweekly until 8 weeks after each operation. The screws and plates were removed after euthanasia 8 weeks after the second operation, the disarticulated third metacarpus sectioned, and stained for evaluation of the healing process.

The three drugs selected for investigation were a glucocorticoid, betamethasone; and two non-steroidal anti-inflammatory drugs, phenylbutazone and indomethacin.

Glucocorticoids are recognized as the most powerful anti-inflammatory drugs. Since, however, they have the

potential to impair wound healing and the defense mechanisms against infection, there is a widespread skepticism towards their use in surgery. Besides to investigate the efficacy of a single glucocorticoid injection in reducing swelling and other inflammatory signs of the limb, it was a main objective of this trial to examine whether the wound and bone healing or, otherwise, the animals were adversely affected by the drug.

Phenylbutazone is probably still the most commonly used non-steroidal anti-inflammatory drug in veterinary medicine. It is a common opinion that phenylbutazone and its congeners, in contrast to the salicylates, are more powerfully anti-inflammatory but less directly analgesic, and that their analgesic efficacy for pain of non-rheumatic origin is inferior to that of salicylates (Flower et al., 1980; Pugh, 1982). The present controlled trial was aimed at determining the ability of phenylbutazone to modulate post-operative swelling and other inflammatory events.

Indomethacin is a non-steroidal anti-inflammatory drug which has been much used in humans, but only infrequently in veterinary medicine. A main reason for including this drug was that it has been reported to delay or inhibit fracture healing (Sudmann and Hagen, 1976; Sudmann et al., 1979).

CHAPTER TWO

LITERATURE REVIEW

2.1 INFLAMMATION - GENERAL INTRODUCTION

Injury, either physical, chemical or bacterial causes an acute inflammatory reaction with release of intracellular materials into the extracellular compartment of injured tissue. Every surgical procedure results in an inflammatory reaction. The surgeon who understands the nature and mechanism of this reaction to injury has within his or her power the ability to minimize the adverse consequences and to utilize the reaction to the benefit of the patient (Peacock and van Winkle, 1976).

Inflammation can be characterized as a vascular and cellular response designed to defend the body against alien substances and to dispose of dead and dying tissues preparatory to the repair process (Peacock and van Winkle, 1976). The term inflammation is derived from a latin word "inflammare", which means "to flame within" On a macroscopic level, Celsus (first century A.D.), precisely described in clinical terms the cardinal signs of inflammation - calor, rubor, tumor and dolor, i.e. heat, redness, swelling and pain. Later, Virchow (1858 A.D.) added a fifth sign, functio laesa, or loss of function. (Fig. 2.1).

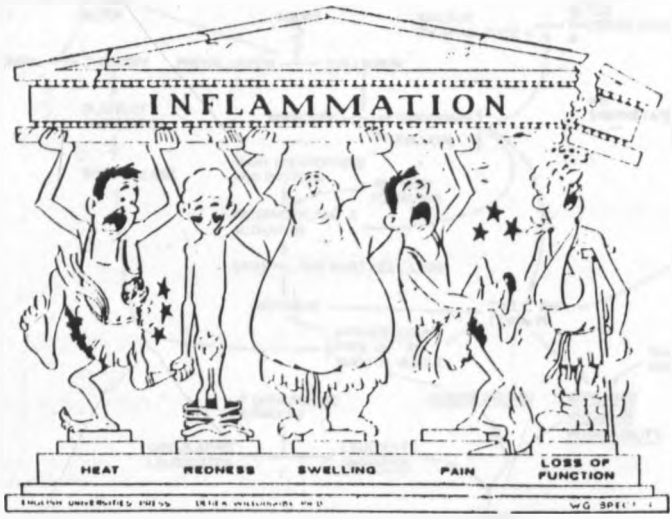


Fig. 2.1 The cardinal signs of inflammation.

While it is relatively simple to describe the inflammatory events on a macroscopic level, it has proved far more difficult to obtain an adequate understanding of the underlying cellular and humoral events in injured tissue. The inter-relationships between some of the putative mediators of the inflammatory response are shown in Fig. 2.2. This figure, which only includes some of the proposed substances and inter-relationships, may serve to illustrate the complexity of the inflammatory phenomenon. Recently a sequential 37-step pathway scheme was presented to describe the actions and events

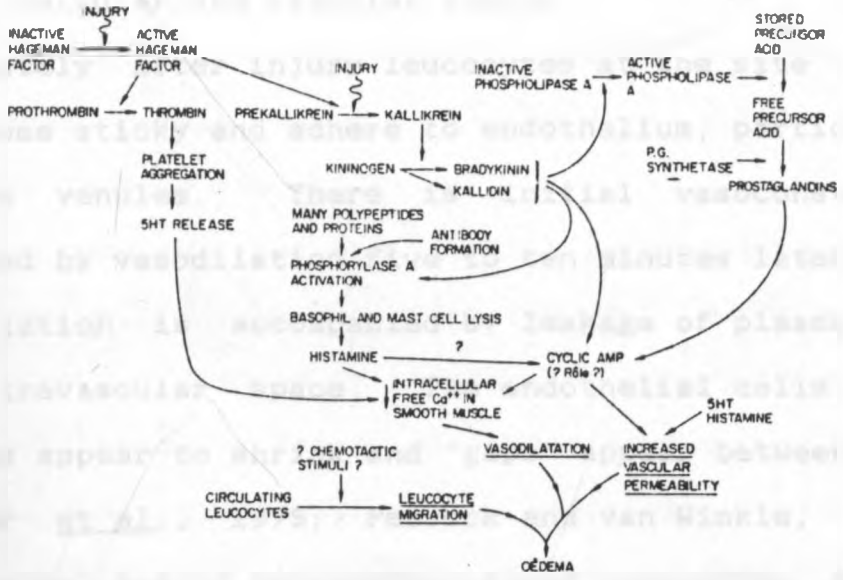


Fig. 2.2 Some of the inter-relationships between putative mediators of the inflammatory response which have been identified in various species (Yoxall, 1979).

in a model of acute inflammation (Vinegar et al., 1982). This was an upgradation of a 12-step scheme devised six years previously (Vinegar et al., 1976). It illustrates the high rate at which new information on the subject continues to be published. While acute inflammation is basically a non-specific and stereotyped reaction which may be initiated by various injurious agents, chronic inflammation is a highly specific response which in most cases includes an immune reaction (Ryan and Majno, 1977).

2.2 ACUTE INFLAMMATION

2.2.1 Cellular and vascular events

Immediately after injury leucocytes at the site appear to become sticky and adhere to endothelium, particularly in the venules. There is initial vasoconstriction followed by vasodilation five to ten minutes later. The vasodilation is accompanied by leakage of plasma into the extravascular space. The endothelial cells in the venules appear to shrink and 'gaps' appear between them (Flower et al., 1975; Peacock and van Winkle, 1976). Leukocytes, mainly polymorphonuclear leucocytes migrate out of the vessels into the interstitium by diapaedesis. They exhibit a positive but somewhat random motion in the direction of the injury.

Factors governing the general pattern of the response of phagocytic cells are not completely understood. In the early stage of inflammation the cellular exudate is mainly composed of polymorphonuclear leucocytes, whereas in the latter stages mononuclear phagocytes predominate. Besides the differences in the numbers of circulating granulocytes and monocytes, it seems likely that motility as well as vascular permeability, chemokinesis, and the chemotactic response, are different for granulocytes and monocytes and may explain why granulocytes predominate in the initial phase. Differences in the survival time of the

cells in tissues may also be of importance. Granulocytes are short lived (4-5 days) and readily disintegrate after phagocytosis, while the mononuclear phagocytes remain alive up to 100 days after ingestion (Schalm, 1975; Furth, 1976).

The complexity of the vascular permeability process has been well reviewed by Böhm (1976). He cites works which show differences in response to injury in various capillary beds. These differences may be due to morphological diversity in the endothelial structure, viz. presence or absence of inter- or intracellular fenestration in the endothelium, presence or absence of a continuous basement membrane and presence or absence of complete investment of pericapillary cells.

After injury there is a short-lived arteriolar constriction due to vasomotor reflex. Thereafter the injured tissue liberates vasoactive compounds which cause vasodilation and increased blood flow. Inflammatory agents and/or the inflammatory reaction stimulate sensory nerve endings by an antidromic reflex, provoke dilation of arterioles in the neighbourhood of the damaged area causing a flare. Arteries and arterioles up to 18 to 30 µm in diameter as well as venules from 20 to 50 µm and veins are innervated, but most investigators agree there are no nerve endings in precapillary and postcapillary vessels. Thus blood flow

through the vascular bed is controlled by nervous influence in larger vessels, and the precapillaries - or sphincters - may regulate the rate of blood flow in response to circulating catecholamines (Furness and Marshall, 1974).

It is accepted that lipid soluble molecules diffuse through the endothelial cytoplasm and the lipid insoluble molecules move out of the vasculature during inflammation by endothelial vesicles, or by crossing the tight endothelial junctions which loosen and then through the basement membrane (Böhm, 1976). Vascular dilation accompanied by changes in the endothelium produce leakage of plasma starts almost immediately after injury and continues for 2 days. Vascular activity in inflammation takes place in the arterioles and venules, and the capillaries, the real functional units of the entire vascular system, are spared. There is not a single pharmacologic agent known capable of modifying the physiologic functions of the capillary wall (Ryan and Majno, 1977).

2.2.2 Endogenous mediators of inflammation

Endogenous mediators are derived from the injured host as opposed to exogenous mediators which are derived from bacteria. Endogenous mediators originate either from plasma or tissues and they may be interrelated as shown in Fig. 2.2.

Plasma factors: These include the kinin system, the complement system and the clotting system.

The kinin system starts by the activation of Hageman's factor (clotting factor XII) which can then follow 3 pathways:

(1) One triggering the clotting cascade by activating clotting factor XI.

(2) One triggering the fibrinolytic system by activating plasminogen proactivator to give plasminogen activator which converts plasminogen to plasmin.

(3) One that stimulates prekallikrein activator activity. Prekallikrein activator converts prekallikrein to kallikrein which converts kininogen to bradykinin, a powerful vasodilator and pain stimulator in presence of other chemicals like prostaglandin E.

Other by-products of Hageman's factor activity which are biologically active in the inflammatory processes are C3, C5 and C567 complement fragments. The complement fragments cause erythema and vascular leakage as well as having chemotactic activity towards leucocytes (neutrophils, monocytes and basophils). Fibrinopeptides (released from fibrinogen molecules by the action of thrombin during clotting) and biologically

active fragments released during proteolysis of fibrin by plasmin are potential inflammatory mediators having been reported to enhance the effect of bradykinin on smooth muscle, induce vascular leakage and cause chemotactic attraction to neutrophils (Ryan and Majno, 1977).

Tissue factors: These include vasoactive amines, acidic lipids, lysosomal components and lymphocyte products.

The vasoactive amines, histamine and 5-hydroxytryptamine (serotonin), are released mainly from mast cells and platelets. They increase vascular permeability and are chemotactic to eosinophils.

Acidic lipids include the end products of arachidonic acid by the two known pathways: the cyclo-oxygenase pathway and the lipoxygenase pathway. Arachidonic acid precursor is stored bound in the cell membranes of most body cells except erythrocytes (Higgins, 1985). Its release is effected by phospholipase A which may be activated by bradykinin. In the cyclooxygenase pathway arachidonic acid gives rise to the unstable cyclic endoperoxidases PGG_2 and PGH_2 (Fig. 2.3). These may be converted to thromboxane (TXA_2), prostacycline (PGI_2) or stable (primary) prostaglandins, PGE_2,

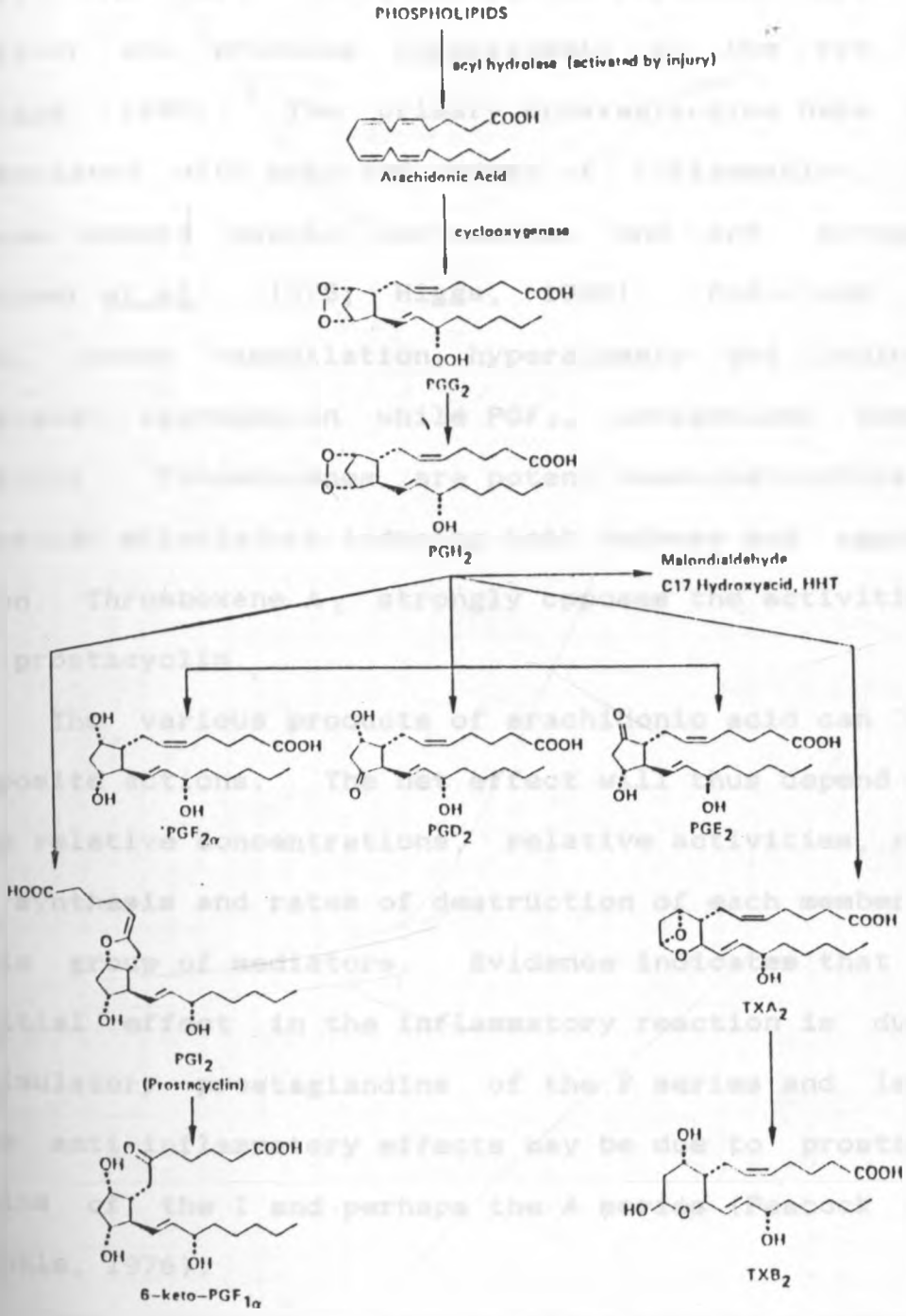


Fig. 2.3 The cyclooxygenase enzyme pathway of arachidonic acid metabolism (Higgins, 1985).

$PGF_{2\alpha}$ and PGD_2. Prostacyclin is a potent vasodilator and produces hyperalgesia in the rat foot (Higgs, 1980). The primary prostaglandins have been associated with pain and oedema of inflammation. They cause smooth muscle contraction and are pyrogenic (Flower et al., 1975; Higgs, 1980). $PGE_{2\alpha}$ and PGD_2 cause vasodilation, hyperalgesia and inhibit platelet aggregation while $PGF_{2\alpha}$ antagonizes these effects. Thromboxanes are potent vasoconstrictors and platelet stimulators inducing both release and aggregation. Thromboxane A_2 strongly opposes the activities of prostacyclin.

The various products of arachidonic acid can have opposite actions. The net effect will thus depend upon the relative concentrations, relative activities, rates of synthesis and rates of destruction of each member of this group of mediators. Evidence indicates that the initial effect in the inflammatory reaction is due to stimulatory prostaglandins of the F series and later, the anti-inflammatory effects may be due to prostaglandins of the I and perhaps the A series (Peacock and van Winkle, 1976).

The lipoxygenase pathway gives rise to a group of mediators called leukotrienes (Fig. 2.4). First, the arachidonic acid gives rise to hydroxyperoxy-eicosatetraenoic acid (HPETE), which is then converted

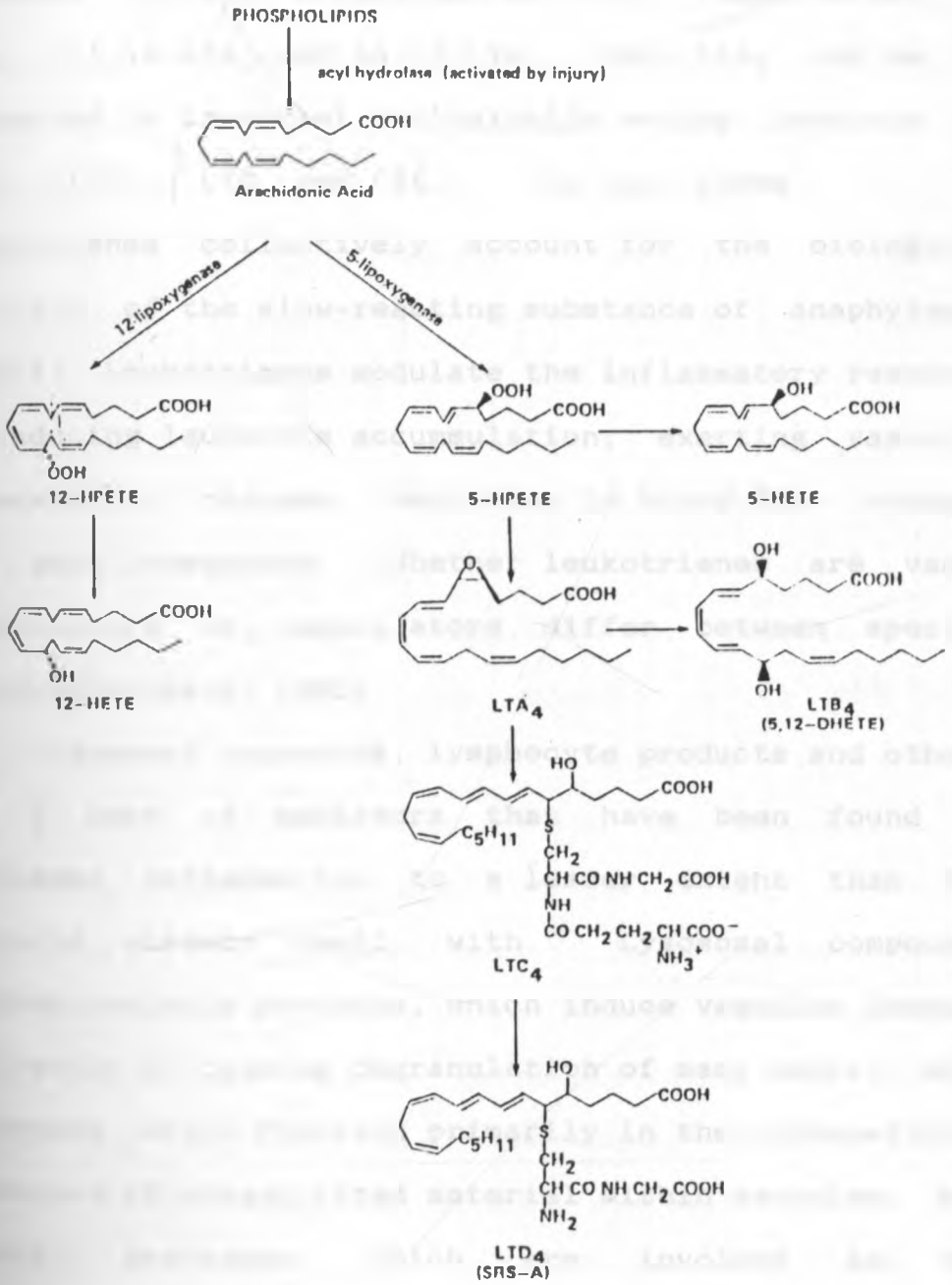


Fig. 2.4 The lipoxygenase enzyme pathway of arachidonic acid metabolism (Higgins, 1985).

either to hydroxyeicosatetraenoic acid (HETE) or to unstable epoxide intermediates known respectively as LTA_4, 11,12-LTA_4 and 14,15-LTA_4. Only LTA_4 can be converted to important biologically active products LTB_4, LTC_4, LTD_4 and LTE_4. The last three leukotrienes collectively account for the biological activity of the slow-reacting substance of anaphylaxis (SRS-A). Leukotrienes modulate the inflammatory response by inducing leucocyte accummulation, exerting vascular permeability changes, mediation in blood flow changes and pain responses. Whether leukotrienes are vaso-constrictors or vasodilators differ between species (Ford-Hutchinson, 1985).

Lysosomal compounds, lymphocyte products and others are a host of mediators that have been found to influence inflammation to a lesser extent than the products already dealt with. Lysosomal compounds include cationic proteins, which induce vascular leakage indirectly by causing degranulation of mast cells; acid proteases, which function primarily in the intracellular digestion of phagocytized material within vacuoles; and neutral proteases, which are involved in the pathogenesis of various tissue damage ranging from simple abscess formation to complex conditions as Arthus reaction, toxic nephritis and various forms of

arthritis. Lymphocyte products, sometimes called lymphokines, are released when sensitized lymphocytes are exposed to specific antigens in vitro or in response to treatment with mitogenic agents. Among the lymphocyte products are the following: macrophage migration inhibitory factor (MIF), chemotactic factors, lymphotoxin, skin reactive factors and mitogenic factors (Ryan and Majno, 1977).

2.3 CHRONIC INFLAMMATION

When wounds become contaminated or contain foreign material that cannot be removed during the acute inflammatory reaction, a condition of chronic inflammation exists (Peacock and van Winkle, 1976). The predominant cell is the monocyte. Monocytes modulate into macrophages some of which coalesce to form multinucleated giant cells which are phagocytic. Mononuclear cells undergo local proliferation. This is necessary because with subsidence of the acute inflammatory reaction, local vascular permeability returns to normal and blood cells cease to pass into the extravascular space. This mononuclear cell proliferation is characteristic of a chronic inflammatory reaction.

When leucocytes die they undergo autolysis. Should accummulation be rapid and emigration from the area fail, they break down and form an abscess. Discharge of enzymes from dead cells may damage more tissue and intensify inflammation (Jones and Hamm, 1977). If leucocytes die without autolysis, a coagulative necrosis involves them and the surrounding tissue, producing caseation as in a tubercle (Peacock and van Winkle, 1976).

There exists chemotaxis between macrophages and fibroblasts. Mesenchymal cells attracted to the site differentiate into fibroblasts. These cells surround tne macrophages. Collagen is laid down outside the fibroblasts eventually enclosing the lesion in a dense fibrous capsule forming a granuloma. This is the fate that foreign bodies undergo. As long as they are present the macrophages persist and eventually a dense fibrous layer forms around them and remains as long as the foreign bodies (Peacock and van Winkle, 1976).

2.4 WOUND AND BONE HEALING

2.4.1 Wound healing

Butterworth's Medical Dictionary (1978) defines a wound as any interruption, by violence or by surgery, in the continuity of the external surface of the body or of the

surface of any internal organ. Further, that legally the whole thickness of the skin must be broken, and an internal injury alone would not qualify. The various types of wounds are then defined, but are self explanatory, viz: lacerated, penetrating, perforating, septic, aseptic, incised, sucking, contused, etc..

Skin is a highly complex organ containing cells, a fibrous network composed of collagen, elastin and an amorphous ground substance which consists of proteins, polysaccharides, glycoproteins, globular proteins, salts and water (Peacock and van Winkle, 1976). Skin healing is therefore very complex and instead of regeneration as happens in fat and connective tissues, damaged skin is replaced by a fibrous scar tissue which, on the surface, is covered by regenerated and remodelled epithelium. In general, skin restores its surface continuity by epithelization, synthesis of dense connective tissue, and contraction (Johnston, 1981). The basic mechanisms involved appear to be essentially the same in all species, only the rates differ and these, in many instances can be accounted for by known metabolic and anatomical differences among animal species (Peacock and van Winkle, 1976).

Immediately after injury there is a vaso-constriction for 5-10 minutes which can cause actual vascular occlusion at the point of injury. This limits bleeding. Vasodilation follows, blood flows out and fills the gap created. It clots uniting the wound edges. Fibrin and protein form a scab at the surface. This protects the tissue underneath against infection and maintains internal homeostasis.

Leukocytes increase in numbers at the site. At first the neutrophils predominate. After 12 hours monocytes begin to arrive at the site. They clear debris, dead leucocytes and tissue, and fluid from vessels. In uncomplicated simple wounds this 'clean-up' job is finished in 3 to 5 days (Johnston, 1981).

Fibroblasts differentiate from mesenchymal cells in the vicinity and use the wound fibrin strands as a scaffold along which they lay down collagen and ground substance that make up scar tissue. Capillary infiltration occurs at the same time as the fibroblast invasion. The capillaries bring in oxygen for the cells which are very active in synthesizing protein for repair.

The epidermis starts the process of bridging the gap within 24 hours of injury. It does so by migration and mitosis of the basal cells. The activity of these epidermal cells leads to loosening and shedding of the

scab in 5 to 6 days.

Healing in a clean, controlled wound occurs rapidly. In a full-thickness skin wound in which skin edges cannot approximate, healing follows the same general steps already outlined. The degree of scar formation differs. Fortunately the size of the scar area is reduced by contraction of the wound. This involves the steady inward movement of wound edges towards a central point, resulting in complete or partial obliteration of the central wound area. The end result depends to a large extent on the degree of mobility of surrounding tissue (Peacock and van Winkle, 1976).

As contraction of a wound proceeds, the skin surrounding the wound is stretched, thinned and under tension. Gradually new collagen is laid down in the dermis so that the tension is relieved and the dermis regains its degree of thickness. New epithelial cells are produced in the area of skin under tension. This compensatory mechanism is called intussusceptive growth (Johnston, 1981).

A wound is said to be infected when the number of organisms exceed the ability of local tissue defences to handle them (Peacock and van Winkle, 1976). Infection by some organisms can be a serious deterrent to wound

healing. Other factors have been found to retard wound healing. These include antiseptics and chemicals used on wounds; oedema and lymphatic obstruction; deficiencies of protein, zinc and vitamin C; anaemia and lowered oxygen tension; anti-inflammatory drugs when given long enough and in high enough doses, as well as cytotoxic drugs.

2.4.2 Bone healing

There are two types of bones, the flat bones found in the head and the long tubular bones found elsewhere. A long bone has two types of structures, the spongiosa or cancellous bone at the ends and the compact bone which makes up the shaft and surrounds the marrow. Spongy bone is composed of columns of bone which are arranged along the lines of stress. Compact bone has numerous microscopic tubules called Haversian canals. Capillaries run within these canals. Bone is deposited around each canal in concentric layers forming a unit called the osteon. Periosteum surrounds bone on the outside. It has a fibrous outer layer and an inner cambrium from which new bone cells arise. A similar active layer, the endosteum, lines the marrow cavity.

Bone is composed of an organic phase called matrix and an inorganic phase. The matrix is made up of fibrous protein and collagen. The inorganic salt of

bone, calcium hydroxyapatite, is deposited and crystallizes around the organic matrix support.

Bone cells are usually classified into three types:

(1) The osteoblast which synthesizes the matrix.

(2) The osteocyte, thought to represent a resting stage of the osteoblast.

(3) The osteoclast which is a multinuclear cell usually found in the periphery of the bone and appears responsible for bone resorption.

The fact that in new bone formation the osteoblast lays down collagen matrix around it, and that eventually it lies in the lacuna of the Harversian system, strongly suggests that the osteocyte is merely a late and functional form of the osteoblast (Peacock and van Winkle, 1976).

A bone fracture is a mechanical discontinuity resulting in instability (Perren, 1981). In spontaneous healing, a sequence of biological events prepares the fracture for final bridging by bone. During each phase of spontaneous bone healing, the fracture is accompanied by a successive decrease in motion due to progressively increasing stiffness of the interfragmentary tissue. The mechanical consequences of a fracture are movement in the fracture zone and disturbance of the transmission of forces along the bone. These consequences are

subsequently counteracted by healing of the fracture which restores normal transmission of forces and abolishes movement.

Prerequisites for healing are a good blood supply, accurate positioning of the fragments, adequate immobilization and early ambulation (Dingwall, 1974). In spontaneous healing most of the above are absent. Healing involves the growth of new tissue around the fracture site. Following trauma the reaction of the periosteum, endosteum and intracortical Haversian system is characterized by infiltration of fibroblastic or fibrocartilargic tissue as well as capillaries in the gaps around the fragment ends. Any movement in the defect delays healing by disturbing and destroying newly formed cells and capillary buds. The thickness of the new tissue, the callus cuff, is in proportion to the extent of the fragment movement (Prieur and Somner-Smith, 1984). External callus develops around the ends of the bone fragments, while the internal callus forms in the medullary cavity and between the ends of the bone. Thickening of the callus increases the transverse lever arms and thus diminishes the extent of fracture movement.

Differentiation of the stem cell is related to its environment. Stress (compression) plus a high oxygen

tension results in osteoid or bone formation; tension plus high oxygen tension results in the formation of fibrous tissue; and stress associated with low oxygen tension results in cartilage formation. Fibroblastic cells that differentiate in the well vascularized area near the bone become osteoblasts forming bony trabeculae. Cells further away from the vascularised area differentiate in an avascular environment, become chondroblasts and form cartilage (Dingwall, 1974). When the external callus and trabeculae of the internal callus bridge the site, the fracture is united. The rigidity of the callus is improved by transformation of the initial inter-fragmentary callus to rigid fibro-cartilage, followed by ossification and consequent fracture stabilization. When the fracture site is bridged by trabecular bone, it is said to have healed. Once the fracture is stabilized a remodelling and substitution of the bony (woven bone) callus with lamellar bone and Haversian systems is possible (Prieur and Somner-Smith, 1984).

Fracture healing in the presence of internal fixation of varying rigidity follows the same pattern as does spontaneous healing, but may be reduced to a single step if the fracture is sufficiently stable (Perren, 1981). In stable motionless contact areas, Haversian

canals cross the discontinuity from fragment to fragment (contact healing), thus restoring the original structure of the bone in a one-step procedure. Where a gap exists, lamellar bone filling is seen in the gap, oriented at right angles with the original bone structure. This is gap healing. The gap is protected from strain by nearby contact areas. This situation is found when a fracture is plated. The bone cortex next to the plate heals by contact healing whereas the cortex furthest from the plate heals by gap healing.

Healing of an infected fracture is characterized by cloudy callus, resorption at the fragment ends, sequestration and patchy bone resorption. If compression is maintained in a rigid internal fixation system, primary bone healing will prevail in spite of infection (Rittman and Perren, 1974)

2.5 HEAT - FEVER

Vasodilation after injury brings warm blood supply to the injured area. This causes local increase in heat which may or may not involve the whole body. Fever, an abnormally high body temperature,is a clinical sign which may accompany inflammatory disease whether due to infections, tissue necrosis, or hypersensitivity (Ryan and Majno, 1977).

Regulation of body temperature requires a delicate balance between the production and loss of heat. The hypothalamus regulates the set point at which body temperature is maintained. In fever, this set point is elevated (Flower et al., 1975). Fever is attributed to the release of factors called endogenous pyrogens from the host cells into the blood. They appear to be proteins with a molecular weight range of 10,000 to 20,000 daltons. Body cells that have been shown to release endogenous pyrogens are neutrophils, blood monocytes, peritoneal macrophages, lung macrophages, eosinophils, phagocytic liver cells (Kupffer cells), as well as cells of the spleen and the lymph nodes. Release of pyrogens from intact cells occurs following phagocytosis or exposure to endotoxins or antigen-antibody complexes. Such processes are involved in clinical inflammatory states.

In the hypothalamus prostaglandins may act as local neuro-humoral transmitters (Ryan and Majno, 1977). Fever is often associated with an inflammatory process and most prostaglandins (except PGI_2) are themselves pyrogenic. In febrile conditions the metabolic rate increases in accordance with Van't Hoff's law, which states that for each degree Fahrenheit the temperature rises, the metabolic rate is increased by 7% (Lumb and Jones, 1973).

2.6 PAIN

Pain is an unpleasant sensory or emotional experience, associated with actual or potential tissue damage, or described in terms of such damage (IASP, 1979). The experience of pain cannot be measured directly. Most authorities agree that pain is a perception, not a physical entity, and that perception of pain depends on a functional cerebral cortex. Unlike most other sensations, no single area of the cerebral cortex seems specifically necessary for the perception of pain. Hyperalgesia is defined as reduced pain threshold to stimuli which are normally not painful. Pain and hyperalgesia are common features of the inflammatory reaction, and are caused by complex events inherent to the inflammatory process.

The term noxious describes stimuli which, if perceived, give rise to the perception of pain. The receptors specifically responsive to noxious stimuli are called nociceptors. Nociceptive threshold is the strength of a stimulus necessary before a nociceptor will generate nerve impulses in the peripheral nerve fibre of which it is a part. This is constant and varies little among humans and animals. Pain detection threshold is the strength at which noxious stimulation is perceived by a human being as pain and shows both inter- and intraindividual variation. Pain tolerance

threshold is the strongest intensity of noxious stimulation that a human being will permit an experimenter to deliver. This is the most variable of the three thresholds. Most clinical veterinary neurologists are amazed by the high tolerance thresholds of some dogs. Assessment of pain in animals has to be based on comparative anatomy, physiology and behaviour (Kitchell and Rickson, 1983).

Different groups of afferent fibres mediate different qualities of pain. Sharp pain is conducted by A-delta fibres, while dull pain is mediated by C-fibres. It is assumed that inflammatory pain is assignable almost entirely to the activation of polymodal nociceptors of C-fibres (Moncada et al., 1978).

The gate control theory of Melzack and Wall (1965) is the basis of modern pain theory (Fig. 2.5).

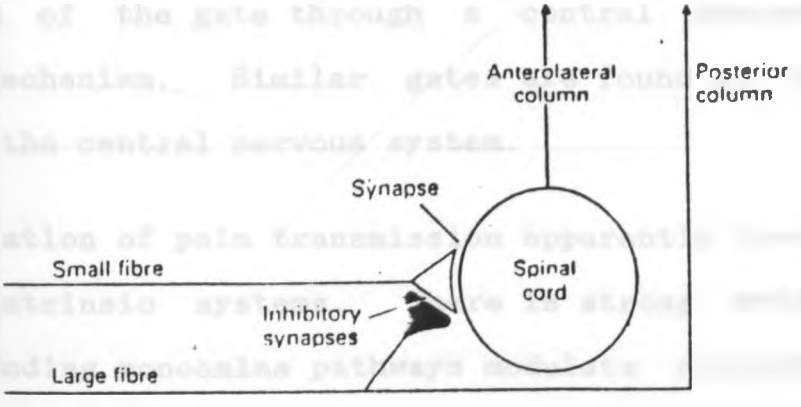


Fig. 2.5 Diagram of gate control (Feldman and Scurr, 1979).

It attempts to explain how an identical stimulation may not even reach conciousness on one occasion, but on another be interpreted as a pinprick and still on another as pain. The theory postulated that large diameter A fibres (A-β fibres), and smaller diameter A fibres (A-δ fibres) and the C fibres, are all activated during noxious stimulation of peripheral receptors. The theory suggests that, at the spinal cord level, there is a 'gate' which under certain circumstances allows pain stimulation to pass through it and impinge on higher centres. Stimulation of large nerve fibres may activate inhibitory synapses, 'close the gate' and thereby block the ascending passage of the pain impulses. This is effected by release of substances such as the encephalins. The gate at the spinal cord level is not only under control from the periphery but there is also modulation of the gate through a central descending control mechanism. Similar gates are found at other levels in the central nervous system.

Modulation of pain transmission apparently involves several intrinsic systems. There is strong evidence that descending monoamine pathways modulate nociceptive input within the dorsal horn via alpha-adrenergic receptors (Wilson and Yaksh, 1980).

Substance P is believed to be the neurotransmitter which is released by small peripheral afferent fibres which transmit painful stimuli (Fig. 2.6). Enkephalins

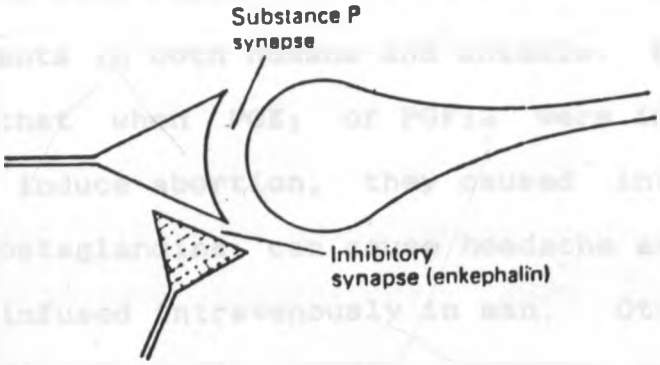


Fig. 2.6 Mode of action of substance P and enkephalin (Feldman and Scurr, 1979).

block the production of substance P thus inhibiting pain transmission. Enkephalins are endogenous pentapeptides which together with the longer chain parent polypeptides (endorphins), have opioid agonist activity. These peptides and exogenous opioids, such as morphin, have a common locus of action, their areas of highest affinity being the dorsal horn, the medullary nuclei and the periaqueductal grey matter of the mid-brain (Wilson and

Yaksh, 1980). Descending control of pain seems to involve a system with serotonin as the transmitter.

The pain produced by prostaglandins is slow in onset but long lasting, and prostaglandins will potentiate pain produced by other agents alone by lowering the pain threshold. This has been demonstrated in experiments in both humans and animals. Karim (1971) observed that when PGE_2 or $PGF_{2\alpha}$ were injected in women to induce abortion, they caused intense local pain. Prostaglandins can cause headache and vascular pain when infused intravenously in man. Other examples quoted by Flower et al. (1985), include long lasting hyperalgesia when PGE_1 was injected intradermally in man, while bradykinin mixed with PGE_1 caused itching. Bradykinin is a particularly effective algogenic agent and has been shown to induce both visceral and cutaneous pain (Lim et al., 1967).

2.7 INFLAMMATION - PROPHYLAXIS AND THERAPY

The signs and symptoms of inflammation are expressions of the disease process that are often used by the physician in diagnosis and in evaluating the effectiveness of treatment. Inflammation is a normal body defence and repair response. However, it can

become excessive and detrimental (Allgöwer and Perren, 1967). To alleviate the unwanted effects of the inflammatory reaction selective therapeutic measures have been developed. These include the application of cold packs to affected areas, compression bandaging where possible, and antiphlogistic drugs.

Anti-inflammatory drugs have been classified as steroidal and non-steroidal. Steroidal anti-inflammatory drugs (SAID) include both natural and synthetic glucocorticoids which reduce oedema, pain and lower elevated body temperature. Most non-steroidal anti-inflammatory drugs (NSAID) are weak organic acids with similar anti-inflammatory properties. The mode of action of both groups includes blocking the arachidonic acid cascade at different points (Fig. 2.7), thus blocking the formation of inflammatory mediators, e.g. prostaglandins and leukotrienes.

2.8 MODE OF ACTION OF ANTI-INFLAMMATORY DRUGS

2.8.1 Steroidal anti-inflammatory drugs

Glucocorticoids suppress the development of the cardinal signs of inflammation. Microscopically they inhibit oedema, fibrin deposition, capillary dilatation and migration of leukocytes. They further stabilize lysosomal membranes, inhibit capillary proli-

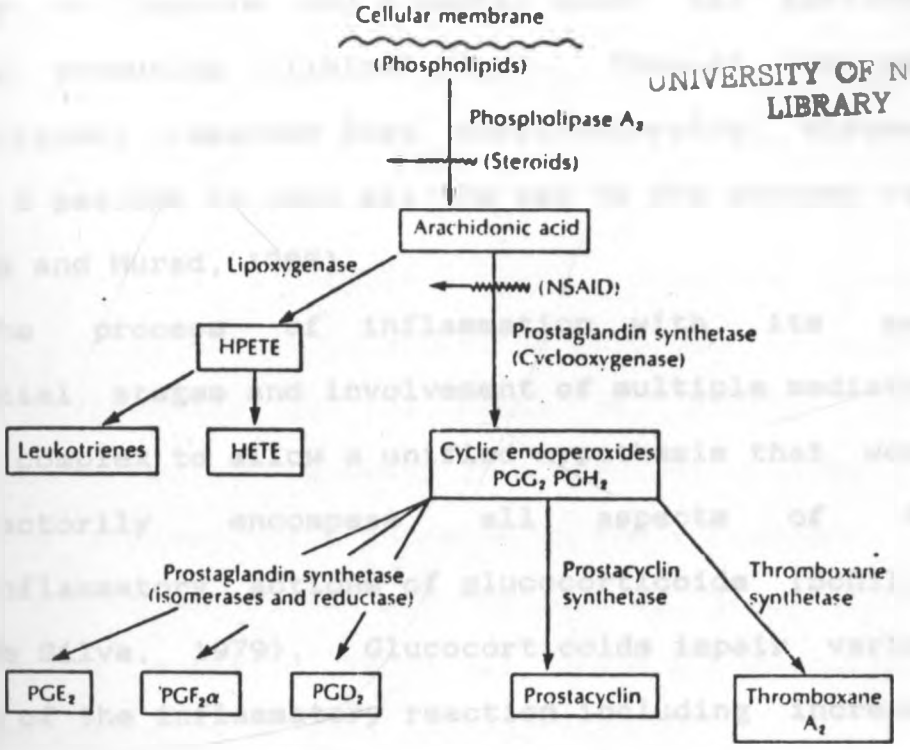


Fig. 2.7 The sites of action of steroidal and non-steroidal anti-inflammatory drugs (Goth, 1984).

feration, fibroblastic proliferation, deposition of collagen and cicatrization. Glucocorticoids inhibit inflammation whether the initiating agent is radiation,

mechanical, chemical, infectious or immunological. Their anti-inflammatory effect is palliative only, and the underlying cause of disease remains. An infection may continue to progress while the patient superficially appears to improve and a peptic ulcer may perforate without producing clinical signs. Thus it has been appropriately remarked that corticosteroids, misused, permit a patient to walk all the way to the autopsy room (Haynes and Murad, 1985).

The process of inflammation with its many sequential stages and involvement of multiple mediators is too complex to allow a unified hypothesis that would satisfactorily encompass all aspects of the anti-inflammatory actions of glucocorticoids (Schiller and De Silva, 1979). Glucocorticoids impair various phases of the inflammatory reaction including increased vascular permeability, exudation, vasodilation, leukocyte migration, phagocytosis and the release of lysosomal enzymes (Danneberg, 1979; Nwangwu, 1981).

The anti-inflammatory activity of glucocorticoids may, in part, be explained by inhibition of eicosanoid synthesis. The glucocorticoids interact with specific membrane receptors, and after transcriptional and translational events lead to the formation of macrocortin which inhibits the phospholipase A_2

(Blackwell et al., 1980; Flower, 1981). The availability of arachidonic acid is thus restricted and the formation of both cyclooxygenase and lipoxygenase products is reduced. Macrocortin may exist in a preformed store within some cells, and the glucocorticoids induce first its release (1-2 hours) then its resynthesis (3-4 hours) (Blackwell and Flower, 1983).

Cortisol is a natural hormone from the adrenal cortex. Synthetic glucocorticoids have been introduced into therapeutics on the basis of having anti-inflammatory potency greater than cortisol without also having a corresponding increase in their tendency to retain sodium and partly also because of their longer duration of action. Table 2.1 gives a comparison of relative potencies of some glucocorticoids.

2.8.2 Non-steroidal anti-inflammatory drugs

Members of this group are assumed to have a similar mode of action, accounting for both their therapeutic and toxic side effects. The prototype is aspirin, hence these compounds are often referred to as aspirin-like or acetylsalicylic acid-like (ASA-like) drugs.

Table 2.1. Comparison of potencies of various steroids (Goth, 1984).

Steroid	Anti-inflammatory potency	Daily dose (mg)	Sodium retention
Cortisone acetate (Cortone)	0.8	50-100	0.8
Cortisol (Cortef)	1	50-100	1
Prednisone (Meticorten)	2.5	10-20	0.8
Prednisolone (Meticortelone)	3	10-20	0.8
Methylprednisolone (Medrol)	4	10-20	0
Triamcinolone (Aristocort)	5	5-20	0
Dexamethasone (Decadron)	20	0.75-3	0
Paramethasone (Haldrone)	6	4-6	0
Betamethasone (Celestone)	20	0.6-3	0
Desoxycorticosterone (DOC)	0	1-3	10-25
Fludrocortisone (Florinef)	12	0.1	100
Aldosterone	0.2		250

Over the years a large number of possible modes of action have been suggested. Among those reviewed by Nickander et al. (1979) are:

(a) Inhibition of chemotaxis of cells implicated in the inflammatory process.

(b) Inhibition of lysosomal membrane labilization.

(c) Antagonist effects on mediators other than PGs (e.g. histamine and bradykinin).

(d) Inhibition of the biosynthesis of mucopolysaccharides.

(e) Uncoupling of oxidative phosphorylation.

(f) Fibrinolytic activity.

(g) Sulfhydryl-disulfide stabilization.

(h) Inhibition of collagenase production.

(i) Suppression of lymphocyte function.

During the 1970s it became evident that inhibition of eicosanoid synthesis is a main mechanism of action of acetylsalicylic acid and related drugs (Flower et al., 1985) They inhibit the conversion of arachidonic acid to the unstable endoperoxide intermediate, PGG_2, which is catalyzed by the cyclooxygenase (Fig. 2.7). Individual agents have differing modes of inhibitory activity on the cyclooxygenase. Acetylsalicylic acid acetylates a serine at the active site of the enzyme. Platelets are especially susceptible to this action

because, unlike most other cells, they do not regenerate the enzyme. In contrast to acetylsalicylic acid, salicylic acid has no acetylating capacity and is almost inactive against cyclooxygenase *in vitro*. Nevertheless, it is as active as acetylsalicylic acid in reducing the synthesis of prostaglandins *in vivo*. The basis of this action and, thus, of the anti-inflammatory effects of salicylic acid is not clearly understood. Since acetylsalicylic acid is rapidly hydrolyzed to salicylic acid *in vivo* (half-life in human plasma, approximately 15 minutes), the acetylated and the nonacetylated species probably act as pharmacologically distinct entities (Flower *et al.*, 1985).

Most of the other common ASA-like drugs are 'irreversible' inhibitors of the cyclooxygenase, although there are some exceptions (Flower *et al.*, 1974). For indomethacin, the mode of inhibition is particularly complex, and probably involves a site on the enzyme different from that which is acetylated by acetylsalicylic acid.

Acetylsalicylic acid and related compounds have been found to have differential effects according to dose (Higgs *et al.*, 1979). At low doses, the entry of leucocytes into inflammed areas was found to be potentiated, while their accumulation was inhibited at higher doses which reduced oedema. The drugs may

possibly at low doses selectively inhibit the cyclooxygenase, resulting in a diversion of arachidonic acid substrate through the lipoxygenase pathway, and consequently an increased production of chemotactic leukotrienes and increased inflammatory reaction. At higher doses there might be a dual cyclooxygenase and lipoxygenase inhibition resulting in reduced inflammatory reaction.

2.9 TOXIC AND ADVERSE EFFECTS

2.9.1 Steroidal anti-inflammatory drugs

A single dose of a glucocorticoid, even a large one,is virtually without harmful effects (Haynes and Murad, 1985). The initial suppression of the hypothalamic-pituitary-adrenal axis is followed by complete return to normal function within a few days after cessation of medication (Williamson et al., 1980).

High doses of glucocorticoids for long periods of time are associated with numerous adverse effects, e.g. adrenal insufficiency upon cessation of treatment, fluid and electrolyte disturbances, hyperglycaemia, osteoporosis, myopathy, behavioral disturbances, posterior subcapsular cataract, arrest of growth and Cushing's syndrome (Haynes and Murad, 1985).

The effect of glucocorticoid treatment on infections is quite complex. Animal experiments suggest that cortisone exerts an adverse effect on the course of a variety of experimental infections, particularly fungal diseases. It must be remembered, however, that very large doses of the steroids are used in such experiments. With reasonable doses, antibody production is not decreased, opsonins remain normal and leukocytes ingest and destroy microorganisms, even in experimental infections. In humans, varicella and herpes of the eye may be more severe, and fungal diseases may develop after prolonged steroid therapy. On the other hand, there is every reason to believe that the danger of using glucocorticoids in infections has been exaggerated. Infection must be viewed as an added factor, rather than as an absolute contraindication, when the risks of using corticosteroids are appraised (Goth, 1984).

Prolonged administration of high doses of glucocorticoids has been shown to inhibit or slow wound and bone healing (Blunt et al., 1950; Sissons and Hadfield, 1951; Peacock and van Winkle, 1976). However, slow-healing wounds are not always undesirable. Glucocorticoid containing ointments have been applied topically to surgical and traumatic wounds of the prepuce in bulls to prevent rapid healing that would

lead to stricture formation by proliferating connective tissue. Healing is delayed, but strictures are thereby prevented (McDonald, 1982).

2.9.2 Non-steroidal anti-inflammatory drugs

In general NSAID share the same potential adverse effects, with gastrointestinal problems being the most common. The relative differences in margin of safety between the many NSAID can only come from careful observation of efficacy versus adverse effects. There is a growing opinion that this difference is very much dependent on individual patient differences resulting in populations that respond more favourably to one drug than to another (Huskisson,1977).

There are occasional reports of more serious adverse effects such as aplastic anaemia, hepatotoxicity, acute anaphylactic reactions, and goiter (Nickander et al., 1979).

Gastrointestinal effects: Gastrointestinal complaints are not only the most commonly reported adverse effects, but also the most common reason for rejecting new NSAID from further clinical consideration. As a group NSAID may cause gastric and intestinal ulcers. Secondary

anaemia may accompany as a result of blood loss. The mechanisms by which NSAID injure mucosal cells are complex. Deleterious effects may result from local actions, which cause injury to the submucosal capillaries with subsequent necrosis and bleeding, and from effects on the secretion of acid and mucus, which appear to be due to systemic inhibition of some prostaglandins (Flower et al., 1985).

Renal and hepatic effects: One of the most common adverse effects reported in animals after prolonged administration of NSAID is that of renal papillary necrosis (Arrigoli-Martelli, 1977). Although the mechanism of this action is not clear, prostaglandins act as intrarenal hormones regulating renal blood flow; therefore, alterations in prostaglandin synthesis may well modify renal function to the extent of pathology.

There is an increasing awareness that NSAID can produce hepatic injury. In general, the hepatotoxicity is dose dependent and not associated with evidence of hypersensitivity. With salicylates there is concern that they might be an important factor in the severe hepatic injury and encephalopathy observed in Reye's Syndrome. This syndrome is a rare but often fatal consequence of infection with certain viruses. Although

a causal relationship with salicylates has not been definitely established, various authorities have advised against the use of salicylates in children with chicken-pox or influenza (Flower et al., 1985).

Haematological effects: Ingestion of acetylsalicylic acid and other NSAID causes a definite prolongation of the bleeding time. This effect is probably due to acetylation of platelet cyclooxygenase and the consequent reduced formation of TXA_2. Because of the antiplatelet action of these drugs, NSAID should not be used in patients with blood clotting problems.

Blood dyscrasia resulting from NSAID-induced injury to the haematopoietic system have been reported including thrombocytopenia, leukocytopenia and agranulo-cytosis (Summy-Long, 1984).

Prolongation of gestation: Both in experimental animals and in the human female, NSAID have been shown to prolong gestation. Furthermore, prostaglandins of the E and F series are potent uterotropic agents (Flower et al., 1985)

Foetal effects: Indomethacin and some other NSAID have been used therapeutically to close the Ductus arteriosus in neonates. This desired action of NSAID could well cause detrimental effects on the fetus. Maternal treatment with NSAID, especially near term could be a potential hazard (Nickander et al., 1979).

Intolerance: Certain individuals display intolerance to acetylsalicylic acid and most NSAID manifested by signs that range from vasomotor rhinitis with profuse watery secretions, angioneurotic oedema, generalized urticaria and bronchial asthma, to laryngeal oedema, bronchoconstriction, hypotension, shock, loss of consciousness and complete vasomotor collapse. Despite the resemblance to anaphylaxis, the underlying mechanism does not appear to be immunological in nature. This has prompted the hypothesis that the reaction reflects the diversion of arachidonic acid metabolism towards the formation of increased amounts of leukotrienes and other products of the lipoxygenase pathway (Szczeklik and Gryglewski, 1983).

2.10 SPECIES DIFFERENCES IN THE RESPONSE TO ANTI-INFLAMMATORY DRUGS

The range of species in which drugs are used and studied is what distinguishes veterinary from human pharmacology. While the mechanism of action of a drug is often the same in humans and other mammalian species, the intensity and duration of the effects produced can vary widely. This implies that species variations in the response produced by a fixed dose of drug can be attributed to differences either in pharmacokinetic processes (absorption, distribution, and elimination) or in the pharmacodynamic sensitivity of tissue receptor sites. Since it has been found that dosage appropriate for the species can offset differences in the intensity of the response produced by a number of drugs, it is generally assumed that the range of therapeutic plasma concentrations in animals is the same as in humans.

In other orders of animals, (e.g. birds, amphibians, fish), these differences may be even more extreme. For example, fish are almost completely lacking in the hepatic microsomal oxygenase system for drug metabolism. As a result, the duration of action of a single dose of many common drugs, if added to the aquarium water, is almost infinite. However, there are few pharmacokinetic data available for non-mammalian species (Baggot, 1984).

2.10.1 Steroidal anti-inflammatory drugs

Glucocorticoids have similar anti-inflammatory effects in various species studied - rats, mice, rabbits, dogs, cats, cattle, horses, pigs and humans. Some species have however shown more sensitivity than others to this group of drugs. One example is the delay of wound and bone healing due to reduction in collagen and fibrous tissue formation when high doses are given long enough. It appears that rats, mice and rabbits require much higher doses than humans to produce this effect (Peacock and van Winkle, 1976).

2.10.2 Non-steroidal anti-inflammatory drugs

Due to widespread use of NSAID in man, most of their adverse effects in this species are assumed to be known. Less is known about adverse effects in other species (von Teelmann, 1983). Inter- and intraspecies variations in drug responses are mostly not considered to be due to differences in receptor sensitivity, but rather to differences in the pharmacokinetics (Ruckebusch and Toutain, 1983).

Humans and dogs differ markedly in their sensitivity to various NSAID (Fig. 2.8). It will be seen that on a body weight basis, only about ten per cent of the therapeutic dose of fenoprofen given to

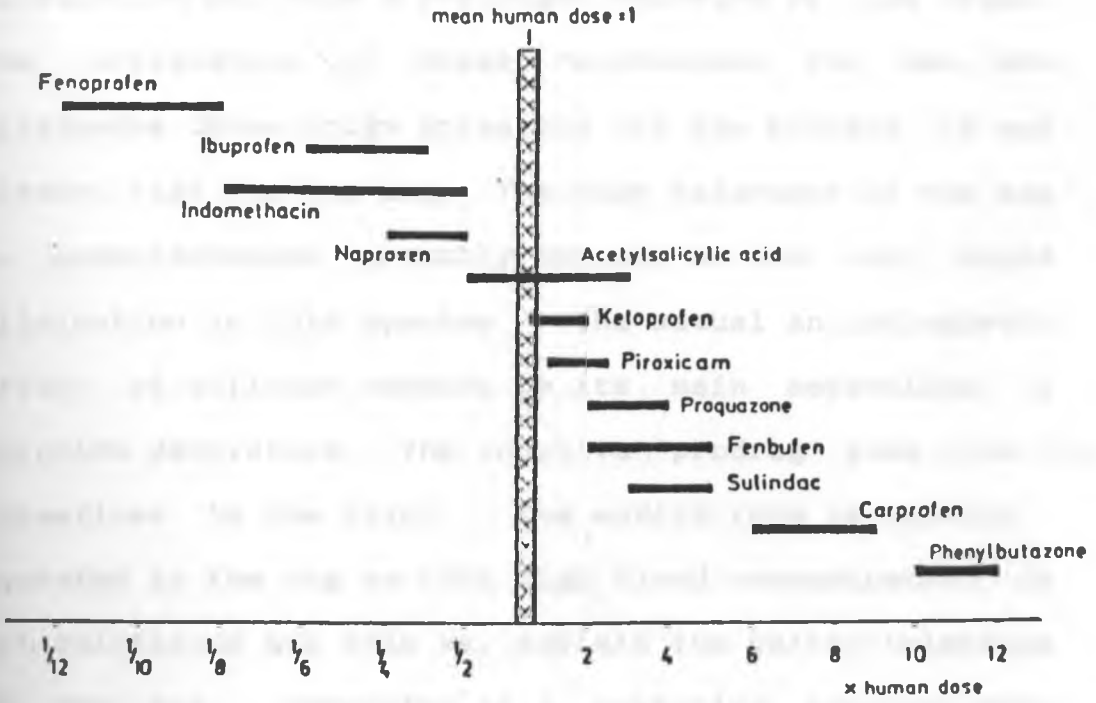


Fig. 2.8 The relationship between ulcerogenic doses in dogs and therapeutic doses of various NSAID in humans (von Teelmann, 1983).

humans will cause gastrointestinal lesions in the dog, while on the other extreme, dogs will tolerate ten times the therapeutic dose of phenylbutazone usually given to humans without resulting in gastrointestinal lesions. The higher sensitivity of the dog's gastrointestinal tract to some of these drugs is a result of their predominantly

biliary elimination, a pronounced entero-hepatic re-circulation and thus a prolonged exposure of this organ. The utilization of doses recommended for man, who eliminates these drugs primarily via the kidneys, is not without risk for the dog. The high tolerance of the dog to phenylbutazone probably depends on its very rapid elimination in this species. The actual antiphlogistic effect of sulindac depends on its main metabolite, a sulphide derivative. The inactive 'prodrug' goes from the intestines to the liver. The active form is rapidly excreted in the dog so that high blood concentration is not maintained and this may explain the better tolerance in the dog. Carprofen is a selective prostaglandin inhibitor. It is particularly active against $PGF_{2\alpha}$ and PGI_2 and less active against PGE_2. Hence the protection of the mucosa by PGE_2 is not markedly affected (von Teelmann, 1983).

Naproxen is an NSAID which is widely used in human medicine. Several cases of naproxen toxicosis have been reported in dogs when recommended dosages in humans have been extrapolated to the dog (Roudebush and Morse, 1981; Smith, 1982). There is an extensive enterohepatic recirculation of naproxen in the dog, which results in a half-life of about 72 hours (Frey and Rieh, 1981). As suggested by Schwartz-Porsche et al. (1982), naproxen

might be a useful and convenient NSAID in the dog, since due to the slow elimination, a therapeutic plasma concentration is maintained with only one daily dose. A dose of 5 mg/kg orally on the first day and 2 mg/kg on the following days gave excellent pain relief in dogs with chronic pain of the spine and/or the extremeties.

That differences in rates and pathways of biotransformation are the main determinants for most species differences, are strikingly demonstrated by experiments performed with two NSAID, salicylate and phenylbutazone. Sodium salicylate was administered intravenously at a dose of 44 mg/kg to goats, ponnies, swine, dogs and cats (Davis and Westfall, 1972). The pharmacokinetic parameters are shown in Table 2.2.

Table 2.2 Pharmacokinetic constants for salicylate after intravenous administration of sodium salicylate (44 mg/kg) (Davis, 1983).

species	no.	B mg/litre	h^{-1}	Vp litre/kg	Clr $ml.kg^{-1}h^{-1}$	t1/2 h
Cat	10	185	0.018	0.244	4.39	37.6
Dog	7	200	0.081	0.220	17.80	8.6
Swine	10	213	0.118	0.207	24.40	5.9
Pony	14	211	0.673	0.209	140.7	1.03
Goat	11	293	0.884	0.150	132.6	0.78

The clearances of salicylates in ponnies and goats were about 30 times greater than in cats. The high clearance in ponnies was due to rapid clearance of salicylates in the alkaline urine of this species, whereas the high clearance in goats was associated with rapid biotransformation coupled with rapid clearance into an alkaline urine.

Phenylbutazone gives a good demonstration of the futility and potential danger of extrapolation of data from one species to another with therapeutic intent (Davis, 1983). Initially, horses were treated with phenylbutazone according to dosage regimes recommended for humans. The drug was considered to be worthless in horses until its pharmacokinetics in this species was studied. As seen in Table 2.3, there are large species variations on the rate of elimination of phenylbutazone. Phenylbutazone, unlike salicylate, is slowly eliminated from ruminants and the rate in the goat is much greater than in the ox.

Species variation may also depend on anatomical and physiological variations. The binding of phenylbutazone to fibrous particles has been given to explain the prevalence of colonic and caecal ulceration in hay fed horses as most of the digestion occurs in the posterior gut in this species (Lees and Higgins, 1985).

Table 2.3 Half-lives of phenylbutazone in several species (Davis, 1983)

Species	$t_{\frac{1}{2}}$ h	Species	$t_{\frac{1}{2}}$ h
Human	72	Rat	6
Ox	55	Dog	6
Goat	42	Swine	4
Female	19	Baboon	5
Male	14.5	Horse	3.5
Cat	18	Rabbit	3

2.11 METHODS FOR ASSESSING INFLAMMATORY EVENTS AND HEALING PROCESSES

An experimental design aimed at testing the anti-inflammatory response to a drug must take into account the various parameters of inflammation: swelling (oedema and exudation), pain, pyrexia (local or systemic) and redness. The method of initiating and assessing the degree of inflammation should be as consistent as possible throughout the experiments.

2.11.1 Swelling

Carrageenin induced paw oedema in rats is classically one of the most used tests, but others are also useful, such as determination of leucocyte migration in pleural exudate induced by turpentine,or cell collection from artificial skin chambers (Mazue et al., 1983). Tissue damage resulting from surgical or accidental trauma gives rise to exudation and oedema.

Various ways of measuring swelling exist. Goldie et al. (1974) used the Archimedes principle in measuring the volume of water displaced by the feet of patients who had sprained their ankles. When measured at the same time of day and at standardized conditions the volume differed by only 0.3%. When not measured at standardized conditions the variation coefficient increased to 2.4%. Colen et al. (1979) assessed oedema in rats whose limbs were replanted following amputation by measuring diameters of the limb at 2 arbitrary points using calipers. Lökken et al. (1975) used a mechanical device which gave exact and reproducible measurements of the post-operative swelling by substraction of pre- from post-operative recordings in bilateral molar teeth extraction procedures.

2.11.1 Pain

The most successful method used to evaluate analgesic activity of NSAID involves assessment of the drugs' ability to modify what might be defined as "inflammatory pain" (Arrigoni-Martelli, 1979). Pain is obtained by all types of inflammatory processes such as in animal models after local injection of brewer's yeast or carrageenin, intraperitoneal administration of 2-phenyl-1-4-benzoquinone or acetic acid in rodents (Mazue, 1983), or after surgical procedures in man and animals.

A modification of Randal-Selitto assay is often used to quantify pain in experimental situations. This involves injecting 5 mg of yeast into the hind paw of rats. After 3 hours the degree of hyperalgesia is determined by applying a force of increasing magnitude to the hind limbs by means of an air driven Teflon plunger. The force (in mm Hg) at which the animals begin to struggle or vocalize is assumed to represent the pain threshold and serves as the end point (Arrigoni-Martelli, 1979). A recent hyperalgesic assay involves injecting 500 µg carrageenin instead of yeast. Inhibition of stretching (writhing, squirming, abdominal constriction) in the mouse or rat has been used as an index of analgesia. Analgesics were found to reduce the number of stretches.

In humans the most successful efforts to quantitate pain in clinical situations have been those accepting the patient's own report. Whichever criteria for analgesia are used, they should be well defined. The observations should be made as close in time as possible to the pain experience, especially when dealing with out-patients.

2.11.3 Erythema and hyperpyrexia

The assay methods that rely on a modification of the intensity and/or the appearance of erythema which have been found to be the most useful for detecting anti-inflammatory drugs have been those utilizing ultra-violet light irradiation of depilated skin causing a direct injury to epidermal and subjacent structures. A close correlation has been suggested to exist between the potency of the drugs in this assay and their anti-inflammatory potency in man (Arrigoni-Martinelli, 1979). Erythema can also be obtained by applying irritant substances directly to the skin, e.g. nicotinic acid (Mazue et al., 1983). The problem has been to find a reliable method of assessing erythema.

There is no widely accepted standard experimental technique which utilizes the increased temperature of an inflammed site for the assessment of the anti-

inflammatory activity (Arrigoni-Martinelli, 1979). In animal models local or systemic hyperpyrexia have been obtained by the administration of, e.g. brewer's yeast and bacterial pyrogens. Measurement of skin temperature may be accomplished by contact thermometers, infrared radiometers or by sophisticated and expensive infrared thermography.

2.11.4 Biochemical markers of inflammation

Assessment of cyclooxygenase inhibition has during the recent years been a standard method for evaluation of anti-inflammatory drugs (Ferreira and Vane, 1979). Other biochemical markers may also prove valuable for assessment of anti-inflammatory activity of drugs, such as γ-glutamyltranspeptidase which plays an important role in the turnover of glutathione and in protein biosynthesis (Singh et al., 1986).

2.11.5 Cellular or repair phase

The cellular or repair phase of the inflammatory process follows the vascular and exudative changes. This is experimentally reproduced by granuloma-induced formation which is achieved by introducing cotton pellets sub-cutaneously in rats, or implantation of pouches containing croton oil or mycobacterial adjuvant in the

back of rats (Mazue et al., 1983). Clinically foreign bodies can induce granuloma formation, e.g. plates and screws, wires, etc.

Cotton pellets implanted subcutaneously stimulate maximum granuloma formation during the first few weeks. Most assessments have been made using 7 to 14 day old granulomas. The assessment of the response is usually based on the dry weight of the granuloma (Arrigoni-Martelli, 1979).

2.11.6 Bone repair

To study and compare bone repair in different therapeutic trials identical and reproducible fractures need to be made. In studies on glucocorticoid effects on bone healing, Blunt et al. (1950) used a mallet to give a sharp blow to the midshaft of rabbit tibia. This created a fracture without breaking the skin. The pattern of fracture could however, not be controlled. Sisson and Hadfield (1951) broke the rabbit tibia at the junction of the upper and middle thirds, but give no details on the method. The mobility of the fractured bone was determined each day. This precluded one of the major requirements of good fracture healing, that of rigid immobilization. Histologic examination of the bones was carried out at the end of the test period.

More recently the potential deleterious effects of indomethacin on bone healing have been studied by a standard femoral fracture technique in rats (Sudmann et al.,1979) and by drill hole defects produced in the body of a caudal vertebra (Elves et al., 1982). Studies on inhibitory effects upon the healing of fractures in the rat have also been done with acetylsalicylic acid (Allen et al., 1980) and ibuprofen (Törnkvist and Lindholm, 1980).

CHAPTER THREE

EFFECT OF A GLUCOCORTICOID ON THE POST-OPERATIVE COURSE FOLLOWING EXPERIMENTAL ORTHOPAEDIC SURGERY IN DOGS

3.1 INTRODUCTION

Steroids or endogenous compounds of the adrenal cortex are divided into two classes, namely the corticosteroids (glucocorticoids and mineralocorticoids) and the androgens. The first clinical use of a steroidal compound (cortisone) was reported by Hench et al. (1949) in arthritic patients who experienced a dramatic improvement of their clinical condition. Glucocorticoids and synthetic analogues thereof have the capability to prevent or suppress the development of the local heat, redness, swelling and tenderness by which the inflammatory process is recognized. The amazing results in patients with rheumatoid arthritis evoked widespread interest, and cortisone was soon reported to cause symptomatic improvement in a variety of diseases and injuries associated with inflammation. It was recognized that cortisone was not a cure for many diseases. It seemed to provide the susceptible tissues with a shield-like buffer against the irritant. After a while, not only therapeutic success, but a large number

of cortisone-induced adverse effects were reported. Serious adverse effects occurred after continued use of large doses and following withdrawal of therapy. Manipulation on the cortisone structure yielded a variety of synthetic analogues with significant therapeutic gains in the ratio of anti-inflammatory activity to effects on electrolyte metabolism. However, hopes for elimination of adverse effects and toxicity have not been fulfilled (Haynes and Murad, 1985).

It has also been recognized for a long time that long-term glucocorticoid therapy may adversely affect wound healing and bone formation (Plotz et al., 1950); Loeb, 1950). On the other hand, short-term therapy with glucocorticoids during oral surgical interventions has been demonstrated to markedly reduce post-operative swelling (Hooley and Hohl, 1974; Skjelbred and Lökken, 1982a,b) as well as pain (Skjelbred and Lökken, 1982a,b; 1983), without any observable adverse effects, e.g. on the wound healing. The control of the inflammatory process following accidental or intentional bone/soft tissue traumas may be a beneficial measure in veterinary as well as human clinic.

The aim of the present study was to investigate whether the promising results obtained with short-term glucocorticoid administration in human oral surgery,

could be reproduced in experimental orthopaedic surgery on the forelimbs of dogs. Further, to study potential deleterious effects on wound and bone healing, and the effect on the endogenous cortisol levels.

3.2 MATERIAL AND METHODS

3.2.1 Experimental design

The trial was carried out as a randomized placebo controlled crossover study with two "identical" surgical bone/soft tissue interventions performed on the forelimbs of each animal with an interval of 28 days to allow a paired comparison of the post-operative courses.

3.2.2 Experimental animals

Eight mongrel dogs of mixed gender with a mean weight of 15.7kg (range 12-20kg) were obtained from the local dog pound. They were housed individually in kennels and provided with water ad libitum. Commercial dog food was fed once a day between 4 and 6 p.m.

On acquisition a complete physical examination was performed and the following laboratory tests carried out to ensure that the obtained values were within the following normal ranges (Benjamin, 1978) : erythrocytes (5.5-8.5 x 10), white blood count (6-18 x

10), packed cell volume (37-55%), blood urea nitrogen (10-30 mg/dl), total protein (5.4-7.1 g/dl), albumin (2.3-3.2 g/dl), globulin (2.7-4.4 g/dl), alkaline phosphatase (3-16 IU/l) and serum glutamate pyruvate transaminase (4.8-24 IU/l).

Blood slides made from capillary blood were examined for babesia and ehrlichia. Only healthy animals, free from blood parasites, were included in the study. The laboratory tests were repeated before the 2nd operation to ensure that the animals were still healthy.

Deworming was done on the chosen animals using piperazine and thenium (Ancaris®, Burroughs Wellcome, Kenya) against hookworms and ascarids, and bunamidine (Scolaban®, Burroughs Wellcome, Kenya) against tape-worms. Faecal samples were examined for parasite eggs 48 hours later. If found positive the animals were dewormed again and another faecal sample tested 48 hours later.

3.2.3 Drug administration

Immediately before each operation a single dose of 3mg betamethasone (0.5 ml Celestone Chronodose®, Schering Corp., USA) or placebo (0.5 ml saline) was injected into the hamstring muscles. The treatments were allocated

according to a randomization list so that half of the dogs received the active drug at the 1st operation. The order was reversed during the 2nd operation. To keep the trial blind to the surgeon, the injections were administered by an assistant who had no other responsibility in the trial. No other medication was given during the trial period.

3.2.4 Surgery

Four dogs were operated on Monday morning and the other 4 on the subsequent Tuesday morning. The 2nd operation was done exactly 4 weeks later. The left leg was operated on the first occasion and the right leg on the second.

Preparation for surgery: Four days before surgery the dog's limb to be operated was shaved from the elbow downwards. An Oster clipper (Oster Corp., Milwaukee, Wisconsin, USA) with a No. 40 blade was used.

Premedication and anaesthesia: On the morning of surgery all 4 dogs were premedicated with subsutaneous atropine sulphate 0.02 mg/kg (Veterinary Drug Co. Ltd., York, UK), and acetylpromazine 0.125 mg/kg (ACP , C-VET

Ltd., Bury, St. Edmunds, UK). Anaesthesia was induced with intravenous thiopentone sodium 20 mg/kg (Intraval®, Dawa Pharmaceuticals Ltd., Nairobi, Kenya). A mixture of halothane (Fluothane®, ICI, Cheshire, UK) and oxygen, given in a semi-closed system, was used to maintain anaesthesia. Once the patient, the surgeon and the assistant were ready for aseptic surgery, the operation site was draped. The same assistant was used for both operations. A tourniquet was applied proximal to the elbow of the limb to be operated.

Surgical procedure: The procedure is illustrated in Fig. 3.1a-i.

a. A vertical skin incision was made dorsally over the 3rd metacarpus. A superficial vein which makes an inverted Y over this bone, was retracted after transection and ligation of one branch. Chromic 2/0 catgut was used for ligation.

b,c. The tendon of the common digital extensor muscle was also retracted. The periosteum covering the 3rd metacarpus was incised with a scapel to allow for stripping of the soft tissue from the bone.

d. A DCP (6 hole mini dynamic compression plate, Synthes®, Waldenburg, Switzerland) was placed

lengthwise on the bone. The middle two holes were eccentrically drilled using a Hall air drill (AMSCO, Hall Surgical, Santa Barbara, California, USA) with a 1.5 mm drill bit. The holes were then tapped with a 2mm tap. A drill guide was used to protect the soft tissue.

e,f. Crystalline benzyl penicillin (Penicillin G, Dawa Pharmaceuticals Ltd.,Nairobi,Kenya), 1 million IU in 50 ml sterile water, was used to irrigate the surgical site. The DCP was removed and the bone transected between the two holes using a micro oscillating saw (AMSCO, Hall Surgical).

g,h. The DCP was then replaced and 2 mm screws (Synthes®) inserted into the predrilled holes and fastened, thus reducing and compressing the fracture. The remaining four holes were drilled, tapped and screwed to complete the fracture stabilization. The torniquet was then removed, the surgical site once more irrigated with the benzyl penicillin solution and bleeding controlled.

i. The skin was sutured with 2/0 monofilament nylon in a simple interrupted pattern.

The mean duration of various anaesthetic and surgical procedures are presented in Table 3.1, and individual values are given in Appendix 3.1.

3.2.5 Assessments

Post-operative readings of swelling, pain, limping and rectal body temperatures were taken between 8 and 9 a.m. on days 1 to 7, 10, 14 and 21, and recorded on printed data sheets (Appendix 3.2). Any abnormalities in wound healing were also recorded, as well as any clinical signs of adverse effects that could be related to the medication.

Swelling: The post-operative volume of the limb was measured by immersion of the limb into a custom made calibrated plastic cylinder. The limb was dipped up to a premarked line on the skin at the junction of the upper and middle 1/3 of the radius on the lateral-posterior aspect of the limb. The cylinder had an outlet with a flexible tube 3cm from the top. It was filled with tap water to overflowing (Fig. 3.2). The limb was thoroughly washed with plain plain water before being dipped into the cylinder. The water displaced was collected into a graduated cylinder until the flow changed from continuous flow to a drip. The average of three successive readings was taken to be the volume

of the day. The volume of swelling was obtained by simple subtraction of pre- from post-operative measurements.

Pain: The surgeon estimated the pain felt by the animal by exerting digital pressure on the fracture site. The pain estimate was marked on a visual analogue scale (VAS) that ran from "no pain" (0 mm) to "pain cannot be worse" (100 mm).

Limping: The surgeon assessed the degree of limping on a 100 mm VAS that ran from "no limping" to "limping cannot be worse".

Temperature: The rectal temperature was recorded by a mercury thermometer. Temperature above 39.3°C was selected as a clinical indicator of hyperthermia.

Bone healing: Radiographs were taken 2, 4, 6 and 8 weeks after each operation. Their evaluation was based on 4 parameters; radiographic union, degree of callus formation, evidence of infection and/or foreign body reaction, and the number of loose screws. The first 3 parameters were graded against a semi-quantitative scale from 0 to ++++, according to the following criteria:

Radiographic union of bones:

0 No evidence of union. A radiolucent line at the fracture site.

\+ Callus bridging the fracture gap is partially ossified.

++ Fracture gap is filled with ossified tissue whose radio-opacity does not yet match the remaining cortex.

+++ Most of the osseous tissue in the fracture gap has the same radio-opacity as the rest of the cortex.

++++ Fracture line undecernible as it is completely filled with radio-opaque bone.

Radiographs showing examples of the grading of the bone union are presented in Fig. 3.3a-d.

Degree of callus formation:

0 No exostosis (new bone formation) at the fracture site and its adjacent area.

\+ Minimal exostosis above the level of the cortex.

++ Exostosis around the fracture site is more extensive giving the cortical surface a rough appearance.

+++ Exostosis has extended to the top of the DCP at the fracture site.

++++ Exostosis has extended beyond and along the surface of the top of the DCP.

Radiographs showing examples of grading of callus formation are presented in Fig. 3.4a-d.

Evidence of infection and/or foreign body reaction:

0 No evidence of infection and/or reaction to the DCP implant.

+ Fracture gap enlarged and there is osteolysis in the distal bone fragment.

++ Fracture gap is wider. There is osteolysis of the proximal and distal bone fragments and new bone is encrouching on the proximal end of the DCP.

+++ Fracture gap is large with osteolysis of the whole bone and exostosis in the proximal fracture segment and on the DCP.

++++ Fracture gap is large with rough cortical surface due to exostosis. There is osteolysis and cavitation of the whole bone, sometimes including bone sequestration.

Radiographs showing examples of the grading are presented in Fig. 3.5a-e.

Number of loose screws:

Screws were deemed loose if they had migrated from the DCP or they had osteolytic areas along their length (Fig. 3.6). The maximum number that could come loose was 6.

Bone sections: - Eight weeks after the second operation the animals were euthanized using 18% pentobarbitone (Euthatal®, May and Baker, Dagenham, UK). The 3rd metacarpi were harvested and the DCP, including screws, recovered. The metacarpi were stored in 10% buffered formalin (BDH Chemicals Ltd., Poole, UK) to await processing. 20µ thick whole bone sagittal sections were cut at 3 levels with a cryomicrotome (PMV 450 MP, PMV, Sweden) at -20 °C and stained with haematoxylin and eosin according to standard procedure. Stained sections were examined for degree of bone healing.

The healing was graded according to a semi-quantitative scale that ran from 0 to ++++ using the following criteria:

0 No bone tissue seen across the fracture gap in any of the three levels of sectioning.

\+ Fracture gap of the cortex nearest to the DCP bridged by a mixture of osseous and cartilagenous tissue, while the opposite cortex has fibrous tissue only.

++ Fracture gap visible and has more tissue across it.

+++ Fracture gap bridged by osseous tissue, though

its position is indicated by the presence of bone tissue in the medullary cavity and osseous callus on the cortex opposite the DCP.

++++ Complete healing of the fracture.

Photographs of sections showing examples of the grading are presented in Fig. 3.7a-e.

Cortisol assessment: 10 ml blood was collected from each dog between 8.30 and 9 a.m., and at 4 p.m. for 4 days before and 5 days after each operation. On the day of operation blood was collected at 4 p.m. The serum from this blood was stored at -20°C for future cortisol analyses. All whole blood tubes were kept at room temperature (18-20°C) for one hour before centrifugation at 2000 g for 10 minutes. Pipetted serum was used for the subsequent cortisol analyses. A Farmos Diagnostica [^{125}I]-cortisol RIA kit (Farmos Group Ltd., Turku, Finland) with tubes marked for standards and unknowns was used. 25 µl of lyophilized cortisol standards in cortisol-free human serum were pipetted into appropriate tubes in concentrations of 0, 20, 50, 150, 400, 1000 and 2000 nmol/l. 25 µl of unknowns were pipetted into appropriate tubes. 100 µl of [^{125}I]-cortisol solution was pipetted into all tubes. Subsequently, 100 µl of rabbit antiserum solution was

pipetted into all tubes. All tubes were mixed thoroughly on a vortex mixer, covered with an adhesive plastic film and incubated for 60 min at 37°C. The tubes were then equilibrated at room temperature (18-20°C) for 10 min. 1.0 ml polyethylene glycol was then dispensed into all tubes except the total tubes, and mixed on a vortex mixer. All tubes were then centrifuged for 15 min at 2000 g. All supernatants were discarded and each tube was counted for one minute in a Packard Auto-Gamma counter (Packard, Downers Grove, Ill., USA).

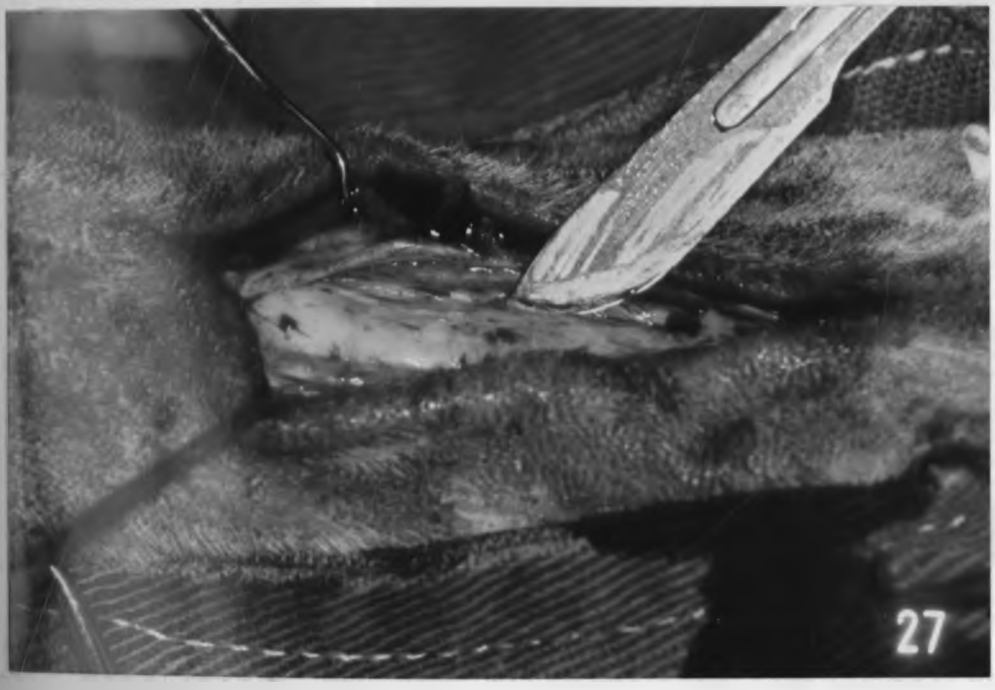
The cortisol results were obtained by calculating the mean of the counts found in the zero-standard tubes. A standard curve was drawn on semi-log graph paper with per cent binding of standard sample count/mean zero-standard count on the ordinate, and cortisol concentrations (nmol/l) of the standards on the abscissa. The cortisol concentration of the unknowns was read from the standard curve.

3.2.6 Statistical analyses

The data were assessed for normality, and the appropriate statistical test was chosen. An example is given in Appendix 3.3. A two-sided Wilcoxon signed

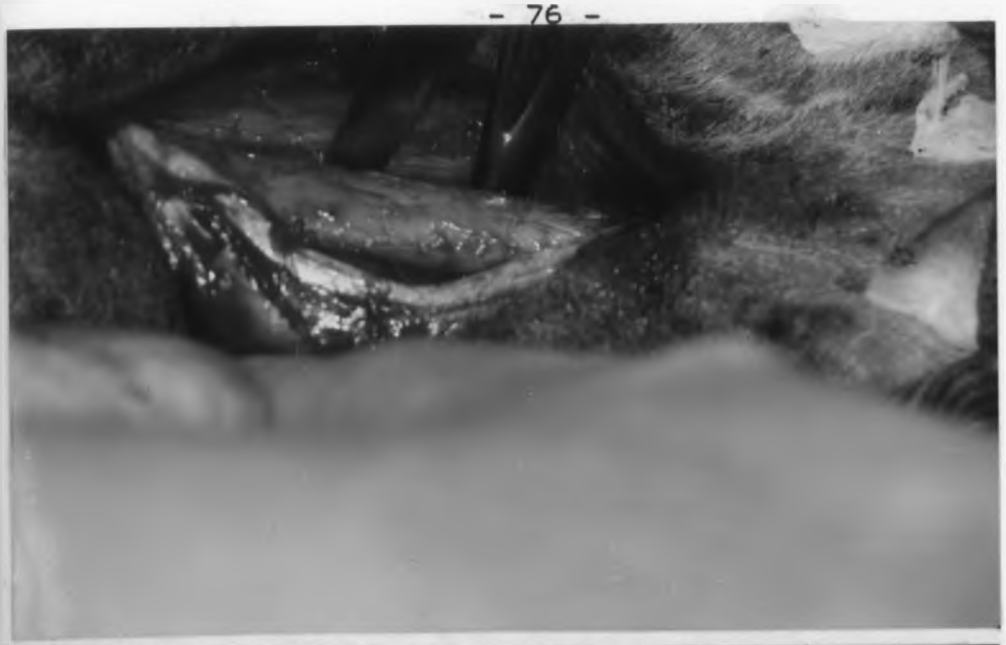
rank test with correction for ties (Lehmann and d'Abrera, 1975) was found appropriate for the analyses of post-operative swelling, pain and limping. A significance level of 5% was used.

a



b

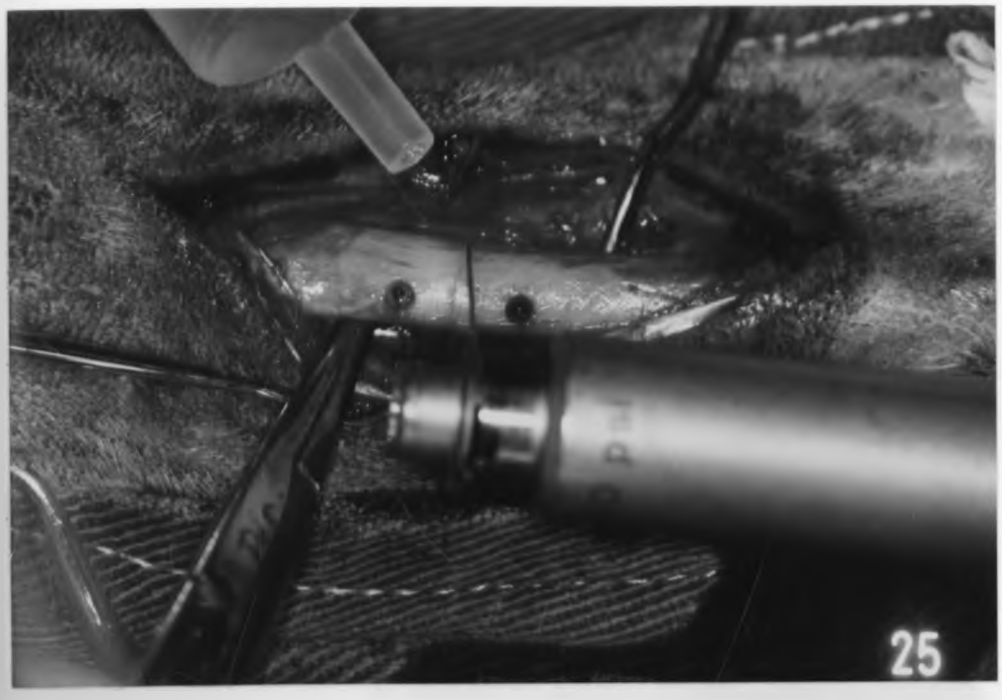
Fig. 3.1 Surgical procedure: a. Incision through the skin and subcutaneous tissues above the 3rd metacarpus. b. The periosteum covering the 3rd metacarpus is incised with a scarpel to allow for stripping of the soft tissue from the bone.



c

d

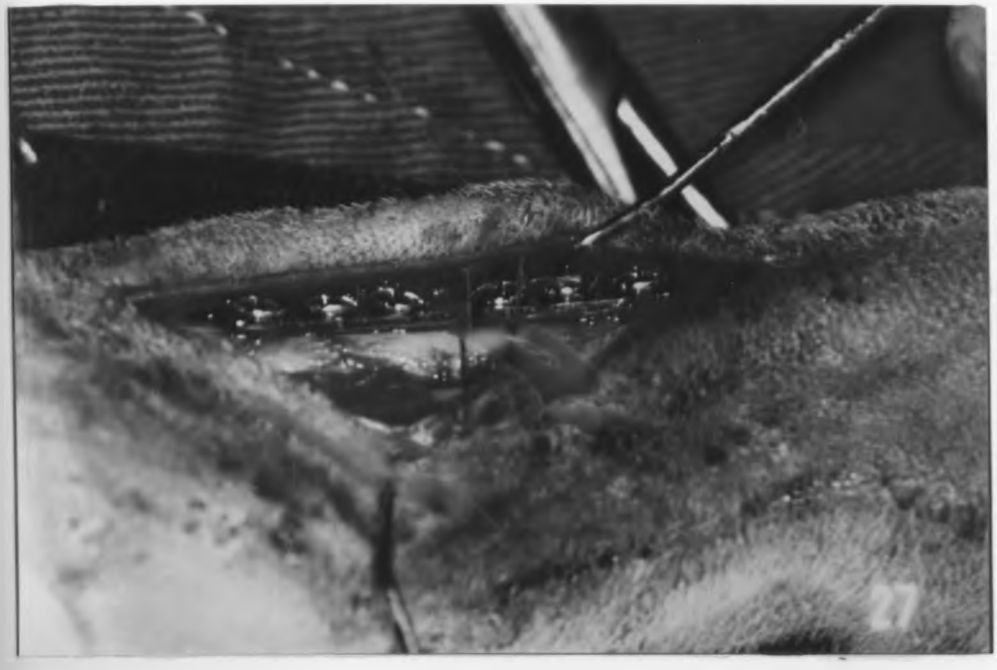
Fig. 3.1 Surgical procedure (continued): c. Most of the soft tissue has been stripped from the dorsal and lateral sides of the 3rd metacarpus. d. Position of the drill and drill guide during the drilling of two eccentric holes through the middle two holes of the dynamic compression plate (DCP) placed dorsally on the 3rd metacarpus.



e

f

Fig. 3.1 Surgical procedure (continued):
e. Transection between the two predrilled holes using micro-oscillating saw while the area is irrigated.
f. Complete transverse 3rd metacarpal fracture.



g

h

Fig. 3.1 Surgical procedure (continued): g. Lateral and h. Dorsal views of the DPC *in situ*.

i

Fig. 3.1 Surgical procedure (continued): i. Skin sutures in place after completion of the surgical procedure.

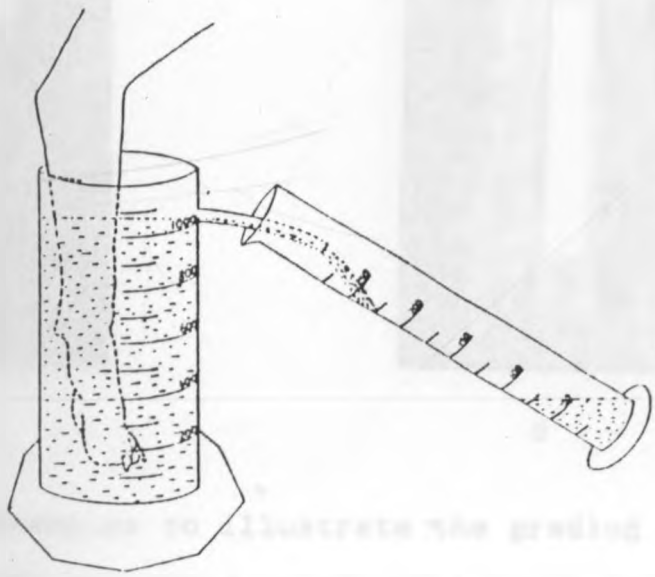
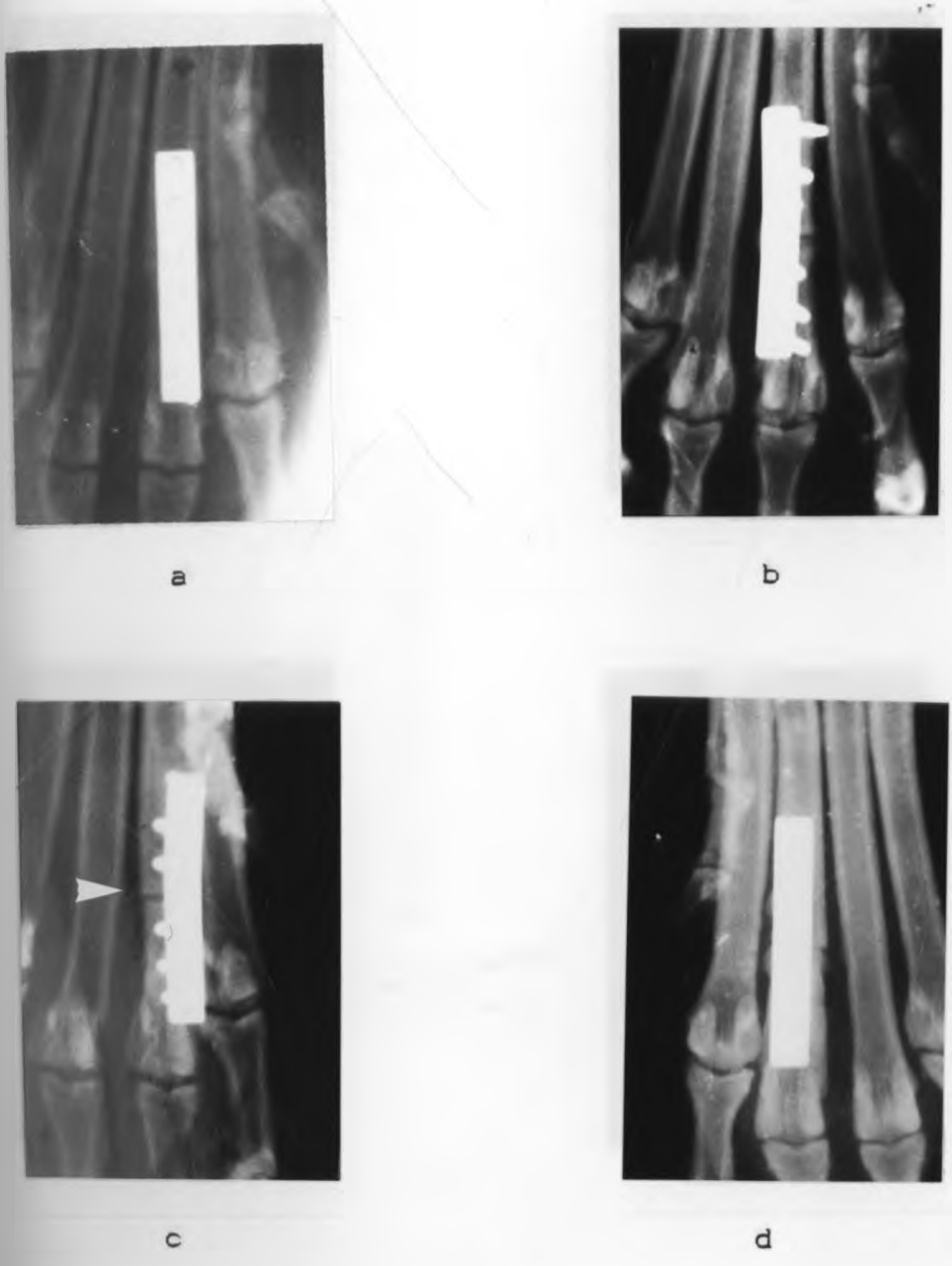
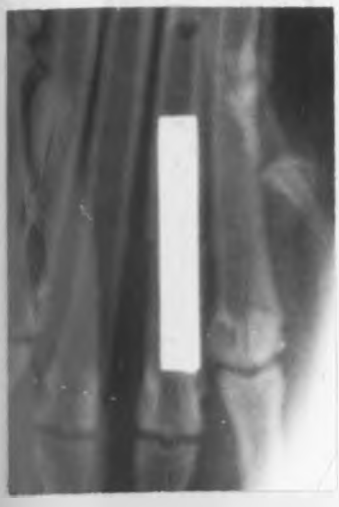


Fig. 3.2 Device for foot volumetry.

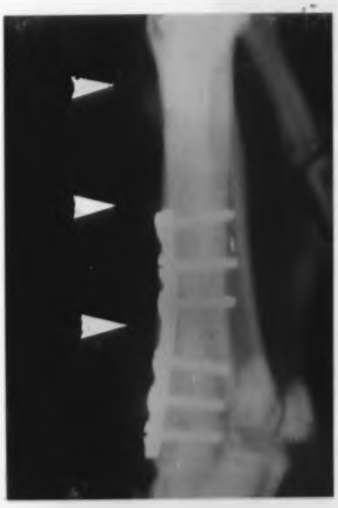


a b c d

Fig. 3.3 Examples to illustrate the grading system for bone union described on p. 69. a. grade 0, b. grade +, c. grade ++, d. grade +++, e. grade ++++, not obtained in the study (The quality of the photographs is inferior to the original radiographs).

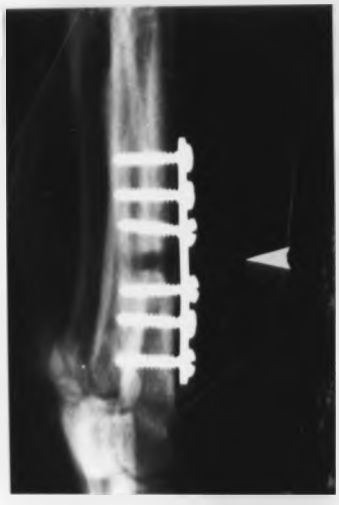


a



b

c



d

Fig. 3.4 Examples to illustrate the grading system for callus formation described on p.69. a. grade 0, b. grade +, c. grade ++, d. grade +++, e. grade ++++, not obtained in the study. (The quality of the photographs is inferior to the original radiographs).

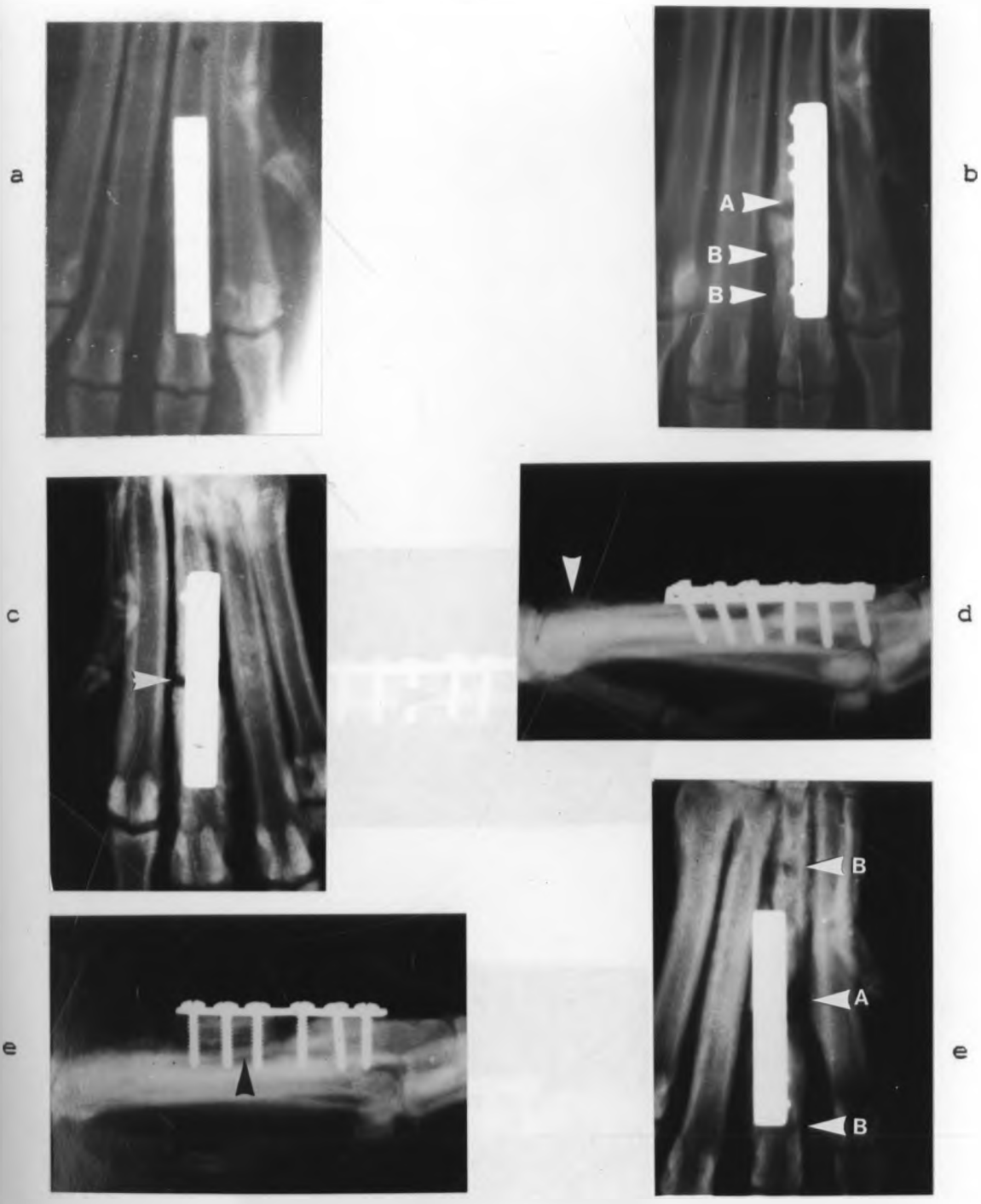
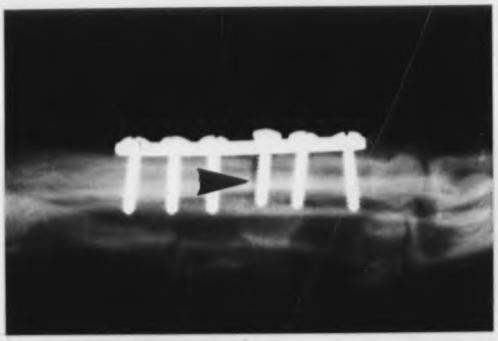
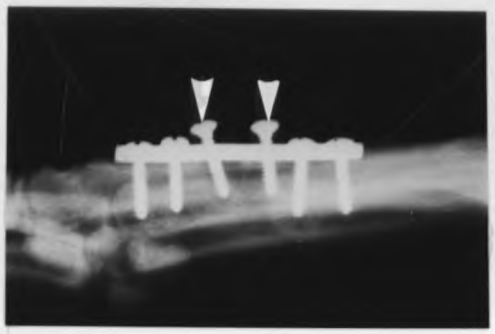


Fig. 3.5 Examples to illustrate the grading system for evidence of infection and/or reaction to implant described on p.70. a. grade 0, b. grade +, c. grade ++, d. grade +++, e. grade ++++. (A = large fracture gap; B = bone lysis and black arrow = sequestrum).

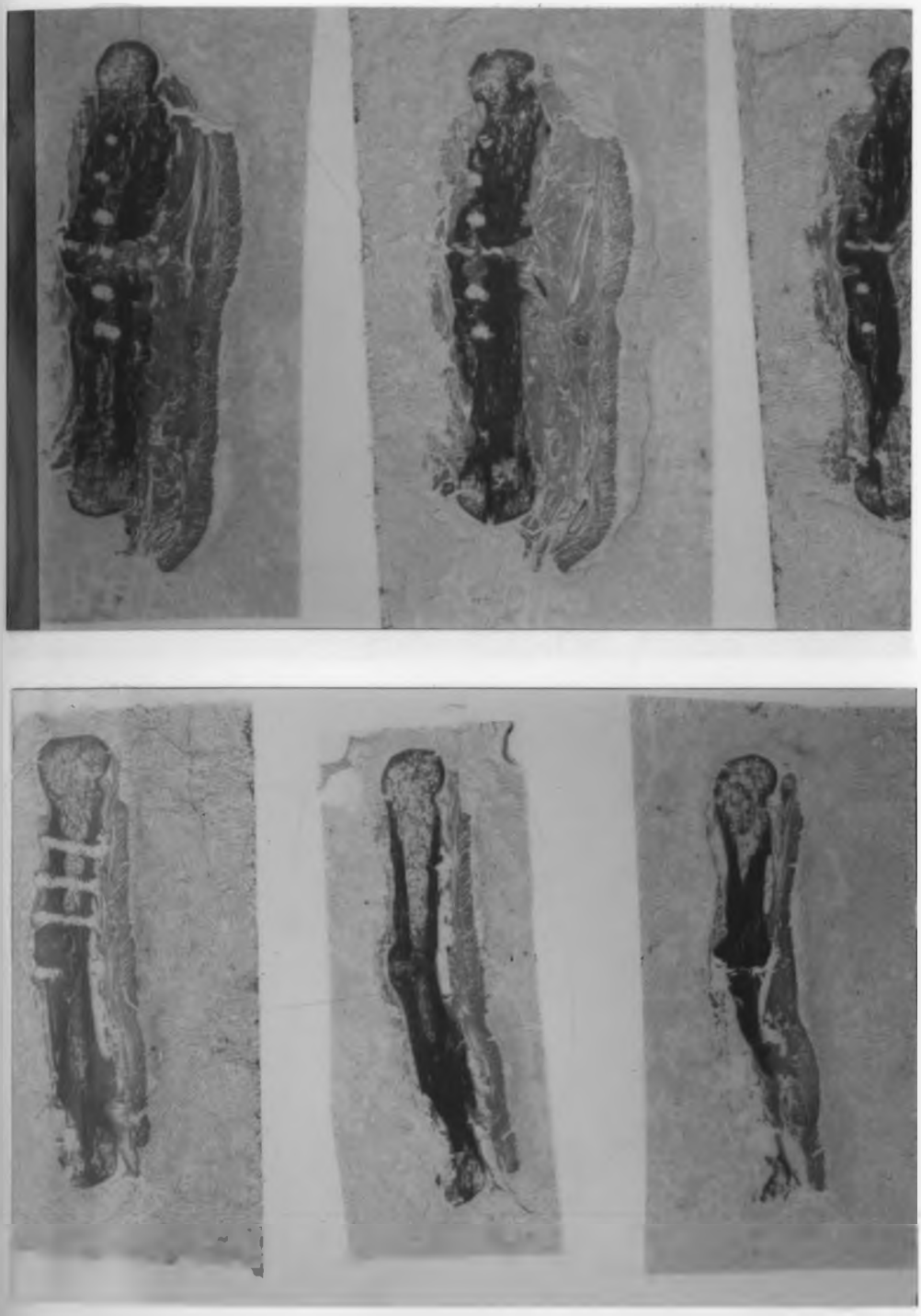


a



b

Fig. 3.6 Examples of loose screws as determined by a. osteolytic areas (black arrow), or b. migration from the DCP (white arrows).



a

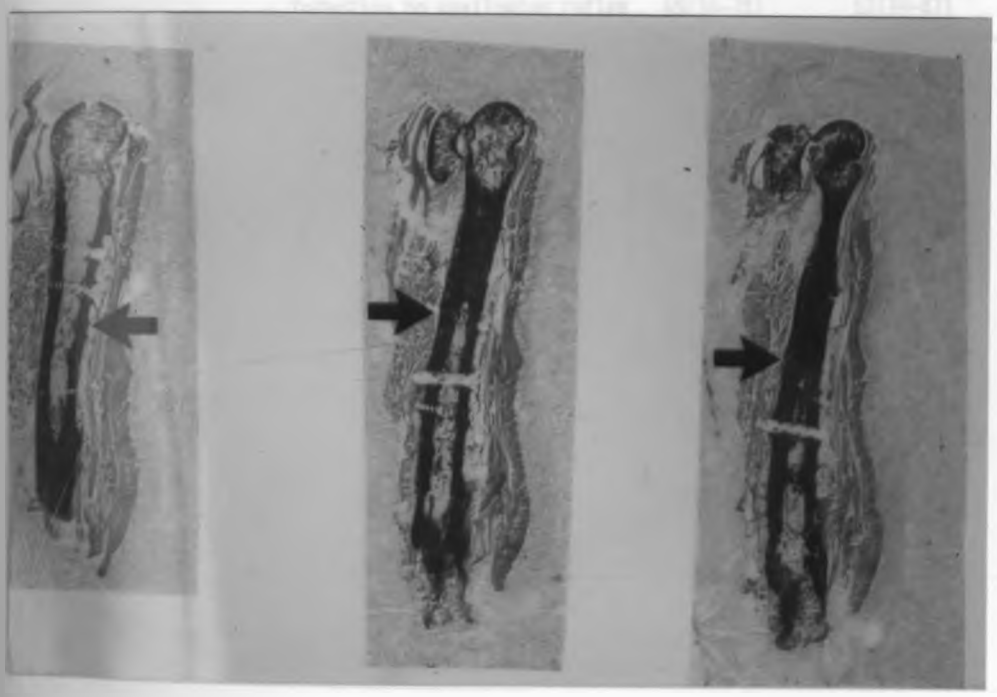
b

Fig. 3.7 Examples to illustrate the grading system of bone healing in H/E stained tissue sections of the 3rd metacarpi as described on p.71. a. grade 0, b. grade +.

c

d

Fig. 3.7 (continued) c. grade ++, d. grade +++.



e

Fig. 3.7 (continued) e. grade ++++ (arrow = fracture site).

Table 3.1 Time in minutes of various anaesthetic and surgical procedures in a crossover trial with a single i.m. injection of 3 mg betamethasone tested against placebo in 8 dogs. Mean (range).

	Betamethasone	Placebo
Induction of anaesthesia to drug injection	1.3(1-2)	1.6(1-3)
Induction to incision	10(5-15)	9(5-15)
Incision to last suture	31(25-36)	29(20-35)
Induction to swallowing reflex	45(34-75)	52(39-87)

3.3 RESULTS

3.3.1 Swelling

There was less swelling post-operatively when betamethasone was administered (Fig. 3.8). The differences were statistically significant for the first 3 days after surgery (Table 3.2). The values for individual dogs on the various days are given in Appendix 3.4. It can be seen that there was considerable individual variation in swelling and response to treatment. The volume of the limb did not return to the pre-operative value, due to the DCP implant.

3.3.2 Pain

There was a tendency towards less pain when betamethasone was administered (Fig. 3.9), but on none of the days of assessment did the difference reach a level of significance (Table 3.3). As for swelling, there was great variation between the dogs in their response to digital pressure applied on the site of surgery (Appendix 3.5).

3.3.3 Limping

There was a tendency to less limping with betamethasone (Fig. 3.10 and Table 3.4). Also this parameter displayed large variations between the dogs (Appendix 3.6).

3.3.4 Temperature

Readings above 39.3°C (the pre-selected clinical cut-off value between normal and elevated temperature) were recorded 4 times after the operation when placebo was given and 6 times after the operation when betamethasone was administered. None of the animals exhibited persistent pyrexia indicative of an on-going systemic infection. The mean and ranges of temperatures recorded over the trial period according to treatment group are presented in Table 3.5. Individual readings are given in Appendix 3.7.

3.3.5 Wound healing

There was no clear-cut difference in wound healing between the two treatment regimes. There was slower healing in one dog during placebo treatment. During betamethasone treatment one dog developed a seroma, and another dog with wound dehiscence had to be resutured on the 4th post-operative day. All these three dogs used to lick their wounds frequently.

3.3.6 Bone healing/radiographs

The assessments of bone healing after 2, 4, 6 and 8 weeks are presented in Table 3.6a-d. The first radiologically detectable bone activity was observed 4 weeks after surgery. The radiographic evaluation did not reveal any apparent difference between the two treatments.

3.3.7 Bone healing/tissue sections

There were no apparent differences in the healing scores between the two treatment groups as shown in Table 3.7.

3.3.8 Cortisol assessment

The endogenous cortisol level was depressed after injection of betamethasone (Fig. 3.11). This depression lasted about 3 days, and then it began to return to normal levels. After the operation when placebo was given there was an increase in the cortisol level. On the 3rd day there was a significant, but temporary, drop in the endogenous cortisol level. On the 4th day the levels were normal. Individual values are presented in Appendix 3.8.

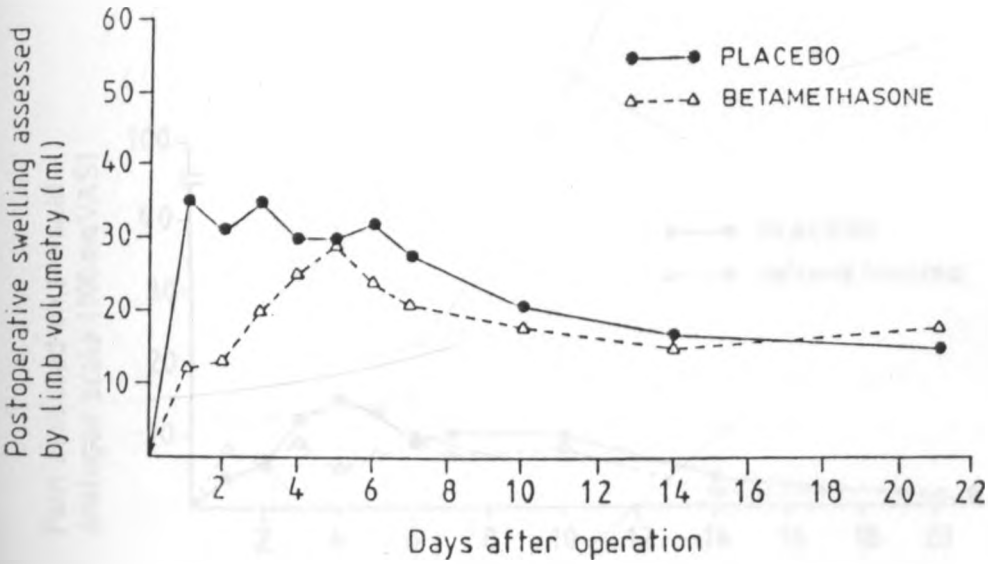


Fig. 3.8 Mean post-operative swelling after orthopaedic surgery in a crossover trial with a single i.m. injection of 3 mg betamethasone tested against placebo in 8 dogs.

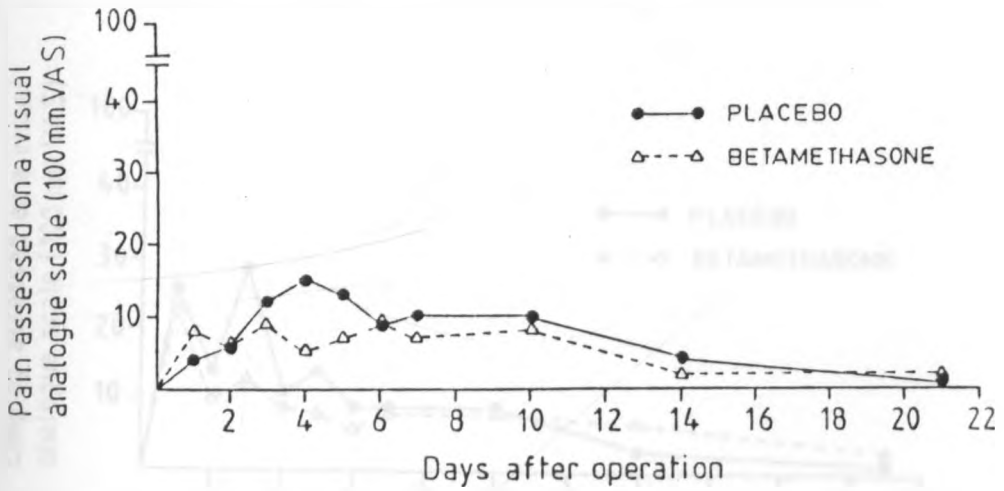


Fig. 3.9 Mean post-operative pain assessed by a VAS after orthopaedic surgery in a crossover trial with a single i.m. injection of 3 mg betamethasone tested against placebo in 8 dogs.

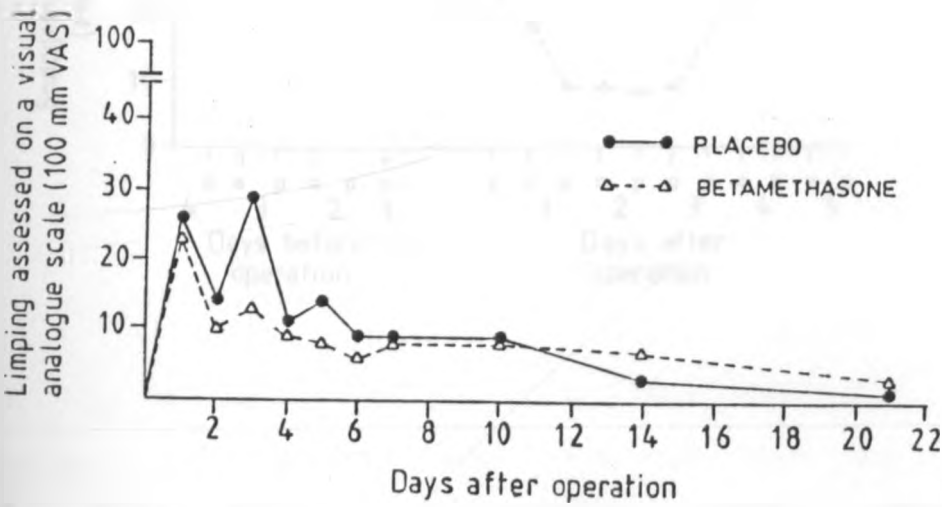


Fig. 3.10 Mean limping values assessed by a VAS after orthopaedic surgery in a crossover trial with a single i.m. injection of 3 mg betamethasone tested against placebo in 8 dogs.

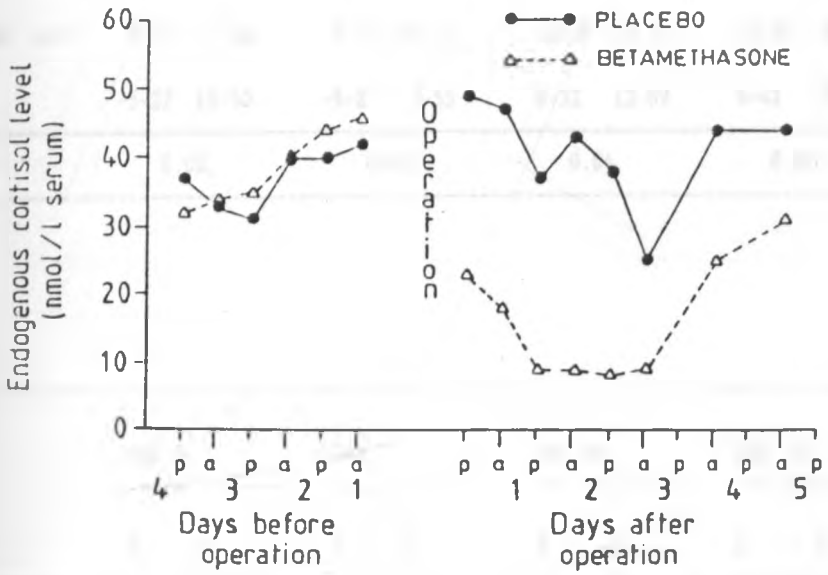


Fig. 3.11 Endogenous cortisol levels after orthopaedic surgery in a crossover trial with a single i.m. injection of 3mg betamethasone tested against placebo in 8 dogs; a = morning values and p = afternoon values.

Table 3.2 Post-operative swelling as ml displaced water measured by limb volumetry after orthopaedic surgery in a crossover trial with a single i.m. injection of 3 mg betamethasone (B) tested against placebo (P) in 8 dogs.

	Day 1		Day 2		Day 3		Day 4		Day 5	
	B	P	B	P	B	P	B	P	B	P
Mean	12	35	13	31	20	35	25	30	29	30
Median	10	30	16	32	22	31	25	29	36	29
95% conf. int.	2-21	23-46	5-22	21-41	13-28	22-48	15-36	18-42	19-39	21-39
Range	-5-27	19-50	-5-27	9-55	8-32	12-69	6-42	10-59	11-42	15-55
P value	0.02		0.03		0.04		0.31		0.47	

	Day 6		Day 7		Day 10		Day 14		Day 21	
	B	P	B	P	B	P	B	P	B	P
Mean	24	32	21	28	18	21	15	17	18	15
Median	28	32	24	29	18	18	13	14	19	13
95% conf. int.	12-35	26-39	9-31	18-38	9-27	11-30	6-25	9-25	8-27	5-25
Range	1-42	22-48	-10-34	10-51	0-34	6-47	1-35	5-36	3-38	-1-37
P values	0.09		0.09		0.31		0.42		0.36	

Table 3.3 Post-operative pain in mm, assessed by a VAS after orthopaedic surgery in a crossover trial with a single i.m. injection of 3 mg betamethasone (B) tested against placebo (P) in 8 dogs.

	Day 1		Day 2		Day 3		Day 4		Day 5	
	B	P	B	P	B	P	B	P	B	P
Mean	8	4	6	6	9	12	5	15	7	13
Median	5	1	3	2	3	8	3	10	6	10
95% conf. int.	1-14	0-10	1-11	0-13	0-21	0-23	1-9	0-31	3-11	5-21
Range	1-20	0-22	0-15	0-21	0-45	0-44	1-15	0-60	3-18	3-36
P value	0.09		0.44		0.29		0.16		0.12	

	Day 6		Day 7		Day 10		Day 14		Day 21	
	B	P	B	P	B	P	B	P	B	P
Mean	9	9	7	10	8	10	2	4	2	1
Median	4	7	6	8	8	10	0	3	0	0
95% conf. int.	1-17	4-14	1-12	5-15	4-13	5-16	0-14	0-8	0-5	0-3
Range	0-28	3-22	0-19	4-18	0-17	2-22	0-6	0-15	0-7	0-4
P value	0.29		0.29		0.36		0.20		0.25	

Table 3.4 Post-operative limping in mm, assessed by a VAS after orthopaedic surgery in a crossover trial with a single i.m. injection of 3 mg betamethasone (B) tested against placebo (P) in 8 dogs.

	Day 1		Day 2		Day 3		Day 4		Day 5	
	B	P	B	P	B	P	B	P	B	P
Mean	23	26	10	14	13	29	9	11	8	14
Median	16	9	7	14	7	14	7	15	10	13
95% conf. int.	7-36	0-53	4-16	7-21	0-28	0-58	2-15	4-18	5-13	5-23
Range	7-47	4-79	4-24	0-24	0-52	6-11	0-25	0-19	1-14	0-34
P values	0.47		0.23		0.24		0.34		0.18	

	Day 6		Day 7		Day 10		Day 14		Day 21	
	B	P	B	P	B	P	B	P	B	P
Mean	6	9	8	9	8	9	7	3	3	1
Median	6	6	8	7	7	6	7	1	0	0
95% conf. int.	5-9	2-15	6-11	3-15	5-10	3-16	3-11	0-7	0-8	0-3
Range	5-11	0-21	5-13	0-21	4-14	0-19	0-14	0-12	0-14	0-6
P values	0.33		0.44		0.27		0.07		0.12	

Table 3.5 Post-operative temperature in °C, mean (range), in a crossover trial after orthopaedic surgery with a single i.m. injection of 3 mg betamethasone tested against placebo in 8 dogs.

	Day 1	Day 2	Day 3
Betamethasone	38.9(38.3-39.6)	38.6(38.0-39.4)	38.8(37.8-39.4)
Placebo	39.1(38.0-40.2)	38.7(38.5-39.1)	38.4(37.9-39.2)

	Day 4	Day 5	Day 6
Betamethasone	38.6(38.0-39.6)	38.8(37.9-39.8)	38.5(37.8-39.4)
Placebo	38.8(37.6-39.9)	38.6(38.0-39.0)	38.5(38.0-39.1)

	Day 7	Day 10	Day 14
Betamethasone	38.7(38.1-39.4)	38.4(37.2-39.3)	38.2(37.0-39.0)
Placebo	39.1(37.8-38.7)	38.1(37.8-38.5)	38.2(37.2-39.1)

	Day 21
Betamethasone	37.9(37.3-38.6)
Placebo	38.0(37.0-38.8)

Table 3.6a.b Radiographic assessment of bone healing in a crossover trial 2 and 4 weeks after orthopaedic surgery with a single i.m. injection of 3 mg betamethasone (B) tested against placebo (P) in 8 dogs.

Dog no.	Radiographic union[a]		Degree of callus[a]		Infection/ reaction to implant[a]		No. of loose screws	
	B	P	B	P	B	P	B	P
2 weeks (a)								
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
4 weeks (b)								
1	0	+	0	+	0	0	0	0
2	+	0	+	+	+	0	1	0
3	0	0	+	+	0	0	0	0
4	0	0	+	+	+	+	0	0
5	+	+	+	+	0	0	0	0
6	0	0	+	+	++	0	2	0
7	0	0	0	+	0	+	0	0
8	0	0	+	0	+	0	0	0

[a] 0 = minimum; ++++ = maximum

Table 3.6c,d Radiographic assessment of bone healing in a crossover trial 6 and 8 weeks after orthopaedic surgery with a single i.m. injection of 3 mg betamethasone (B) tested against placebo (P) in 8 dogs.

Dog	Radiographic union[a]		Degree of callus[a]		Infection/ reaction to implant[a]		No. of loose screws	
	B	P	B	P	B	P	B	P
6 weeks (c)								
1	+	0	0	0	0	+	0	0
2	+	+	++	+	+	0	0	0
3	++	+	+	++	+	0	0	0
4	+	+	+	+	0	+	0	0
5	++	+	+	0	0	0	0	0
6	0	++	++	+	+	0	2	0
7	0	+	0	+	0	0	0	0
8	0	0	+	0	+	0	0	0
8 weeks (d)								
1	++	0	+	++	0	++	0	0
2	0	+	++	+	+	0	3	0
3	+++	+++	+	+	0	0	0	0
4	+++	0	0	++	0	+	0	2
5	++	+++	+	+	0	0	0	0
6	+	+	++	+	+++	0	2	0
7	++	+	0	0	0	0	0	0
8	0	++	+	0	+	0	2	0

[a] 0 = minimum; ++++ = maximum.

Table 3.7 Assessment of bone healing using H/E stained sagittal sections of 3rd metacarpi 8 and 12 weeks after orthopaedic surgery in a crossover trial with a single i.m. injection of 3 mg betamethasone tested against placebo in 8 dogs.

Dog	Betamethasone		Placebo	
no	8 weeks	12 weeks	8 weeks	12 weeks
1	+			0
2		0	++	
3		+++	+++	
4	+++			0
5	+++			++++
6		0	++	
7	+++			++
8		+	+++	

0 = minimum bone healing; ++++ = maximum bone healing.

3.4 DISCUSSION

A single injection of 3 mg betamethasone reduced the post-operative swelling significantly. On the 3rd day the mean reduction was 43% compared to placebo. Review of the literature shows few controlled clinical trials on the effect of short term glucocorticoid administration on the post-surgical or post-traumatic course (Swartz and Dluhy, 1978; Schiller and De Silva, 1979). The reduction in swelling, recorded in the present study agrees well with earlier reports, which showed that glucocorticoid administration reduced the swelling after oral surgery by about 50% (Skjelbred and Lökken, 1982a,b; 1983). In some other experiments with animals, glucocorticoids have also been shown to reduce post-traumatic swelling. Traumatic oedema of the rat ear pinna was reduced by 29% following medication with hydrocortisone (Marek and Blaha, 1980),and betamethasone has been found to significantly reduce the swelling in replanted rat legs (Colen et al., 1979).

While the reduction of swelling was significant, neither the reduction of pain nor the reduction of limping reached a level of significance, although the pain estimates tended to be lower after glucocorticoid administration. The lack of significant pain relief is in accordance with the common view that glucocorticoids

do not exert an analgesic effect (Huskisson, 1984), but contrasts the striking pain relief reported in human oral surgery (Messer and Keller, 1975; Huffman, 1977; Skjelbred and Lökken, 1982a,b; 1983). The analgesic effect of glucocorticoids might be secondary to other anti-inflammatory actions such as reduction of swelling, but there is both theoretical and experimental support of the view that a dissociation may exist between pain and other inflammatory events, such as swelling (Moncada et al., 1978; Ferreira and Nakamura, 1979; Skjelbred and Lökken, 1982a). In the dogs it was found difficult to obtain reliable and consistent estimates for pain and limping, in spite of the dogs being used as their own controls. These results should therefore be interpreted with caution. The limping assessments showed the largest variations, both inter- and intraindividually, and it was decided to omit this parameter in the subsequent studies.

The present methods of assessment did not reveal any adverse effect of the glucocorticoid on wound or bone healing. This agrees with the view that a single dose of a glucocorticoid is not likely to impair the healing process and is essentially safe, while long term treatment is associated with numerous adverse effects (Schiller and De Silva, 1979; Haynes and Murad,

1985). In rabbits, multiple doses of cortisone have been demonstrated to delay the healing of experimental fractures (Blunt et al.. 1950; Sissons and Hadfield, 1951). Peacock and van Winkle (1976) reviewed a number of studies in which glucocorticoids had been found to inhibit wound healing in laboratory animals, but considered the data inadequate, since in most instances the doses used were considerably higher than those normally administered in the clinic.

The glucocorticoid injected, Celeston Chronodose®, is a suspension of equal parts of betamethasone disodium-phosphate and betamethasone acetate. Since the easily soluble phosphate ester is quickly absorbed, and the acetate is only slightly soluble and claimed to provide a sustained action, a single injection has been found adequate to curb post-traumatic sequelae (Hooley and Frances, 1969; Skjelbred and Lökken, 1982a).

Before both operations a slight rise occurred in the endogenous cortisol levels. This might be related to a certain stress caused by the pre-operative preparation, e.g. blood sampling and shaving. With one exception, the dogs uniformly exhibited a marked supression of the serum cortisol level after the glucocorticoid injection. The reasons for the

exception remain obscure. On the 3rd day the level started to return to normal which was reached on the 5th day. The relatively rapid return to normal levels supports the view that the function of hypothalamic - pituitary - adrenal axis is only temporarily affected by short-term glucocorticoid administration and is still capable of responding to stress (Hooley et al., 1973; Williamson et al., 1980). The cortisol levels were somewhat elevated after the operation when placebo was injected, then fluctuated and showed a marked drop on the 3rd day, and may reflect the complex mechanisms taking place within the hypothalamic - pituitary - adrenal system.

There are several in vitro and in vivo experimental models that have achieved popularity for their ability to select drugs known to exert beneficial anti-inflammatory effects in rheumatoid conditions (Arrigoni-Martelli, 1979). It should be kept in mind, however, that the mechanisms and pattern of reactions of an acute inflammatory process after, for example a surgical trauma, differ from the conditions in rheumatoid arthritis. Accordingly, anti-inflammatory drug effects are not necessarily the same in these different conditions. It has been questioned whether the results obtained with glucocorticoids in oral surgery, would

also apply to surgical interventions and accidental traumas in other parts of the body, e.g. the extremeties (Skjelbred and Lökken, 1982a). The present model with a standardized soft tissue and bone injury on the fore limbs of dogs, revealed a similar effect of a short-term glucocorticoid administration, at least with the reduction of the post-traumatic swelling as an indicator of anti-inflammatory efficacy. The study gave no evidence of any glucocorticoid-induced adverse effects. This adds some support to the view that short term glucocorticoid administration might be of value in alleviating suffering and excessive inflammatory responses in surgery and traumatology. This applies to the veterinary as well as the human clinic.

CHAPTER FOUR

EFFECTS OF PHENYLBUTAZONE AND INDOMETHACIN ON THE POST-OPERATIVE COURSE FOLLOWING EXPERIMENTAL ORTHOPAEDIC SURGERY IN DOGS

4.1 INTRODUCTION

Phenylbutazone was introduced in 1949 for the treatment of rheumatoid arthritis and allied diseases, while indomethacin was introduced in 1963 for treatment of the same disorders. Both are non-steroidal anti-inflammatory drugs (NSAID), and they have similar or identical modes of action related to their ability to interfere with the formation of mediators of inflammation, such as the prostaglandins.

NSAID are widely used in veterinary practice, although not as frequently as in human medicine. They are, as stated by Higgins (1985) prescribed for conditions as clinically diverse as equine exertional myopathy ("azoturia"), spasmodic colic, arthroses and arthritic conditions, tendonitis, and as post-operative prophylaxis to control untoward inflammatory sequelae.

The present placebo-controlled studies with bilateral limb surgery were designed to obtain information on how phenylbutazone and indomethacin might modulate the course and healing process after a

standardized surgical soft tissue/bone injury on the fore limbs of dogs. Phenylbutazone is probably still the most widely used NSAID in veterinary practice (Lees and Higgins, 1985). Indomethacin is another NSAID which has been commonly used in human, but only infrequently in veterinary medicine. A main reason for including indomethacin was that it has been reported to delay or inhibit fracture healing in experimental models in rats (Sudmann et al., 1979; Elves et al., 1982).

4.2 MATERIAL AND METHODS

4.2.1 Experimental design

The experimental design was on a placebo-controlled crossover basis as described in Chapter 3.2.1. In a group of 8 dogs phenylbutazone was tested against placebo; while in two other groups of 8 dogs indomethacin in high and low dosage was tested against placebo.

4.2.2 Experimental animals

Twenty four mongrel dogs were obtained from the local dog pound. The mean weight of the dogs in the phenylbutazone group was 15 kg (range 12-18), of those given

indomethacin in high dose 18 kg (15-21) and those given a low dose 18 kg (16-21). They were housed individually in kennels and provided with water ad libitum. Commercial dog food was fed once a day between 4 and 6 p.m.

Only healthy animals which fulfilled the examination criteria described in Chapter 3.2.2 were included.

In this study the animals were dewormed with the broad spectrum anthelmintic nitroscanat (Lopatol®, Ciba-Geigy Ltd., Basle, Switzerland).

4.2.3 Drug administration

Phenylbutazone: For one operation phenylbutazone (Mac's Pharmaceuticals, Nairobi, Kenya) was given orally, for the other matching placebo tablets. 300 mg phenylbutazone was given twice daily (7.30 - 8 a.m. and 6 - 6.30 p.m.) for 8 days, starting on the day before surgery. The treatments were allocated according to a randomization list. Half of the dogs received the active drug at the 1st operation.

Indomethacin/high dose: For one operation indomethacin (Indocid®, Merck-Sharp & Dohme, N.J., USA) was given orally, at the other matching placebo

tablets. 25 mg indomethacin was given twice daily as described for phenylbutazone. The medication had to be discontinued after 2 1/2 days, however, since the dogs showed signs of acute toxicity, mainly gastrointestinal haemorrhage.

Indomethacin/low dose: For one operation indomethacin (Confortid®, Dumex, Copenhagen, Denmark) was given orally, at the other a matching placebo syrup. 5 mg indomethacin (1 ml of 0.5% indomethacin syrup) was given twice daily as described for phenylbutazone.

4.2.4 Surgery

Pre-operative preparation, anaesthesia and surgery were as described in Chapter 3.2.4.

The duration of the various anaesthetic and surgical procedures are given in Tables 4.1-3, while individual values are given in Appendices 4.1 and 4.2.

4.2.5 Assessments

Assessment and evaluation of swelling, pain, temperature, bone healing and bone sections were done as described in Chapter 3.2.5.

Table 4.1 Time in minutes of various anaesthetic and surgical procedures in a crossover trial with 8 dogs given placebo and 300 mg phenylbutazone orally twice daily for 8 days starting the day before surgery. Mean (range).

	Phenylbutazone	Placebo
Induction to incision	9(6-14)	7(5-11)
Incision to last suture	33(28-38)	34(28-38)
Induction to swallowing reflex	49(38-60)	45(33-56)

Table 4.2 Time in minutes of various anaesthetic and surgical procedures in a crossover trial with 7 dogs given placebo and 25 mg indomethacin orally twice daily for 2 1/2 days starting the day before surgery. Mean (range).

	Indomethacin	Placebo
Induction to incision	6(3-8)	7(4-13)
Incision to last suture	30(23-39)	28(21-33)
Induction to swallowing reflex	45(33-58)	41(30-56)

Table 4.3 Time in minutes of various anaesthetic and surgical procedures in a crossover trial with 8 dogs given placebo and 5 mg indomethacin orally twice daily for 8 days starting the day before surgery. Mean (range).

	Indomethacin	Placebo
Induction to incision	6(2-9)	7(1-9)
Incision to last suture	29(24-33)	27(23-31)
Induction to swallowing reflex	49(37-66)	45(34-54)

4.3 RESULTS

4.3.1 Swelling

Phenylbutazone: Phenylbutazone did not result in any significant difference in swelling as compared to placebo (Fig. 4.1, Table 4.4, Appendix 4.3).

Indomethacin/high dose: High dose indomethacin did not result in any significant difference in swelling as compared to placebo (Fig. 4.2, Table 4.5, Appendix 4.4).

Indomethacin/low dose: There was a tendency towards less swelling after the operation when indomethacin was given. The differences were significant on days 7, 14 and 21 (Fig. 4.3, Table 4.6, Appendix 4.5).

4.3.2 Pain

Phenylbutazone: Less pain was assessed during the first days after the operation when phenylbutazone was administered. The difference was significant on Day 3 (Fig. 4.4, Table 4.7,). Variation among the animals was evident (Appendix 4.6).

Indomethacin/high dose: The pain assessments were significantly lower after the operation when indomethacin was given (Fig. 4.5, Table 4.8).As for phenylbutazone variation among the animals was evident (Appendix 4.7).

Indomethacin/low dose: There was no consistent difference in the pain assessments after the two operations (Fig. 4.6, Table 4.9, Appendix 4.8).

4.3.3 Temperature

Phenylbutazone: Readings above 39.3°C were recorded 14 times after the operation when phenylbutazone was given and 16 times after the operation when placebo was administered. Mean values and ranges are presented in Table 4.10, and individual values in Appendix 4.9.

Indomethacin/high dose: After the operations with indomethacin and placebo temperatures above 39.3°C were recorded 2 and 5 times respectively. Mean values and ranges are presented in Table 4.11, and individual values in Appendix 4.10.

Indomethacin/low dose: After the operations with indomethacin and placebo, temperatures above 39.3°C were recorded 1 and 2 times respectively. Mean values and ranges are presented in Table 4.12, and individual values in Appendix 4.11.

4.3.4 Wound healing

Phenylbutazone: According to subjective evaluation 7 days post-operatively, four dogs showed better wound healing with placebo, one with phenylbutazone, while three dogs showed no difference.

Indomethacin/high dose: There was no noticeable difference in wound healing between the two operations.

Indomethacin/low dose: As with indomethacin in high dose there appeared to be no difference in wound healing between the two operations.

4.3.5 Bone healing/radiographs

Phenylbutazone: Comparison of the two sets of radiographs taken 4 and 6 weeks after surgery revealed tendencies in favour of placebo both with regard to bone

union, callus formation and the tissue acceptance of the plates and screws (Table 4.13b,c). After 8 weeks, however, there were no noticeable differences (Table 4.13d).

Indomethacin/high dose: Comparison of the two sets of radiographs revealed no consistent difference between the two operations with regard to bone union (Table 4.14a-d). After the operation when indomethacin was given, infection and/or reaction to the implants occurred more frequently and at 8 weeks there was more callus formation, compared to when placebo was given.

Indomethacin/low dose: The radiographic evaluation revealed no consistent differences between indomethacin and placebo (Table 4.15a-d).

4.3.6 Bone healing/tissue sections

Phenylbutazone: According to the tissue sections there appeared to be somewhat better bone healing after the operation when placebo was given (Table 4.16).

Indomethacin/high dose: No bone sections were made in this trial.

Indomethacin/low dose: Evaluation of the bone sections revealed no consistent difference between indomethacin and placebo (Table 4.17).

4.3.7 Adverse drug reactions

Phenylbutazone: In no dog was there any clinically apparent sign of an adverse drug reaction after either of the two operations.

Indomethacin/high dose: Medication was discontinued on the 1st post-operative day since signs of toxicity developed, such as lethargy, vomiting and bloody stool. The dogs had then received a total dose of 125 mg indomethacin each. More or less severe signs proved to occur in all dogs when indomethacin was administered, and one died on the 5th post-operative day. At autopsy 8 weeks after the 2nd operation the gastrointestinal mucosa was hyperaemic in all dogs and there were ulcers in various stages of healing, especially in the duodenum and jejunum. The Peyer's patches were prominent and some of them were ulcerated.

Indomethacin/low dose: One dog developed bloody stool on the 5th post-operative day when indomethacin

was given. Otherwise, there were no evident clinical signs of toxicity. At autopsy 8 weeks after the 2nd operation the Peyer's patches were not as pronounced as after the high dosage of indomethacin and only a few minor ulcers were noted. The hyperaemia of the gastrointestinal mucosa was, however, even more marked than when indomethacin had been given at high dosage.

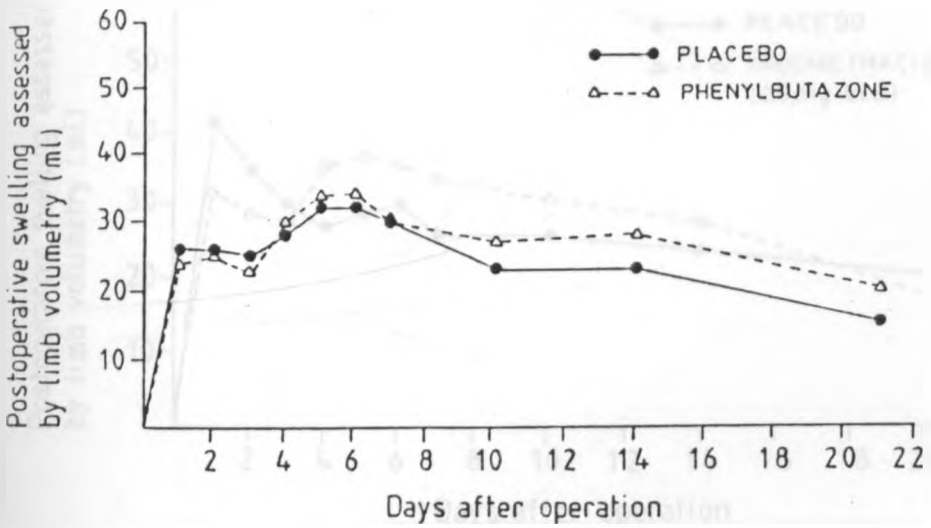


Fig. 4.1 Mean post-operative swelling after orthopaedic surgery in a crossover study with 8 dogs given placebo and 300 mg phenylbutazone orally twice daily for 8 days starting the day before surgery.

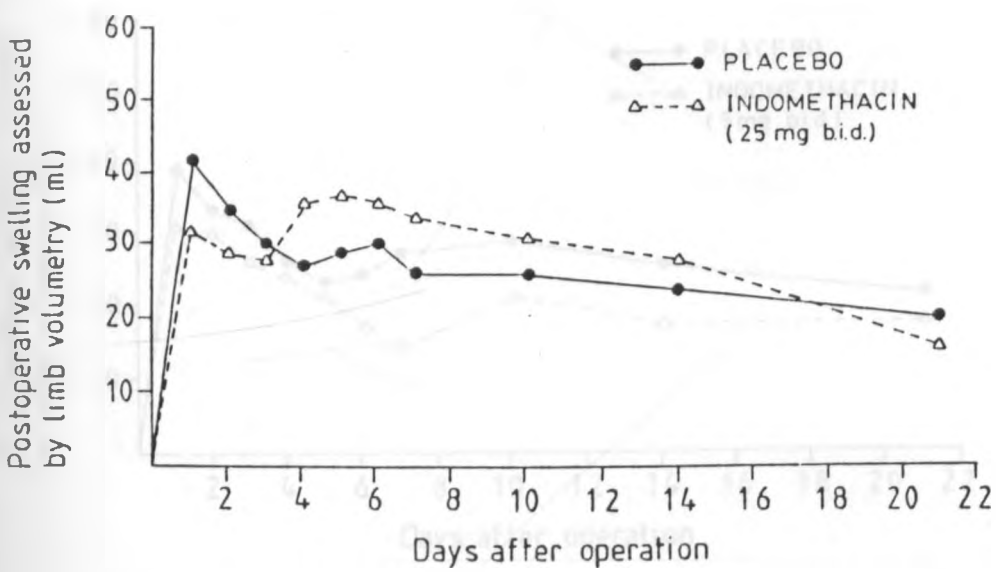


Fig. 4.2 Mean post-operative swelling after orthopaedic surgery in a crossover study with 7 dogs given placebo and 25 mg indomethacin orally twice daily for 2 1/2 days starting the day before surgery.

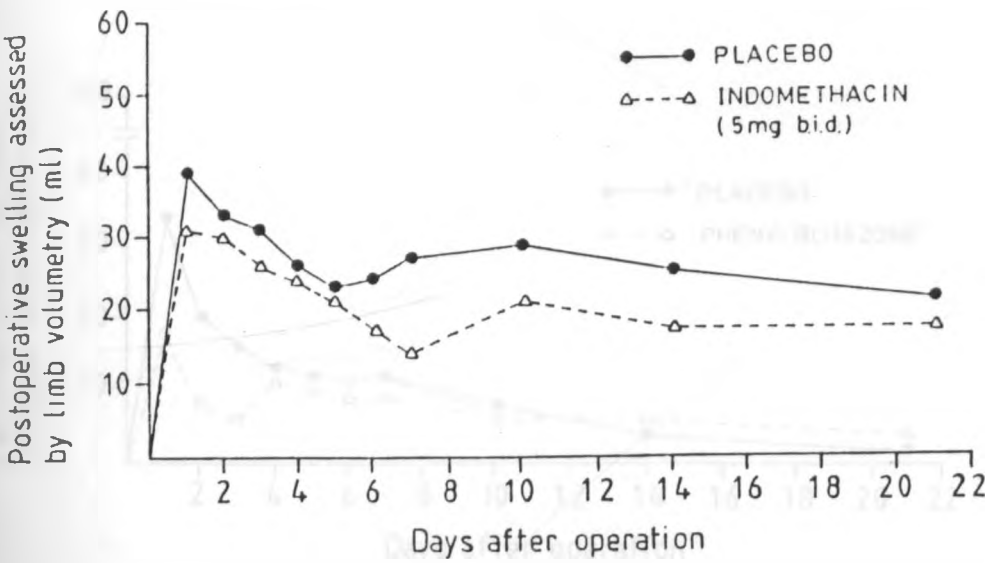


Fig. 4.3 Mean post-operative swelling after orthopaedic surgery in a crossover study with 8 dogs given placebo and 5 mg indomethacin orally twice daily for 8 days starting the day before surgery.

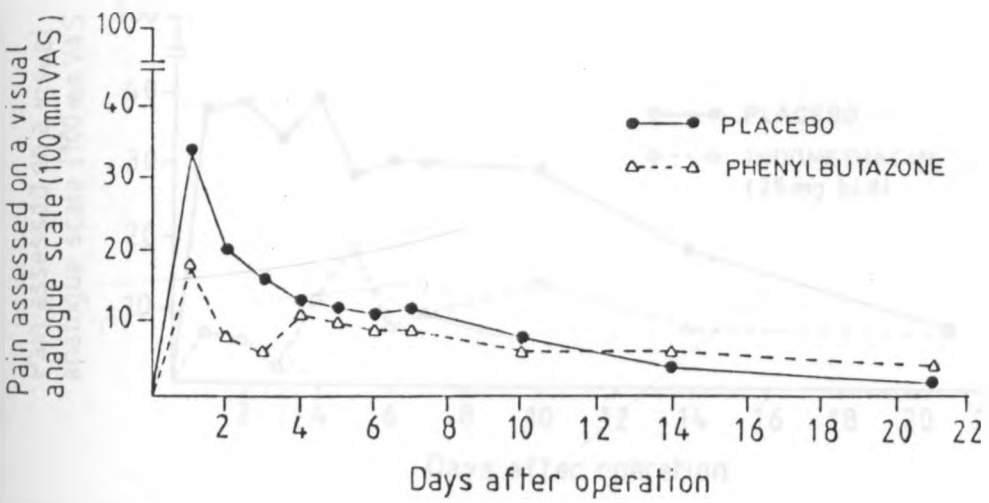


Fig. 4.4 Mean post-operative pain assessed by a VAS after orthopaedic surgery in a crossover study with 8 dogs given placebo and 300 mg phenylbutazone orally twice daily for 8 days starting the day before surgery.

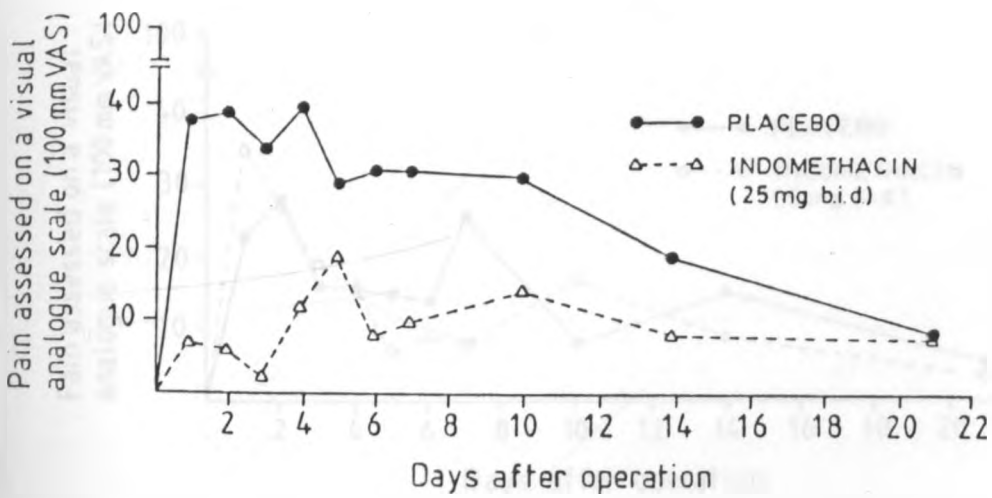


Fig. 4.5 Mean post-operative pain assessed by a VAS after orthopaedic surgery in a crossover study with 7 dogs given placebo and 25 mg indomethacin orally twice daily for 2 1/2 days starting the day before surgery.

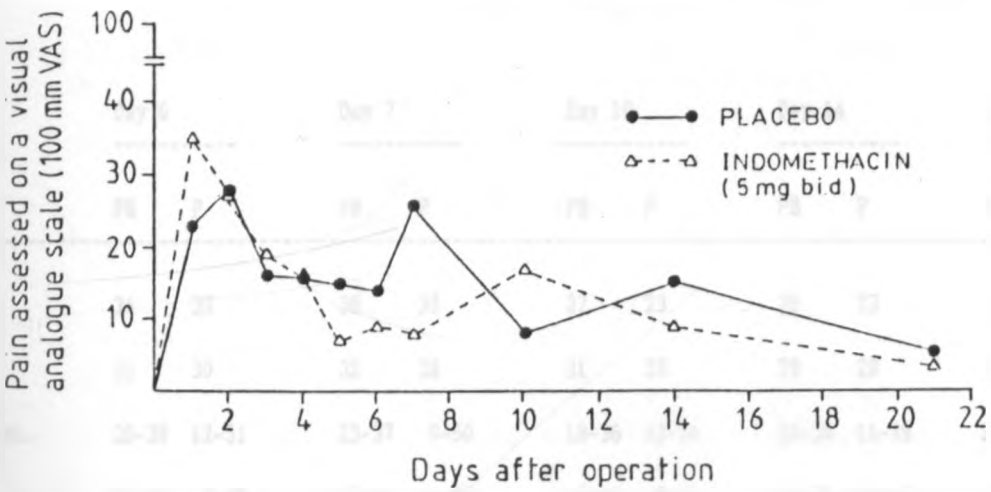


Fig. 4.6 Mean post-operative pain assessed by a VAS after orthopaedic surgery in a crossover study with 8 dogs given placebo and 5 mg indomethacin orally twice daily for 8 days starting the day before surgery.

Table 4.4 Post-operative swelling as ml displaced water measured by limb volumetry after orthopaedic surgery in a crossover trial with oral administration of 300 mg phenylbutazone (PB) twice daily tested against placebo (P) in 8 dogs.

	Day 1		Day 2		Day 3		Day 4		Day 5	
	PB	P	PB	P	PB	P	PB	P	PB	P
Mean	24	26	25	26	23	25	30	28	34	32
Median	21	15	23	28	26	27	30	28	31	31
95% conf. int.	17-31	13-39	16-33	16-36	16-31	13-27	24-36	17-39	27-41	19-45
Range	13-40	4-50	9-38	0-43	6-37	-6-48	18-42	2-51	24-50	6-61
P value	0.46		0.42		0.43		0.43		0.44	

	Day 6		Day 7		Day 10		Day 14		Day 21	
	PB	P	PB	P	PB	P	PB	P	PB	P
Mean	34	32	30	30	27	23	28	23	20	15
Median	32	30	32	28	31	25	29	29	21	16
95% conf. int.	28-39	12-51	23-37	9-50	18-36	12-34	23-34	11-35	11-29	3-26
Range	25-48	3-88	15-40	-4-87	6-42	-6-41	18-39	-10-45	2-39	-15-36
P value	0.45		0.48		0.44		0.36		0.24	

Table 4.5 Post-operative swelling as ml displaced water measured by limb volumetry after orthopaedic surgery in a crossover trial with oral administration of 25 mg indomethacin (I) twice daily tested against placebo (P) 7 dogs.

	Day 1		Day 2		Day 3		Day 4		Day 5	
	I	P	I	P	I	P	I	P	I	P
Mean	32	42	29	35	28	30	36	27	37	29
Median	31	38	29	29	30	29	37	22	36	31
95% conf. int.	23-42	33-50	20-38	28-42	18-38	24-36	22-49	20-35	23-52	20-38
Range	18-55	33-64	13-47	27-52	11-52	17-38	11-61	20-46	13-69	14-43
P value	0.08		0.16		0.41		0.08		0.08	

	Day 6		Day 7		Day 10		Day 14		Day 21	
	I	P	I	P	I	P	I	P	I	P
Mean	36	30	34	26	31	26	28	24	16	20
Median	33	28	31	24	28	23	28	19	15	23
95% conf. int.	23-49	19-40	20-49	14-38	19-42	16-36	19-38	16-32	9-23	12-28
Range	17-71	12-48	14-73	9-49	14-62	10-46	17-53	12-43	4-29	8-33
P value	0.14		0.11		0.11		0.22		0.28	

Table 4.6 Post-operative swelling as ml displaced water measured by limb volumetry after orthopaedic surgery in a crossover trial with oral administration of 5 mg indomethacin (I) twice daily tested against placebo (P) in 8 dogs.

	Day 1		Day 2		Day 3		Day 4		Day 5	
	I	P	I	P	I	P	I	P	I	P
Mean	31	39	30	33	26	31	24	26	21	23
Median	29	37	33	34	27	30	15	30	20	26
95% conf. int.	24-39	34-45	22-37	25-41	20-32	23-40	19-30	17-36	16-26	11-34
Range	16-49	28-52	11-45	15-52	9-35	15-49	14-34	3-44	13-30	-4-41
P value	0.07		0.26		0.18		0.42		0.47	

	Day 6		Day 7		Day 10		Day 14		Day 21	
	I	P	I	P	I	P	I	P	I	P
Mean	17	24	14	27	21	29	17	25	17	21
Median	17	26	14	23	25	30	14	27	18	20
95% conf. int.	13-21	14-34	10-18	18-37	16-27	20-39	10-23	19-32	11-23	15-28
Range	10-24	2-41	5-25	8-49	12-32	9-44	6-30	12-38	4-29	8-34
P value	0.10		0.02		0.04		0.02		0.19	

Table 4.7 Post-operative pain in mm, assessed by a VAS after orthopaedic surgery in a crossover trial with oral administration of 300 mg phenylbutazone (PB) twice daily tested against placebo (P) in 8 dogs.

	Day 1		Day 2		Day 3		Day 4		Day 5	
	PB	P	PB	P	PB	P	PB	P	PB	P
Mean	18	34	8	20	6	16	11	13	10	12
Median	15	28	5	14	3	14	5	14	10	8
95% conf. int.	5-31	10-58	2-14	4-36	1-11	7-26	1-21	2-18	5-14	6-17
Range	0-45	2-99	1-22	1-67	0-15	3-41	0-36	0-22	2-22	4-22
P value	0.20		0.09		0.04		0.31		0.36	

	Day 6		Day 7		Day 10		Day 14		Day 21	
	PB	P	PB	P	PB	P	PB	P	PB	P
Mean	9	11	9	12	6	8	6	4	4	2
Median	5	8	7	11	4	8	4	5	3	2
95% conf. int.	1-18	2-21	3-18	4-21	2-10	3-13	1-11	2-6	0-8	0-4
Range	0-32	0-38	0-26	2-35	0-15	0-16	0-17	0-6	0-18	0-7
P value	0.47		0.34		0.28		0.24		0.26	

Table 4.8 Post-operative pain in mm, assessed by a VAS after orthopaedic surgery in a crossover trial with oral administration of 25 mg indomethacin (I) twice daily tested against placebo (P) in 7 dogs.

	Day 1		Day 2		Day 3		Day 4		Day 5	
	I	P	I	P	I	P	I	P	I	P
Mean	7	38	6	39	2	34	12	40	19	29
Median	0	21	0	17	0	18	0	46	0	11
95% conf. int.	0-16	12-65	0-11	8-69	0-5	10-58	0-28	13-66	0-47	6-52
Range	0-26	0-100	0-16	0-96	0-7	8-95	0-58	5-100	0-100	0-75
P value	0.02		0.02		0.03		0.07		0.08	

	Day 6		Day 7		Day 10		Day 14		Day 21	
	I	P	I	P	I	P	I	P	I	P
Mean	8	31	10	31	14	30	8	19	7	8
Median	7	7	7	18	16	10	6	5	7	5
95% conf. int.	1-14	0-64	1-18	2-59	6-22	1-59	2-15	0-39	2-13	1-15
Range	0-20	0-100	0-29	0-100	0-29	0-100	0-23	0-64	0-19	0-26
P value	0.05		0.04		0.37		0.34		0.43	

Table 4.9 Post-operative pain in mm, assessed by a VAS after orthopaedic surgery in a crossover trial with oral administration of 5 mg indomethacin (I) twice daily tested against placebo (P) in 8 dogs.

	Day 1		Day 2		Day 3		Day 4		Day 5	
	I	P	I	P	I	P	I	P	I	P
Mean	35	23	27	28	19	16	16	16	7	15
Median	27	8	17	27	14	9	8	17	0	7
95% conf. int.	12-57	2-45	13-41	11-44	5-33	5-28	0-33	5-27	0-17	0-30
Range	8-64	0-83	0-59	0-70	0-49	0-40	0-70	0-38	0-40	0-61
P value	0.22		0.42		0.42		0.50		0.24	

	Day 6		Day 7		Day 10		Day 14		Day 21	
	I	P	I	P	I	P	I	P	I	P
Mean	9	14	8	26	17	8	9	15	3	5
Median	4	4	0	0	9	4	0	0	0	0
95% conf. int.	0-18	0-33	0-21	0-58	0-36	0-17	0-25	0-33	0-10	0-12
Range	0-36	0-78	0-49	0-100	0-84	0-38	0-64	0-72	0-27	0-24
P value	0.44		0.25		0.14		0.47		0.50	

Table 4.10 Post-operative temperature in °C, mean (range), in a crossover trial after orthopaedic surgery with oral administration of 300 mg phenylbutazone twice daily tested against placebo in 8 dogs.

	Day 1	Day 2	Day 3
Phenylbutazone	38.9(38.2-39.7)	39.1(38.5-39.7)	38.9(38.2-40.2)
Placebo	39.3(38.5-41.0)	39.0(38.5-39.4)	38.9(38.0-39.4)

	Day 4	Day 5	Day 6
Phenylbutazone	38.8(37.5-39.8)	38.6(37.8-39.6)	38.9(38.0-40.0)
Placebo	38.9(37.8-39.7)	38.5(38.0-39.6)	38.5(37.6-39.5)

	Day 7	Day 10	Day 14
Phenylbutazone	38.3(37.5-39.0)	38.4(37.5-39.2)	38.6(37.7(39.2)
Placebo	38.9(37.8-40.0)	38.0(37.2-38.8)	38.4(39.9-39.5)

	Day 21
Phenylbutazone	38.0(37.4-38.3)
Placebo	37.6(37.2-38.0)

Table 4.11 Post-operative temperature in °C, mean (range), in a crossover trial after orthopaedic surgery with oral administration of 25 mg indomethacin twice daily tested against placebo in 7 dogs.

	Day 1	Day 2	Day 3
Indomethacin	38.6(38.1-39.0)	38.6(38.2-39.2)	38.6(38.2-39.0)
Placebo	38.8(38.4-39.4)	38.8(38.3-39.6)	38.4(38.1-39.4)

	Day 4	Day 5	Day 6
Indomethacin	38.4(37.5-39.3)	38.2(37.5-39.3)	38.3(37.5-39.3)
Placebo	38.4(37.9-39.3)	38.4(37.8-39.0)	38.4(37.7-39.2)

	Day 7	Day 10	Day 14
Indomethacin	38.5(37.4-39.3)	38.4(37.7-39.4)	38.7(38.2-39.2)
Placebo	38.5(38.2-39.0)	38.4(38.0-39.0)	38.4(37.5-39.2)

	Day 21
Indomethacin	38.4(37.8-39.4)
Placebo	38.3(37.6-39.4)

Table 4.12 Post-operative temperature in °C, mean (range), in a crossover trial after orthopaedic surgery with oral administration of 5 mg indomethacin twice daily tested against placebo in 8 dogs.

	Day 1	Day 2	Day 3
Indomethacin	38.3(37.8-38.5)	38.1(37.8-38.5)	38.0(37.3-38.4)
Placebo	38.6(37.8-39.6)	38.1(37.5-38.7)	38.0(37.1-38.9)

	Day 4	Day 5	Day 6
Indomethacin	38.1(37.5-39.3)	38.2(37.6-39.8)	38.1(37.6-39.0)
Placebo	37.7(36.8-38.4)	37.7(36.6-38.5)	38.3(36.9-39.4)

	Day 7	Day 10	Day 14
Indomethacin	37.8(37.2-38.4)	38.0(36.8-38.6)	37.9(36.8-38.8)
Placebo	37.6(36.0-38.2)	38.0(36.8-38.8)	38.1(36.0-39.2)

	Day 21
Indomethacin	37.1(36.5-38.0)
Placebo	37.4(37.0-38.1)

Table 4.13a,b Radiographic assessment of bone-healing in a crossover trial 2 and 4 weeks after orthopaedic surgery with oral administration of 300 mg phenylbutazone (PB) twice daily tested against placebo (P) in 8 dogs.

Dog no.	Radiographic union[a]		Degree of callus[a]		Infection/ reaction to implant[a]		Number of loose screws	
	PB	P	PB	P	PB	P	PB	P
2 weeks (a)								
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
4 weeks (b)								
1	++	+	+	+	0	+	0	1
2	0	+	++	0	+++	0	1	0
3	++	++	0	+	0	+	0	0
4	0	+	+	+	+	0	0	0
5	+	+	0	+	0	+	0	0
6	0	++	+	0	+++	0	2	0
7	+	+	+	+	+	0	0	0
8	+	++	++	+	++	+	0	0

[a] 0 = minimum; ++++ = maximum.

Table 4.13c,d Radiographic assessment of bone-healing in a crossover trial 6 and 8 weeks after orthopaedic surgery with oral administration of 300 mg phenylbutazone (PB) twice daily tested against placebo (P) in 8 dogs.

Dog no.	Radiographic union[a]		Degree of callus[a]		Infection/ reaction to implant[a]		Number of loose screws	
	PB	P	PB	P	PB	P	PB	P
6 weeks (c)								
1	+	+	0	++	+	+	0	1
2	0	+	++	0	++	+	3	0
3	+	+	+	+	+	0	1	0
4	0	++	+	+	+	0	1	0
5	++	++	+	+	+	0	0	0
6	++	+++	+++	0	+++	0	3	0
7	0	+++	+++	+	++	+	0	0
8	+	+	+++	+	++	+	2	0
8 weeks (d)								
1	+++	+	0	+	+	0	3	1
2	+	+++	++	+	+	0	2	0
3	+++	+++	0	+	0	0	0	0
4	+++	0	0	+++	0	+++	0	0
5	+	+++	0	+	0	+	0	0
6	+++	+++	+++	0	+++	0	5	0
7	+++	+++	++	+	+	0	0	0
8	++	++	+	+++	+	+++	0	5

[a] 0 = minimum; ++++ = maximum.

Table 4.14a,b Radiographic assessment of bone-healing in a crossover trial 2 and 4 weeks after orthopaedic surgery with oral administration of 25 mg indomethacin (I) twice daily tested against placebo (P) in 7 dogs

Dog no.	Radiographic union*		Degree of callus*		Infection/ reaction to implant*		Number of loose screws	
	I	P	I	P	I	P	I	P
2 weeks (a)								
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
4 weeks (b)								
1	+	0	+	+	++	0	0	0
2	++	+	+	0	+	0	0	0
3	+	0	+	0	+	+	1	0
5	+	+	0	0	+	0	0	0
6	+	0	0	+	0	+	0	0
7	++	0	0	+	0	0	0	0
8	0	+	+	+	0	0	0	0

* 0 = minimum; ++++ = maximum.

Table 4.14 c,d Radiographic assessment of bone-healing in a crossover trial 6 and 8 weeks after orthopaedic surgery with oral administration of 25 mg indomethacin (I) twice daily tested against placebo (P) in 7 dogs.

Dog no.	Radiographic union[a]		Degree of callus[a]		Infection/ reaction to implant[a]		Number of loose screws	
	I	P	I	P	I	P	I	P
6 weeks (c)								
1	0	+	+	+	++	0	3	0
2	0	++	0	0	++	0	0	0
3	0	0	++	++	+++	+	6	3
5	0	++	0	0	++	0	1	0
6	+	0	+	+	+++	+	1	0
7	++	0	+	+	0	+	0	0
8	+++	++	0	+	0	0	0	0
8 weeks (d)								
1	0	+	++	+	+++	0	4	0
2	++	+++	+	+	++	0	1	0
3	+	0	+++	++	++++	++++	6	6
5	0	+++	++	0	+++	0	2	0
6	0	0	+++	+	++++	++	4	0
7	+++	0	+	+	+	+++	0	2
8	+++	+++	+	+	0	0	0	0

[a] 0 = minimum; ++++ = maximum.

Table 4.15a,b Radiographic assessment of bone-healing in a crossover trial 2 and 4 weeks after orthopaedic surgery with oral administration of 5 mg indomethacin (I) twice daily tested against placebo (P) in 8 dogs.

Dog no.	Radiographic union*		Degree of callus*		Infection/ reaction to implant*		Number of loose screws	
	I	P	I	P	I	P	I	P
2 weeks (a)								
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
4 weeks (b)								
1	0	0	0	0	0	+	0	1
2	+	+	0	0	0	+	2	0
3	+	0	0	0	+	+	0	2
4	+	0	0	++	+	+	0	0
5	0	+	0	0	0	+	0	0
6	+	0	+	0	++	0	0	0
7	+	0	+	0	0	+	0	0
8	0	0	0	+	0	+	0	0

* 0 = minimum; ++++ = maximum.

Table 4.15c,d Radiographic assessment of bone-healing in crossover trial 6 and 8 weeks after orthopaedic surgery with oral administration of 5 mg indomethacin (I) twice daily tested against placebo (P) in 8 dogs.

Dog no.	Radiographic union[a]		Degree of callus[a]		Infection/ reaction to implant[a]		Number of loose screws	
	I	P	I	P	I	P	I	P
6 weeks (c)								
1	0	0	++	0	+	+	0	0
2	0	0	0	0	+	+	3	0
3	0	+	+	+	++	++	3	3
4	0	++	++	+	++	+	0	0
5	++	0	0	0	0	++	0	0
6	0	+	0	0	+++	0	0	0
7	+	0	+	0	+	+++	0	3
8	0	++	++	++	++	0	0	0
8 weeks (d)								
1	0	+	++	+	+	0	2	0
2	0	+	+	++	++	+	3	0
3	0	0	++	+	++	+++	0	6
4	++	+	+	+++	0	++	0	0
5	+	+	+	+	+	0	0	0
6	+	+	0	+	++	0	0	0
7	++	0	+	++	0	++	0	2
8	+	+++	+	+++	+	0	0	0

[a] 0 = minimum; ++++ = maximum.

Table 4.16 Assessment of bone-healing using H/E stained sagittal sections of 3rd metacarpi 8 or 12 weeks after orthopaedic surgery in a crossover trial with oral administration of 300 mg phenylbutazone twice daily tested against placebo in 8 dogs.

Dog	Phenylbutazone		Placebo	
no.	8 weeks	12 weeks	8 weeks	12 weeks
1		+++	++	
2	0			++++
3	+++			+++
4		0	++++	
5		0	++	
6	0			0
7	++++			+++
8		0	++	

0 = minimum; ++++ = maximum bone healing.

Table 4.17 Assessment of bone-healing using H/E stained sagittal sections of 3rd metacarpi 8 or 12 weeks after orthopaedic surgery in a crossover trial with oral administration of 5 mg indomethacin twice daily tested against placebo in 8 dogs.

Dog	Indomethacin		Placebo	
no	8 weeks	12 weeks	8 weeks	12 weeks
1		++	+	
2	0			++
3	0			0
4		++++	++	
5		+++	++	
6	++			++
7		++++	0	
8	0			+++

0 = minimum; ++++ = maximum bone healing.

4.4 DISCUSSION

Many traumatic painful swellings have been treated with phenylbutazone. Results from clinical trials with the drug range from excellent to no effect (Album et al., 1975). Most of these reports, however, were based on subjective impression. The present controlled experiment in dogs did not reveal any phenylbutazone-induced reduction of the post-operative swelling, although the drug gave a significant pain relief. These findings agree well with some studies in humans based on objective measurements of swelling, according to which phenylbutazone or its bioactive metabolite oxyphenbutazone have little or no capability of reducing an acute post-traumatic swelling (Olesen and Zachariae, 1965; Goldie et al., 1974; Album et al., 1975).

In veterinary medicine, especially among equine practitioners, NSAID such as phenylbutazone are being increasingly used as an integral part of post-operative management. They are assumed to reduce swelling and other inflammatory events, hopefully without delaying wound healing. Lees and Higgins (1985) state that, although some surgeons are convinced of the value of NSAID based on their clinical experience, it is difficult to give an objective assessment of such usage since well controlled clinical trials are lacking. The

apparent clinical improvement may reflect the pain relief obtained with the drug.

With low dose indomethacin there was a tendency towards less swelling, which became significant after one week. With high dose indomethacin there was no consistent effect on the swelling. As for many other NSAID, the reports regarding the effects of indomethacin on acute inflammatory reactions are conflicting. Some investigators have reported the drug to significantly reduce post-surgical oedema, in rats (Penners, 1971) and in humans (Amin et al., 1983), while others have found no decrease in post-traumatic swelling (e.g. Huskisson et al., 1973; Petersen, 1975).

Indomethacin in high dosage gave a marked reduction of pain, and on the 3rd post-operative day a significant reduction was also recorded with phenylbutazone. The inconsistent pain assessments with indomethacin in low dosage may reflect that this dosage was too low to provide adequate pain relief. A similar observation was made by Mburu et al. (1987), who, using the same model, obtained a significant and marked pain reduction with acetylsalicylic acid in high dosage, while the recordings fluctuated at a lower dosage.

When the trials started it was decided to regard temperatures at or above 39.3 °C to be indicative of

hyperthermia. When phenylbutazone was tested against placebo, such temperatures were recorded 14 and 16 times after the two operations respectively, a frequency which was considerably higher than in any of the other trials. A likely explanation was the high ambient temperature which prevailed when this study was undertaken.

With regard to the effects of the two drugs on fracture and wound healing, it has been suggested on the basis of clinical observations in humans (Pfeifer, 1967), as well as from experiments in animals (Lindner, 1967), that oxyphenbutazone does not disturb the healing of bone injuries. Actually, oxyphenbutazone has been reported to accelerate repair of damaged muscular fibres (Morger, 1967), and in contrast to indomethacin the drug has been found to increase the tensile strength of experimental wounds in animals (Zederfeldt and Gruber, 1967). So far, there seems to be only one report which suggests that human fracture repair is inhibited by indomethacin (Sudmann and Hagen, 1976) but there is also evidence from experimental models in rats that indomethacin may delay or inhibit fracture healing (Sudmann et al.. 1979; Elves et al.. 1982).

With the present methods of assessment it was neither possible to detect any beneficial effect of phenylbutazone on wound and bone healing, nor any deleterious effect of indomethacin on these processes.

These findings with indomethacin agree with those of Allgöwer et al. (1963), who concluded that short-term treatment with indomethacin has no effect upon the healing of fractures. It might be that the period of drug administration was too short to reveal effects on bone healing, since it appears that the effect of indomethacin is short-lived and cessation of treatment soon after a fracture will allow normal healing to occur (Elves et al., 1982).

Difficulties were encountered in selecting an appropriate dosage of indomethacin. While the present mongrel dogs developed gastrointenstinal lesions with a total dose of about 4-7 mg indomethacin/kg bwt, beagles have been reported to tolerate a single dose of 20mg/kg bwt without visible gastrointestinal lesions, and even a total dose of 100-200mg/kg bwt over a 5 to 10 day period was tolerated without excessive signs of toxicity (Tabata et al., 1984). Other investigators have also found indomethacin to be more toxic in mongrel dogs than in beagles (Ruckenbusch and Toutain, 1983). Beagles are known to metabolize drugs more rapidly than most other dogs (Frey et al., 1979) and this may explain why indomethacin is less ulcerogenic in beagles.

The much higher ulcerogenic potential of indomethacin in carnivores compared to many other

species, is likely to depend on their predominantly biliary excretion and enterohepatic recirculation, and thus prolonged exposure of these organs. Duggan et al. (1979) reported the accumulative biliary secretion of indomethacin through the Ductus choledochus of the dog to be 362% of the originally administered dose, while the corresponding value for man was only 10%.

In contrast to indomethacin, phenylbutazone has a shorter half-life in dogs, 6 hours compared to 72 hours in humans (Dayton et al.. 1973). These pharmacokinetic differences may explain why calculated as mg/kg bwt/day, the ulcerogenic dose of indomethacin for dogs is only 1/8 to 1/2 of the therapeutic dose usually given to humans, while for phenylbutazone the ulcerogenic dose in dogs is 10 to 20 times the usual therapeutic dose in humans (Von Teelmann, 1983, Fig. 2.8).

For dogs, indomethacin does not appear to be recommendable, while phenylbutazone presents a relatively wide margin of safety. The short half-life of phenylbutazone in dogs is, however, a disadvantage if it is intended to maintain relatively constant plasma concentrations as the drug then has to be dosed 4 times daily (Kaergaard et al., 1969).

In the present trial, phenylbutazone did not reduce the swelling significantly, and the reduction obtained

with indomethacin was not impressive. Similar conclusions were obtained in human oral surgery with NSAID such as indomethacin (Petersen, 1975), ibuprofen (Lökken et al., 1975), oxyphenbutazone (Album et al., 1977) and acetylsalicylic acid (Skjelbred, 1984). As reported in Chapter 3, betamethasone reduced the swelling by about 40%, which corresponds rather well with the about 50% reduction found in human oral surgery (Skjelbred and Lökken, 1982a,b). When paracetamol was tested against placebo in the experimental model with dogs (Mburu et al., 1987), the reduction in swelling with paracetamol was 33% on the 3rd post-operative day, and the drug also proved to give a very good analgesic effect, without any adverse effects being registered. The 33% reduction in swelling compares nicely with the 29 and 31% reductions reported on the 3rd post-operative day with paracetamol in oral surgery, by Lökken and Skjelbred (1980) and Skjelbred et al. (1984).

Accordingly, the results obtained in the experimental model with bilateral orthopaedic surgery on the fore limbs of dogs, seem to agree well with the results in human oral surgery. As a further conclusion, neither phenylbutazone nor indomethacin seems to be as efficient as betamethasone or paracetamol in curbing an acute post-traumatic inflammatory reaction.

CHAPTER FIVE

GENERAL CONCLUSIONS

1. An experimental model with bilateral orthopaedic surgery on the fore limbs of dogs performed with an interval of 28 days, was established to allow placebo-controlled crossover studies on how steroidal and non-steroidal anti-inflammatory drugs might modulate the signs of an acute post-traumatic inflammatory reaction and the healing process. It was to be ascertained whether results obtained in surgery of the extremities of dogs would be comparable to those obtained in human oral surgery.

2. In a group of 8 dogs a single pre-operative injection of a glucocorticoid (3 mg betamethasone) was tested against placebo. The post-operative swelling was significantly reduced by the glucocorticoid, being 43% less on the 3rd day. The pain assessments were lower after glucocorticoid administration, but neither the reduction in pain nor that of limping reached a level of significance.

3. Any adverse effects of the glucocorticoid on wound or bone healing were not revealed with the present

methods of assessment: (a) clinical inspection; (b) comparison of radiographs taken 2, 4, 6 and 8 weeks after the two operations for bone union, callus formation, signs of infection and foreign body acceptance; and (c) comparison of bone sections from the sites of surgery obtained when the animals were euthanized 8 weeks after the 2nd operation.

4. With one exception, all animals exhibited a marked suppression of the serum cortisol levels after the glucocorticoid injection. The levels remained low for 3 days and then returned to normal.

5. The results obtained with the glucocorticoid add support to the view that short-term glucocorticoid administration may be beneficial in alleviating excessive inflammatory responses in surgery and traumatology, and is essentially safe.

6. In another group of 8 dogs oral administration of 300 mg phenylbutazone twice daily for 8 days starting the day before surgery, was tested against placebo. Phenylbutazone did not reduce the post-operative swelling, but the drug gave a significant pain relief.

7. Clinically wound healing appeared to proceed somewhat better after the operation when placebo was given, and comparison of radiographs 4 and 6 weeks after surgery also revealed tendencies in disfavour of phenylbutazone. After 8 weeks however, there were no noticeable differences. Evaluation of the bone sections also indicated a somewhat better healing with placebo.

8. In a further trial, a group of 8 dogs was to have tested against placebo, 25 mg of indomethacin given orally twice daily for 8 days starting the day before surgery. After 2 1/2 days, however, the medication had to be discontinued since the dogs developed signs of toxicity, e.g. lethargy, vomiting and bloody stool. One dog died on the 5th post-operative day when indomethacin had been given.

9. The pain assessments were significantly lower after the operation when indomethacin was given, but there was no consistent difference in swelling compared to placebo.

10. There appeared to be no difference in wound healing, but the radiographical evaluation revealed tendencies in disfavour of indomethacin.

11. A trial was then undertaken with a lower dosage of indomethacin tested against placebo in 8 dogs. Twice daily 5 mg indomethacin was given per os for 8 days starting on the day before surgery. With indomethacin there was a tendency towards less swelling, which became significant after one week. Pain assessments showed no consistent difference to placebo.

12. Evaluation of the progress of wound and fracture healing revealed no noticeable difference between indomethacin and placebo. It might be that the period of drug administration was too short to detect the indomethacin-induced inhibition of fracture healing reported by some investigators.

13. The difficulties encountered in selecting an appropriate dosage of indomethacin give a striking example of how differences in the pharmacokinetics may explain differences in drug response both within as well as between species.

14 Although the dogs served as their own controls, it was found difficult to obtain reliable and consistent estimates for pain and limping. These results should therefore be interpreted with caution.

15 The device designed for foot/limb volumetry functioned well. There was a good correlation between the effects of steroidal and non-steroidal anti-inflammatory drugs on post-traumatic swelling recorded in this experimental model with limb surgery on dogs and corresponding results obtained in human oral surgery.

16. Therefore, the present studies provide evidence that the results obtained with anti-inflammatory drugs in human oral surgery, also apply to acute traumatic swellings in other parts of the body.

17. While the glucocorticoid showed a marked anti-inflammatory effect, the anti-inflammatory and analgesic properties exhibited by phenylbutazone and indomethacin were not impressive. In the same model paracetamol has been proved to efficiently

and significantly reduce swelling and pain, without any observable adverse effects. All these observations agree with the findings with steroidal and non-steroidal drugs in human oral surgery.

18. As a final conclusion, non-steroidal anti-inflammatory drugs such as phenylbutazone and indomethacin do not seem to be as efficient and recommendable as a glucocorticoid or paracetamol in curbing the sequelae of an acute post-traumatic inflammatory reaction.

REFERENCES

ALBUM, B., OLSEN, I. AND LÖKKEN, P. (1977) Bilateral surgical removal of impacted mandibular 3rd molar teeth as a model for drug evaluation. A test with oxyphenbutazone (Tanderil®). Int. J. Oral Surg., 16, 177-189.

ALLEN, H.L.; WASE, A. AND BEAR, W.T. (1980) Indomethacin and aspirin: effect of non-steroidal anti-inflammatory agents on the rate of fracture repair in the rat. Acta Orthop. Scand., 51, 595-600.

ALLGÖWER, M.; BURRI, C.; GRAFFENRIED, P.; GUBER, U.F.; HEIM, U.; MENG, J.; SEGMUELLER, G.; SIEGRIST, J. AND STUDER, E. (1963) Quantitative analysis of the effect of anti-phlogistic substances in lower leg fractures. Schweiz. Med. Wochenschr., 93, 565-567.

ALLGÖWER, M. AND PERREN, S. (1967) The importance of inflammation in surgery. In Inflammationen inom Kirurgin. Bengmark, S. (ed.), pp. 10-20. A. Lindgren & Söner AB, Goteborg.

AMIN, M.M. AND LASKIN, D.M. (1983) Prophylactic use of indomethacin for prevention of postsurgical complications after removal of impacted third molars. Oral Surg., 55, 448-451.

AMIN, M.M.; ENGEL, M.B. AND LASKIN, D.M. (1983) Effect of indomethacin on postsurgical oedema in rats. Oral Surg., 55. 244-246.

ARRIGONI-MARTELLI, E. (1977) Inflammation and antiinflammatories. Spectrum, New York.

ARRIGONI-MARTELLI, E. (1979) Screening and assessment of anti-inflammatory drugs. Meth. Find. Expt. Clin. Pharmacol., I, 157-177.

BAGGOT, J.D. (1984) Some aspects of veterinary pharmacology. In: Basic and Clinical Pharmacology. Katzuna, B.G. (ed.), 2nd ed., p. 798. Lange Med. Publ., Los Altos.

BENJAMIN, M.M. (1978) Outline of Veterinary Clinical Pathology. 3rd ed., Iowa State Univ. Press, Ames.

BLACKWELL, G.J.; CARNUCCIO, R.; Di ROSA, M.; FLOWER, R.J.; PARENTE, L. AND PERSICO, P. (1980) Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocorticoids. Nature. 287. 147-149.

BLACKWELL, G.J. AND FLOWER, R.J. (1983) Inhibition of phospholipase. Br. Med. Bull., 39, 260-264.

BLUNT, J.W.; PLOTZ, C.M.; LATTES, R.; HOWES, E.L.; MEYER, K. AND RAGAN, C. (1950) Effect of cortisone on experi-

mental fractures in the rabbit. Soc. Exp. Bio. Med. Proc., 73, 678-681.

BÖHM, G.M. (1976) Vascular events in inflammation. Agents Actions (Suppl.) 3, 31-50.

BUTTERWORTH'S MEDICAL DICTIONARY (1978) Critchley, M. (ed.), 2nd ed., p. 1845. Butterworths, London.

COLEN, L.B.; CRAWLEY, W.A. AND BUNCKE, H.J. (1979) Effect of parenteral steroids on edema in replanted rat legs. Plastic Reconst. Surg., 63, 830-833.

DANNEBERG, A.M. Jr. (1979) The anti-inflammatory effects of glucocorticosteroids. Inflammation, 3, 329-343.

DAVIS, L.E. AND WESTFALL, B.A. (1972) Species differences in biotransformation and excretion of salicylate. Am. J. Vet. Res., 33, 1253-1262.

DAVIS, L.E. (1983) Species differences in drug disposition as factors in alleviation of pain. In Animal Pain: Perception and Alleviation. Kitchell, R.L. and Erickson, H.H. (eds.), pp. 161-178. Am. Physiol. Soc., Bethesda.

DAYTON, P.G., CUCINELL, S.A.; WEISS, M. AND PEREL, J.M. (1967). Dose-dependence of drug plasma level decline in dogs. J. Pharmacol. Exp. Ther., 158, 305-316.

DAYTON, P.G.; ISRAILI, Z.H. AND PEREL, J.M. (1973) Influence of binding on drug metabolism and distribution. Ann. NY Acad. Sci., 226, 172-194.

DINGWALL, J.S. (1974) Fractures. In Canine Surgery. Archibald (ed.), 2nd ed., pp. 949-957. Am. Vet. Pub. Inc., Santa Barbara.

DUGGAN, D.E.; HOOKE, K.F.; NOLL, R.M. AND CHIN KWAN, K. (1975) Enterohepatic circulation of indomethacin and its role in intestinal irritation. Biochem. Pharmacol., 25, 1749-1754.

ELVES, M.W.; BAYLEY, I. AND ROYLANCE, P.J. (1982) The effect of indomethacin upon experimental fractures in the rat. Acta Orthop. Scand., 53, 35-41.

FELDMAN, S.A. AND SCURR, C.F. (1979) The gate control theory of pain. In Current Topics in Chronic Pain Feldman, S.A. and Scurr, C.F. (eds.), pp. 6-21. Edward Arnold Pub., Chicago.

FERREIRA, S.H. AND VANE, J.R. (1979) Mode of action of anti-inflammatory agents which are prostaglandin synthetase inhibitors. In Anti-Inflammatory Drugs Vane, J.R. and Ferreira, S.H. (eds.), Vol. 50/II, p. 348. Springer-Verlag, Berlin.

FERREIRA, S.H.; AND NAKAMURA, M. (1979) Pathogenesis and pharmacology of pain. In Advances in Inflammation Research. Weissmann, G.; Samuelsson, B.and Paoletti, R. (eds.), pp. 317-329. Raven Press, New York.

FLOWER, R. (1981) Glucocorticoids, phospholipase A and inflammation. Trends Pharmacol. Sci. 2, 186-189.

FLOWER, R.J.; MONCADA, S. AND VANE, J.R. (1980) Analgesic - antipyretics and anti-inflammatory agents; Drugs employed in the treatment of gout. In Goodman and Gilman's The Pharmacological Basis of Therapeutics. Gilman, A.G.; Goodman, L.S. and Gilman, A. (eds.), 6th ed., pp. 682-728. Bailliere Tindall, London.

FLOWER, R.J.; MONCADA, S. AND VANE, J.R. (1985) Analgesic - antipyretics and anti-inflammatory agents; Drugs employed in the treatment of gout. In Goodman and Gilman's The Pharmacological Basis of Therapeutics. Gilman, A.G.; Goodman, L.S.; Rall, T.W. and Murad, F. (eds.), 7th ed., pp. 674-715. MacMillan Publ. Co., New York.

FORD-HUTCHINSON, A.W. (1985) Leukotrienes: Their formation and role as inflammatory mediators. Fed. Am. Soc. Exper. Biol. Proc., 44, 25-29.

FREY, H.H. AND RIEH, B. (1981) Pharmacokinetics of naproxen in the dog. Am. J. Vet. Res., 42, 1615-1617.

FURNESS, J.B. AND MARSHALL, J.M. (1974) Correlation of the directly observed responses of mesenteric vessels of the rat to nerve stimulation and noradrenaline with the distribution of adrenergic nerves. J. Physiol., 239, 75-88.

GOLDIE, I.F.; GUNTERBERG, B. AND JACOBSON, C. (1974) Foot volumetry as an objective test of the effect of antiphlogistic drugs in ankle sprains. Rheumatol. Rehabil., 13, 204-207.

GOTH, A. (1984) Adrenal steroids. In Medical Pharmacology: Principles and Concepts. Goth, A. (ed.), pp. 527-538. The C.V. Mosby Co., St. Louis.

HAYNES, R.C. AND MURAD, F. (1980) Adrenocorticotropic hormone: adrenocortical steroids and their synthetic analogs, inhibitors of adrenocortical steroid biosynthesis. In Goodman and Gilman's The Pharmacological Basis of Therapeutics. Gilman, A.G.; Goodman, L.S. and Gilman, A. (eds.), 6th ed., pp. 1479-1480. Bailliere Tindall, London.

HENCH, P.S.; KENDALL, E.C.; SLOCUMB, C.H. AND POLLEY, H.F. (1949). The effect of a hormone of the adrenal cortex (17-hydroxy-11-dehydro-corticosterone; compound E) and of pituitary adrenocorticotropic hormone on rheumatoid arthritis. Proc. Staff Meet. Mayo Clin., 24, 181-197.

HIGGINS, A.J. (1985) The biology, pathophysiology and control of eicosanoids in inflammation. J. Vet. Pharmacol. Therap., 8, 1-18.

HIGGS, G.A.; FLOWER, R.J. AND VANE, J.R. (1979) A new approach to anti-inflammatory drugs. Biochem. Pharmacol., 28, 1959-1961.

HIGGS, G.A.; EAKINS, K.E.; MUGRIDGE, K.G.; MONCADA, S. AND VANE, J.R. (1980) The effects of non-steroid anti-inflammatory drugs on leukocyte migration in carrageenin-induced inflammation. Europ. J. Pharmacol., 66, 81-86.

HOOLEY, J.R. AND FRANCIS, F.H. (1969) Betamethasone in traumatic oral surgery. J. Oral Surg., 27, 398-403.

HOOLEY, J.R.; BRADLEY, P.B. AND HAINES, M. (1973) Plasma cortisol levels following short-term betamethasone therapy for oral surgical procedures. Trans. 4th Int. Conf. Oral Surg., Copenhagen.

HOOLEY, J.R. AND HOHL, T.H. (1974) Use of steroids in the prevention of some complications after traumatic oral surgery. J. Oral Surg., 32, 864-866.

HUFFMAN, G.G. (1977) Use of methyl-prednisolone sodium succinate to reduce post-operative oedema after removal of impacted third molars. J. Oral Surg., 35, 198-199.

HUSKISSON, E.C.; BERRY, H.; STREET, F.G. AND MEDHURST, H.E. (1973) Indomethacin for soft-tissue injuries. A double-blind study in football players. Rheumatol. Rehabil., 12, 159-160.

HUSKISSON, E.C. (1977) Anti-inflammatory drugs. Semin. Arth. Rheumatol., 7, 1-20.

IASP (Int. Assoc. Study of Pain) Subcommittee on Taxonomy (1979) Pain terms : A list of definitions and notes on usage. Pain, 6, 249-252.

JOHNSTON, D.E. (1981) Skin and subcutaneous tissue. In Pathophysiology in Small Animal Surgery. Bojrab, M.J. (ed.), 1st ed., pp. 405-414. Lea and Febiger, Philadelphia.

JONES, E.W. AND HAMM, D. (1977) Steroidal and non-steroidal anti-inflammatory drugs for wounds and traumatic inflammation. N.Z. Vet. J., 25, 317-319.

KAERGAARD, N.C.; STERGAARD, E.H. AND FREY, H.H. (1969) Versuche zur Dosierung von Phenylbutazon beim Hund. DTW, 76, 378-381.

KARIM, S.M.M. (1971) Action of prostaglandin in the pregnant woman. Ann. N.Y. Acad. Sci., 180, 483-498.

KITCHELL, R.I. AND ERICKSON, H.H. (1983) Introduction. What is pain? In Animal Pain: Perception and Alleviation. Kitchell, R.I. and Erickson, H.H. (eds.), pp. VII-VIII. Am. Physiol. Soc., Bethesda.

LEES, P. AND HIGGINS, A.J. (1985) Clinical pharmacology and therapeutic uses of non-steroidal anti-inflammatory drugs in the horse. Eq. Vet. J., 17, 83-96.

LEHMANN, E.L. AND D'ABRERA, H.J.M. (1975) Non-parametrics: Statistical Methods Based on Ranks. Holden-Day Inc., San Francisco.

LIM, R.K.S.; MILLER, D.G.; GUZMAN, F.; ROGERS, D.W.; RODGERS, R.W.; WANG, S.K.; SHAO, P.Y. AND SHIH, T.Y. (1967) Pain and analgesia evaluated by the intra-peritoneal bradykinin-evoked pain method in man. Clin. Pharm. Ther., 8, 521.

LINDNER, J. (1967) In Die Posttraumatische Entzündung.

Lindner, J. and Wilhelmi, E. (eds.), p. 221. Hans Huber, Bern-Stuttgart.

LOEB, R.F. (1950) Trans. First Adrenal Cortex Conference. 91. Macy Foundation, Josiah.

LÖKKEN, P.; OLSEN, I.; BRUASET, I. AND NORMAN-PEDERSEN, K. (1975) Bilateral surgical removal of impacted lower third molar teeth as a model for drug evaluation : A test with ibuprofen. Eur. J. Clin. Pharmacol., 8, 209-216.

LÖKKEN, P. AND SKJELBRED, P. (1981) Aspirin or paracetamol? Lancet, ii, 1346-1347.

LÖKKEN, P. AND SKJELBRED, P. (1981) Analgesic and anti-inflammatory effects of paracetamol evaluated by bilateral oral surgery. Br. J. Clin. Pharmacol., 10, 253S-260S.

LUMB, W.V. AND JONES, E.W. (1973) Metabolic rate. In Veterinary Anaesthesia. Lumb, W.V. and Jones, E.W. (eds.), 1st ed. p. 9 Lea and Febiger, Philadelphia.

MAREK, J. AND BLAHA, V. (1980) Traumatic oedema of the rat ear pinna. Its morphology and affection by some anti-inflammatory agents. Int. J. Tiss. React. 11, 95-206.

MAZUE, G.; RICHEZ, P. AND BERTHE, J. (1983) Pharmacology and comparative toxicology of non-steroidal anti-inflammatory agents. In Veterinary Pharmacology and Toxicology. Ruckebusch, Y.; Toutain, P.L. and Koritz, G.D. (eds.), pp. 321-331. MTP Press Ltd., London.

MBURU, D.N.; MBUGUA, S.W.; SKOGLUND, L.A. AND LÖKKEN, P. (1987) Effects of paracetamol and acetylsalicylic acid on the post-operative course after experimental orthopaedic surgery in dogs. In manuscript.

McDONALD, L. (1982) Hormones influencing metabolism. In Veterinary Pharmacology and Therapeutics. Booth, N.H. and McDonald, L.E. (eds.), 5th ed., pp.553-592. Iowa St. Univ. Press, Ames.

MELZAC, R. AND WALL, P.D. (1965) Pain mechanisms: A new theory. Science, 150. 971-978.

MERKER, P.C. (1984) Inflammation and wound repair. Introduction to and philosophy of the symposium. Fed. Proc., 43, 2791-2792.

MESSER, E.J. AND KELLER, J.J. (1975) The use of intraoral dexa-methasone after extraction of mandibular third molars. Oral Surg., 40, 594-598.

MONCADA, S.; FERREIRA, S.H. AND VANE, J.R. (1978) Pain and inflammatory mediators. In Inflammation. Handb. Exp. Pharm.. Vane, J.R.; Ferreira, S.H. (eds.), pp.588-616. Springer-Verlag, New York.

MORGER, R. (1967) Oxyphenbutazon im Kaninchen-Modellversuch beim Volkmann-Syndrom. In Die Posttraumatische Entzündung. Lindner, J. and Wilhelmi, E. (eds.), pp 45-50. Hans Huber, Bern-Stuttgart.

NICKANDER, R.; McMAHON, F.G. AND RIDOLFO, A.S. (1979) Non-steroidal anti-inflammatory agents. Ann. Rev. Pharmacol. Toxicol.. 19, 469-490.

NWANGWU, P.U. (1981) The pharmacodynamics of inflammation and perspectives on use of steroids. Curr. Ther. Res.. 30, 253-264.

OLESEN, E. AND ZACHARIAE, L. (1965) Absent effect of oxyphenbutazone (Tanderil®) upon oedema following operation for Dupuytren's contracture. Acta Chir. Scand.. 129. 352-358.

PEACOCK, E.E. AND VAN WINKLE, J.R. (1976) Wound Repair. 2nd ed., pp. 1-270. W.B. Saunders Co., Philadelphia.

PENNERS, R. (1971) Die Wirkung von Indometacin auf das postoperative Ödem und die Wundheilung. Arznei.-Forsch.. 21, 1842-1843.

PERREN, S.M. (1981) Primary bone healing. In Pathophysiology in Small Animal Surgery. Bojrab, M.J. (ed.), 1st ed., pp 519-527. Lea and Febiger, Philadelphia.

PETERSEN, J.K. (1975) Anti-inflammatory and analgesic effects of indomethacin following removal of impacted mandibular third molars. Int. J. Oral. Surg., 4, 267-276.

PFEIFER, H. (1967) Klinische Beobachtungen über Knochenwundheilung unter Oxyphenbutazon. In Die Posttraumatische Entzündung. Lindner, J. and Wilhelmi, E. (eds.), pp. 213-220. Hans Huber, Bern-Stuttgart.

PLOTZ, C.M.; HOWES, E.L.; MEYER, K.; BLUNT, J.W.; LATTES, R. AND RAGAN, C. (1950). The effect of ACTH and cortisone on connective tissue. Am. J. Path., 26, 709.

PRIEUR, W.D. AND SUMNER-SMITH, G. (1984) General considerations. In Manual of Internal Fixation in Small Animals. Brinker, W.O.; Hohn, R.B. and Prieur, W.D. (eds.), pp. 3-33, Springer-Verlag, New York.

PUGH, D.M. (1982) The hormones. V: Mediators of allergy, anaphylaxis, inflammation, pain and shock. In

Veterinary Applied Pharmacology and Therapeutics Brander, G.C.; Pugh, D.M. and Bywater, R.J. (eds.), 4th ed., pp. 216-231. Bailliere Tindall, London.

RITTMANN, W.W. AND PERREN, S.M. (1974) In Cortical Bone Healing after Internal Fixation and Infection. Springer Verlag, New York.

ROUDEBUSH, P. AND MORSE, G.G. (1981) Naproxen toxicosis in a dog. J. Am. Vet. Med. Assoc., 179, 805-806.

RUCKEBUSCH, Y. AND TOUTAIN, P.L. (1983) Nonsteroidal anti-inflammatory agents: Species differences in pharmacodynamics. Vet. Res. Com., 7, 359-368.

RYAN, G.B. AND MAJNO, G. (1977) Acute inflammation. Am. J. Pathol., 86, 185-276.

SCHALM, O.W.; JAIN, N.C. AND CARROLL, E.J. (1975) The leukocytes: structure, kinetics, function and clinical interpretation. In Veterinary Haematology, 3rd ed., pp. 471-538. Lea & Febiger, Philadelphia.

SCHILLER, R. AND DE SILVA, J.A. (1979) Post-operative steroid injection: the first seventy-two hours of bone healing. J. Am. Podiatry Assoc., 69, 364-366.

SCHWARTZ-PORSCHE, VON D.; FITZEK, A.; RIEH, B. AND FREY, H.H. (1982) Klinische Prüfung von Naproxen beim Hund. Kleintier Praxis, 27, 73-78.

SINGH, J.; CHANDER, J.; SINGH, S.; SINGH, G. AND ATAL, C.K. (1986) γ-glutamyl transpeptidase : a novel biochemical marker in inflammation. Biochem. Pharmacol., 35, 3753-3760.

SISSONS, H.A. AND HADFIELD, G.J. (1951) The influence of cortisone on the repair of experimental fractures in the rabbit. Br. J. Surg, 39, 172-178.

SKJELBRED, P. AND LÖKKEN, P. (1982a) Post-operative pain and inflammatory reaction reduced by injection of a corticosteroid. A controlled trial with bilateral oral surgery. Eur. J. Clin. Pharmacol., 21, 391-396.

SKJELBRED, P. AND LÖKKEN, P. (1982b) Reduction of pain and swelling by a corticosteroid injected 3 hours after surgery. Eur. J. Clin. Pharmacol., 23, 141-146.

SKJELBRED, P. AND LÖKKEN, P. (1983) Effects of naloxone on post-operative pain and steroid-induced analgesia. Br. J. Clin. Pharmacol., 15, 221-226.

SKJELBRED, P.; LÖKKEN, P. AND SKOGLUND, L.A. (1984) Post-

operative administration of acetaminophen to reduce swelling and other inflammatory events. Curr. Ther. Res., 35, 377-385.

SMITH, R.E. (1981) Naproxen toxicosis. J. Am. Vet. Med. Assoc., 180, 107.

SUDMANN, E. AND HAGEN, T. (1976) Indomethacin-induced delayed fracture healing. Arch. Orthop. Unfallchir., 85, 151-154.

SUDMANN, E.; DREGELID, E.; BESSESEN, A. AND MÖRLAND, J. (1979) Inhibition of fracture healing by indomethacin in rats. Eur. J. Clin. Invest., 9, 333-339.

SUMMY-LONG, J.Y. (1984) Non-steroidal anti-inflammatory analgesics. In Medical Pharmacology: Principles and Concepts. Goth, A. (ed.), pp. 366-381. C.V. Mosby Co., St. Louis.

SWARTZ, S.L. AND DLUHY, R.G. (1978) Corticosteroids: Clinical pharmacology and therapeutic use. Drugs, 16, 238-255.

SZCZEKLIK, A. AND GRYGLEWSKI, R.J. (1983) Asthma and anti-inflammatory drugs. Mechanisms and clinical patterns. Drugs, 25, 533-543.

TABATA, K.; OHTSUKI, H. AND OKABE, S. (1984) Role of lymphoid nodules in pathogenesis of indomethacin-induced gastric lesions in dogs. Dig. Dis. Sci., 29, 346-352.

TÖRNKVIST, H. AND LINDHOLM, T.S. (1980) Effect of ibuprofen on mass and composition of fracture callus and bone. Scand. J. Rheumatol., 9, 167-171.

VAN FURTH, R. (1976) Cell kinetics during inflammation. Agents Actions, (Suppl.): 3, 51-59.

VINEGAR, R.; TRUAX, J.F. AND SELPH, J.L. (1976) Quantitative comparison of the analgesic anti-inflammatory activities of aspirin, phenacetin and acetaminophen in rodents. Eur. J. Pharmacol., 37, 23-30.

VINEGAR, R. AND TRUAX, J.F. (1982) Pathways to inflammation II. Introduction to and philosophy of the symposium. Fed. Proc., 41, 2567-2568.

VINEGAR, R.; TRUAX, J.F.; SELPH, J.L. AND VOELKER, F.A. (1982) Pathway of onset, development and decay of carrageenan pleurisy in the rat. Fed. Proc., 41, 2588-2595.

VON TEELMANN, K. (1982) Die Magen-Darm-Empfindlichkeit des Hundes gegenüber nichtsteroidalen Anti-phylogistika/ antirheumatika. Kleintier Praxis, 28, 177-188.

WILLIAMSON, L.W.; LORSON, E.L. AND OSBON, D.B. (1980) Hypothalamic-pituitary-adrenal suppression after short term dexamethasone therapy for oral surgical procedures. J. Oral Surg., 38, 20-28.

WILSON, P.R. AND YAKSH, T.L. (1980) Pharmacology of pain and analgesia. Anaesth. Intens. Care, 8, 248-256.

YOXALL, A.T. (1979) Pain and inflammation : analgesic and anti-inflammatory agents. In Pharmacological Basis of Small Animal Medicine. Yoxall, A.T. and Hird, J.F.R. (eds.), 1st ed., pp. 119-137. Blackwell Sci. Pub., London.

ZEDERFELDT, B. AND GRUBER, U.F. (1967) Der Einfluss von Indomethacin auf die Wundheilung. In Die Posttraumatische Entzündung. Lindner, J. and Wilhelmi, E. (eds.), pp. 51-55. Hans Huber, Bern-Stuttgart.

APPENDICES

Appendix 3.1 Details of patients, anaesthetic and surgical procedures, in a crossover trial with a single i.m. injection of 3 mg betamethasone (B) tested against placebo (P) in 8 dogs.

Dog no.	Sex	Pre-operative weight (kg)		Induction to drug injection (min)		Induction to incision (min)		Incision to last suture (min)		Induction to swallowing reflex (min)	
		B	P	B	P	B	P	B	P	B	P
1	M	20	21	1	2	15	15	35	32	75	59
2	F	15	15	1	2	10	6	25	35	42	54
3	F	16	15	2	2	10	7	31	26	43	46
4	M	12	15	2	3	8	11	36	34	40	50
5	F	16	15	1	1	13	10	26	31	34	87
6	F	16	18	1	1	10	5	34	20	46	39
7	F	16	16	1	1	9	10	33	25	43	39
8	F	16	14	1	1	5	8	30	31	39	40
Mean		16	16	1.3	1.6	10	9	31	29	45	52

Appendix 3.2

BILATERAL ORTHOPAEDIC SURGERY ON DOGS

RESEARCH PROTOCOL

Dog. no. .....

Age ..... Weight ..... Sex .....

Blood sample: .....day, / , ... day, /

Deworming: .....day / , ... day, /

Comments - blood/physical examination:

1st operation: ..... day, /

2nd operation: ..... day, /

	1st operation	2nd operation
Min from induction of anaesthesia to "drug" injection		
Min from induction of anaesthesia to incision		
Min from incision to last suture		
Min from induction of anaesthesia to swallowing reflexes		

Comments - 1st operation

Comments - 2nd operation

Swelling - Measurements

(ml water displaced)

	First operation					Second operation				
	Measurement				Diff.	Measurement				Diff.
	1	2	3	Mean	pre-op.	1	2	3	Mean	pre-op.
Preoperatively										
Day 1 after										
Day 2 after										
Day 3 after										
Day 4 after										
Day 5 after										
Day 6 after										
Day 7 after										
Day 10 after										
Day 14 after										
Day 21 after										

Wound healing

1st operation:

2nd operation:

Radiographs - dates

1st day after		2 weeks after		4 weeks after		6 weeks after		8 weeks after	
1st op.	2nd op.	1st op.	2nd op.	1st op.	2nd op.	1st op.	2nd op.	1st op.	2nd op.

Euthanized: ..... day, /

Time	Date		Pain - Digital pressure		Body temp (°C)	
	Op. 1	Op.2	Op.1	Op.2	Op.1	Op.2
Day of op.						
Day 1 after						
Day 2 after						
Day 3 after						
Day 4 after						
Day 5 after						
Day 6 after						
Day 7 after						
Day 10 after						
Day 14 after						
Day 21 after						

Digital pressure

0 = no evidence of pain

\+ = slight pain

++ = withdrawal

+++ = pain, screams, withdrawal of the leg on moderate pressure

++++ = extreme pain, screams, withdrawal of the leg on light pressure

PAIN ASSESSMENTS - VAS

	FIRST OPERATION		SECOND OPERATION	
	No pain	Pain cannot be worse	No pain	Pain cannot be worse
Day 1 after	.....		.....	
Day 2 after	.....		.....	
Day 3 after	.....		.....	
Day 4 after	.....		.....	
Day 5 after	.....		.....	
Day 6 after	.....		.....	
Day 7 after	.....		.....	
Day 10 after	.....		.....	
Day 14 after	.....		.....	
Day 21 after	.....		.....	

FIRST OPERATION

No limping

Day 1 after .....

Day 2 after .....

Day 3 after .....

Day 4 after .....

Day 5 after .....

Day 6 after .....

Day 7 after .....

Day 10 after .....

Day 14 after .....

Day 21 after .....

LIMPING - VAS

SECOND OPERATION

Limping cannot be worse	No limping	Limping cannot be worse
.....	.....	
.....	.....	
.....	.....	
.....	.....	
.....	.....	
.....	.....	
.....	.....	
.....	.....	
.....	.....	
.....	.....	

Appendix 3.3a Distribution assessment of the individual post-operative swelling measurements in 8 dogs treated with a single i.m. injection of 3mg betamethasone.

Day 1

DATA VERIFICATION

OBSERVATION NUMBER 1 = 22
OBSERVATION NUMBER 2 = 12.3
OBSERVATION NUMBER 3 = 6.7
OBSERVATION NUMBER 4 = 5
OBSERVATION NUMBER 5 =-5.3
OBSERVATION NUMBER 6 = 1
OBSERVATION NUMBER 7 = 25
OBSERVATION NUMBER 8 = 27.3

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 11.75

GEOMETRIC MEAN = 0

HARMONIC MEAN NOT COMPUTED SINCE ZERO OBSERVATION WAS ENCOUNTERED

MEDIAN = 9.5

QUADRATIC MEAN = 16.2194328
(ROOT MEAN SQUARE)

ASCENDING ORDER SORT

OBSERVATION NO. 5 =-5.3
OBSERVATION NO. 6 = 1
OBSERVATION NO. 4 = 5
OBSERVATION NO. 3 = 6.7
OBSERVATION NO. 2 = 12.3
OBSERVATION NO. 1 = 22
OBSERVATION NO. 7 = 25
OBSERVATION NO. 8 = 27.3

MEASURES OF DISPERSION

VARIANCE = 125.9075
STANDARD DEVIATION = 11.1806753
MEAN DEVIATION = 9.9
SEMI-INTERQUARTILE RANGE = 10.25
COEFFICIENT OF VARIATION = 95.15468341
MAXIMUM VALUE = 27.3
MINIMUM VALUE =-5.3
RANGE OF THE DATA = 32.6

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 3
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 9.5
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 23.5
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 10.25

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS = .603720243

QUARTILE COEFFICIENT OF SKEWNESS = .365853659

MOMENT COEFFICIENT OF SKEWNESS = .0472376025

MOMENT COEFFICIENT OF KURTOSIS = 1.60714965

THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3a (continued)

Day 2

DATA VERIFICATION

OBSERVATION NUMBER 1 = 17.3
OBSERVATION NUMBER 2 = 22
OBSERVATION NUMBER 3 = 9
OBSERVATION NUMBER 4 = 3.7
OBSERVATION NUMBER 5 =-5
OBSERVATION NUMBER 6 = 14
OBSERVATION NUMBER 7 = 27.7
OBSERVATION NUMBER 8 = 18.6

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 13.4125

GEOMETRIC MEAN = 0

HARMONIC MEAN NOT COMPUTED SINCE
ZERO OBSERVATION WAS ENCOUNTERED

MEDIAN = 15.65

QUADRATIC MEAN = 16.6291536
(ROOT MEAN SQUARE)

ASCENDING ORDER SORT

OBSERVATION NO. 5 =-5
OBSERVATION NO. 4 = 3.7
OBSERVATION NO. 3 = 9
OBSERVATION NO. 6 = 14
OBSERVATION NO. 1 = 17.3
OBSERVATION NO. 8 = 18.6
OBSERVATION NO. 2 = 22
OBSERVATION NO. 7 = 27.7

MEASURES OF DISPERSION

VARIANCE = 96.8333938
STANDARD DEVIATION = 9.83923875
MEAN DEVIATION = 8.134375
SEMI-INTERQUARTILE RANGE = 6.975
COEFFICIENT OF VARIATION = 73.2916216%
MAXIMUM VALUE = 27.7
MINIMUM VALUE =-5
RANGE OF THE DATA = 32.7

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 6.35
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 15.65
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 20.3
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 6.975

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT
OF SKEWNESS =-.682842009

QUARTILE COEFFICIENT
OF SKEWNESS =-.333333333

MOMENT COEFFICIENT
OF SKEWNESS =-.460026045

MOMENT COEFFICIENT
OF KURTOSIS = 2.30606945

THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3a (continued)

Day 3

DATA VERIFICATION

OBSERVATION NUMBER 1 = 25.7
OBSERVATION NUMBER 2 = 29.6
OBSERVATION NUMBER 3 = 10.4
OBSERVATION NUMBER 4 = 12.7
OBSERVATION NUMBER 5 = 7.7
OBSERVATION NUMBER 6 = 18
OBSERVATION NUMBER 7 = 26.7
OBSERVATION NUMBER 8 = 32.3

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 20.3875
GEOMETRIC MEAN = 18.1945938
HARMONIC MEAN = 15.9544687
MEDIAN = 21.85
QUADRATIC MEAN (ROOT MEAN SQUARE) = 22.2080228

ASCENDING ORDER SORT

OBSERVATION NO. 5 = 7.7
OBSERVATION NO. 3 = 10.4
OBSERVATION NO. 4 = 12.7
OBSERVATION NO. 6 = 18
OBSERVATION NO. 1 = 25.7
OBSERVATION NO. 7 = 26.7
OBSERVATION NO. 2 = 29.6
OBSERVATION NO. 8 = 32.3

MEASURES OF DISPERSION

VARIANCE = 77.5460937
STANDARD DEVIATION = 8.806026
MEAN DEVIATION = 8.1875
SEMI-INTERQUARTILE RANGE = 8.30000001
COEFFICIENT OF VARIATION = 43.1932605%
MAXIMUM VALUE = 32.3
MINIMUM VALUE = 7.7
RANGE OF THE DATA = 24.6

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 11.55
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 21.85
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 28.15
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 8.30000001

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS = -.498238363
QUARTILE COEFFICIENT OF SKEWNESS = -.240963855
MOMENT COEFFICIENT OF SKEWNESS = -.115816786
MOMENT COEFFICIENT OF KURTOSIS = 1.43563388

THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3a (continued)

Day 4

DATA VERIFICATION

OBSERVATION NUMBER 1 = 28
OBSERVATION NUMBER 2 = 41
OBSERVATION NUMBER 3 = 12.4
OBSERVATION NUMBER 4 = 15.4
OBSERVATION NUMBER 5 = 6
OBSERVATION NUMBER 6 = 41.7
OBSERVATION NUMBER 7 = 38.3
OBSERVATION NUMBER 8 = 21

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 25.475
GEOMETRIC MEAN = 21.4078823
HARMONIC MEAN = 17.0198697
MEDIAN = 24.5
QUADRATIC MEAN (ROOT MEAN SQUARE) = 28.5891151

ASCENDING ORDER SORT

OBSERVATION NO. 5 = 6
OBSERVATION NO. 3 = 12.4
OBSERVATION NO. 4 = 15.4
OBSERVATION NO. 8 = 21
OBSERVATION NO. 1 = 28
OBSERVATION NO. 7 = 38.3
OBSERVATION NO. 2 = 41
OBSERVATION NO. 6 = 41.7

MEASURES OF DISPERSION

VARIANCE = 168.361875
STANDARD DEVIATION = 12.9754339
MEAN DEVIATION = 11.775
SEMI-INTERQUARTILE RANGE = 12.875
COEFFICIENT OF VARIATION = 50.9339883%
MAXIMUM VALUE = 41.7
MINIMUM VALUE = 6
RANGE OF THE DATA = 35.7

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 13.9
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 24.5
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 39.65
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 12.875

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS = .225423994
QUARTILE COEFFICIENT OF SKEWNESS = .176699029
MOMENT COEFFICIENT OF SKEWNESS = -.0340567719
MOMENT COEFFICIENT OF KURTOSIS = 1.49171189
THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3a (continued)

Day 5

DATA VERIFICATION

OBSERVATION NUMBER 1 = 37.7
OBSERVATION NUMBER 2 = 41.6
OBSERVATION NUMBER 3 = 11.4
OBSERVATION NUMBER 4 = 12.7
OBSERVATION NUMBER 5 = 37.7
OBSERVATION NUMBER 6 = 40.3
OBSERVATION NUMBER 7 = 33.3
OBSERVATION NUMBER 8 = 19

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 29.2125
GEOMETRIC MEAN = 26.1449843
HARMONIC MEAN = 22.7904762
MEDIAN = 35.5
QUADRATIC MEAN = 31.5403908
(ROOT MEAN SQUARE)

ASCENDING ORDER SORT

OBSERVATION NO. 3 = 11.4
OBSERVATION NO. 4 = 12.7
OBSERVATION NO. 8 = 19
OBSERVATION NO. 7 = 33.3
OBSERVATION NO. 1 = 37.7
OBSERVATION NO. 5 = 37.7
OBSERVATION NO. 6 = 40.3
OBSERVATION NO. 2 = 41.6

MEASURES OF DISPERSION

VARIANCE = 141.426094
STANDARD DEVIATION = 11.8922703
MEAN DEVIATION = 11.134375
SEMI-INTERQUARTILE RANGE = 11.575
COEFFICIENT OF VARIATION = 40.7095262%
MAXIMUM VALUE = 41.6
MINIMUM VALUE = 11.4
RANGE OF THE DATA = 30.2

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 15.85
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 35.5
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 39
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 11.575

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT
OF SKEWNESS =-1.38611429

QUARTILE COEFFICIENT
OF SKEWNESS =-.69762419

MOMENT COEFFICIENT
OF SKEWNESS =-.495286331

MOMENT COEFFICIENT
OF KURTOSIS = 1.46996196

THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3a (continued)

Day 6

DATA VERIFICATION

OBSERVATION NUMBER 1 = 29.3
OBSERVATION NUMBER 2 = 41.6
OBSERVATION NUMBER 3 = .7
OBSERVATION NUMBER 4 = 7
OBSERVATION NUMBER 5 = 15
OBSERVATION NUMBER 6 = 35
OBSERVATION NUMBER 7 = 33.3
OBSERVATION NUMBER 8 = 27.3

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 23.65
GEOMETRIC MEAN = 15.2007635
HARMONIC MEAN = 4.46554442
MEDIAN = 28.3
QUADRATIC MEAN (ROOT MEAN SQUARE) = 27.2550913

ASCENDING ORDER SORT

OBSERVATION NO. 3 = .7
OBSERVATION NO. 4 = 7
OBSERVATION NO. 5 = 15
OBSERVATION NO. 8 = 27.3
OBSERVATION NO. 1 = 29.3
OBSERVATION NO. 7 = 33.3
OBSERVATION NO. 6 = 35
OBSERVATION NO. 2 = 41.6

MEASURES OF DISPERSION

VARIANCE = 183.5175
STANDARD DEVIATION = 13.5468631
MEAN DEVIATION = 12.0625
SEMI-INTERQUARTILE RANGE = 11.575
COEFFICIENT OF VARIATION = 57.2886051%
MAXIMUM VALUE = 41.6
MINIMUM VALUE = .700000003
RANGE OF THE DATA = 40.9

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 11
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 28.3
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 34.15
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 11.575

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS = -1.02973869
QUARTILE COEFFICIENT OF SKEWNESS = -.494600431
MOMENT COEFFICIENT OF SKEWNESS = -.45138716
MOMENT COEFFICIENT OF KURTOSIS = 1.81919382
THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3a (continued)

Day 7

DATA VERIFICATION

OBSERVATION NUMBER 1 = 25.3
OBSERVATION NUMBER 2 = 34.3
OBSERVATION NUMBER 3 =-1
OBSERVATION NUMBER 4 = 13.4
OBSERVATION NUMBER 5 = 17.3
OBSERVATION NUMBER 6 = 31
OBSERVATION NUMBER 7 = 27.3
OBSERVATION NUMBER 8 = 22.6

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 21.275

GEOMETRIC MEAN = 0

HARMONIC MEAN NOT COMPUTED SINCE ZERO OBSERVATION WAS ENCOUNTERED

MEDIAN = 23.95

QUADRATIC MEAN = 23.7525788
(ROOT MEAN SQUARE)

ASCENDING ORDER SORT

OBSERVATION NO. 3 =-1
OBSERVATION NO. 4 = 13.4
OBSERVATION NO. 5 = 17.3
OBSERVATION NO. 8 = 22.6
OBSERVATION NO. 1 = 25.3
OBSERVATION NO. 7 = 27.3
OBSERVATION NO. 6 = 31
OBSERVATION NO. 2 = 34.3

MEASURES OF DISPERSION

VARIANCE	= 111.359375
STANDARD DEVIATION	= 10.5621672
MEAN DEVIATION	= 8.53125
SEMI-INTERQUARTILE RANGE	= 6.9
COEFFICIENT OF VARIATION	= 49.6459091%
MAXIMUM VALUE	= 34.3
MINIMUM VALUE	=-1
RANGE OF THE DATA	= 35.3

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 19.35
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 23.95
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 29.15
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 6.9

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS	=-.759787255
QUARTILE COEFFICIENT OF SKEWNESS	=-.246376812
MOMENT COEFFICIENT OF SKEWNESS	=-.868596636
MOMENT COEFFICIENT OF KURTOSIS	= 2.9086331

THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3a (continued)

Day 10

DATA VERIFICATION

OBSERVATION NUMBER 1 = 22.7
OBSERVATION NUMBER 2 = 33.6
OBSERVATION NUMBER 3 = .4
OBSERVATION NUMBER 4 = 10.4
OBSERVATION NUMBER 5 = 6.3
OBSERVATION NUMBER 6 = 32.7
OBSERVATION NUMBER 7 = 16
OBSERVATION NUMBER 8 = 20.6

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 17.8375
GEOMETRIC MEAN = 11.0069437
HARMONIC MEAN = 2.69330933
MEDIAN = 18.3
QUADRATIC MEAN (ROOT MEAN SQUARE) = 21.8413581

ASCENDING ORDER SORT

OBSERVATION NO. 3 = .4
OBSERVATION NO. 5 = 6.3
OBSERVATION NO. 4 = 10.4
OBSERVATION NO. 7 = 16
OBSERVATION NO. 8 = 20.6
OBSERVATION NO. 1 = 22.7
OBSERVATION NO. 6 = 32.7
OBSERVATION NO. 2 = 33.6

MEASURES OF DISPERSION

VARIANCE = 124.562344
STANDARD DEVIATION = 11.1607301
MEAN DEVIATION = 9.5625
SEMI-INTERQUARTILE RANGE = 9.675
COEFFICIENT OF VARIATION = 62.5690267%
MAXIMUM VALUE = 33.6
MINIMUM VALUE = .400000006
RANGE OF THE DATA = 33.2

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 8.35
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 18.3
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 27.7
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 9.675

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS = -.1243196
QUARTILE COEFFICIENT OF SKEWNESS = -.0284237728
MOMENT COEFFICIENT OF SKEWNESS = 7.17521965E-03
MOMENT COEFFICIENT OF KURTOSIS = 1.80774838

THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3a (continued)

Day 14

DATA VERIFICATION

OBSERVATION NUMBER 1 = 11
OBSERVATION NUMBER 2 = 34.6
OBSERVATION NUMBER 3 = 1.4
OBSERVATION NUMBER 4 = 2.4
OBSERVATION NUMBER 5 = 7.7
OBSERVATION NUMBER 6 = 24
OBSERVATION NUMBER 7 = 26.3
OBSERVATION NUMBER 8 = 14.6

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 15.25
GEOMETRIC MEAN = 9.87932629
HARMONIC MEAN = 5.23288773
MEDIAN = 12.8
QUADRATIC MEAN (ROOT MEAN SQUARE) = 18.9275593

ASCENDING ORDER SORT

OBSERVATION NO. 3 = 1.4
OBSERVATION NO. 4 = 2.4
OBSERVATION NO. 5 = 7.7
OBSERVATION NO. 1 = 11
OBSERVATION NO. 8 = 14.6
OBSERVATION NO. 6 = 24
OBSERVATION NO. 7 = 26.3
OBSERVATION NO. 2 = 34.6

MEASURES OF DISPERSION

VARIANCE = 125.69
STANDARD DEVIATION = 11.2111552
MEAN DEVIATION = 9.7875
SEMI-INTERQUARTILE RANGE = 10.05
COEFFICIENT OF VARIATION = 73.5137716%
MAXIMUM VALUE = 34.6
MINIMUM VALUE = 1.40000001
RANGE OF THE DATA = 33.2

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 5.05
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 12.8
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 25.15
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 10.05

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS = .655597027
QUARTILE COEFFICIENT OF SKEWNESS = .228853721
MOMENT COEFFICIENT OF SKEWNESS = .352899903
MOMENT COEFFICIENT OF KURTOSIS = 1.88877659
THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3a (continued)

Day 21

DATA VERIFICATION

OBSERVATION NUMBER 1 = 22
OBSERVATION NUMBER 2 = 38.3
OBSERVATION NUMBER 3 = 5
OBSERVATION NUMBER 4 = 6
OBSERVATION NUMBER 5 = 3.3
OBSERVATION NUMBER 6 = 27.3
OBSERVATION NUMBER 7 = 22
OBSERVATION NUMBER 8 = 16.3

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 17.525
GEOMETRIC MEAN = 13.0018065
HARMONIC MEAN = 9.04265982
MEDIAN = 19.15
QUADRATIC MEAN (ROOT MEAN SQUARE) = 20.9695017

ASCENDING ORDER SORT

OBSERVATION NO. 5 = 3.3
OBSERVATION NO. 3 = 5
OBSERVATION NO. 4 = 6
OBSERVATION NO. 8 = 16.3
OBSERVATION NO. 1 = 22
OBSERVATION NO. 7 = 22
OBSERVATION NO. 6 = 27.3
OBSERVATION NO. 2 = 38.3

MEASURES OF DISPERSION

VARIANCE = 132.594375
STANDARD DEVIATION = 11.5149631
MEAN DEVIATION = 9.875
SEMI-INTERQUARTILE RANGE = 9.575
COEFFICIENT OF VARIATION = 65.7039235%
MAXIMUM VALUE = 38.3
MINIMUM VALUE = 3.3
RANGE OF THE DATA = 35

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 5.5
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 19.15
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 24.65
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 9.575

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS = -.429362189
QUARTILE COEFFICIENT OF SKEWNESS = -.425587467
MOMENT COEFFICIENT OF SKEWNESS = .303226879
MOMENT COEFFICIENT OF KURTOSIS = 1.98637247

THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3b Distribution assessment of the individual post-operative swelling measurements in 8 dogs treated with a single i.m. injection of placebo.

Day 1

DATA VERIFICATION

OBSERVATION NUMBER 1 = 53.1
OBSERVATION NUMBER 2 = 31
OBSERVATION NUMBER 3 = 24
OBSERVATION NUMBER 4 = 28
OBSERVATION NUMBER 5 = 45
OBSERVATION NUMBER 6 = 20.6
OBSERVATION NUMBER 7 = 56
OBSERVATION NUMBER 8 = 18.7

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 34.35
GEOMETRIC MEAN = 31.9035937
HARMONIC MEAN = 29.5671664
MEDIAN = 29.3
QUADRATIC MEAN (ROOT MEAN SQUARE) = 37.2082988

ASCENDING ORDER SORT

OBSERVATION NO. 8 = 18.7
OBSERVATION NO. 6 = 20.6
OBSERVATION NO. 3 = 24
OBSERVATION NO. 4 = 28
OBSERVATION NO. 2 = 31
OBSERVATION NO. 5 = 45
OBSERVATION NO. 1 = 53.1
OBSERVATION NO. 7 = 56

MEASURES OF DISPERSION

VARIANCE = 190.755
STANDARD DEVIATION = 13.8114083
MEAN DEVIATION = 12.6125
SEMI-INTERQUARTILE RANGE = 13.375
COEFFICIENT OF VARIATION = 39.97513268
MAXIMUM VALUE = 56
MINIMUM VALUE = 18.7
RANGE OF THE DATA = 37.3

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 22.9
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 29.3
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 49.05
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 13.375

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS = 1.09691928
QUARTILE COEFFICIENT OF SKEWNESS = .461682243
MOMENT COEFFICIENT OF SKEWNESS = .436352278
MOMENT COEFFICIENT OF KURTOSIS = 1.57127095

THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3b (continued)

Day 2

DATA VERIFICATION

OBSERVATION NUMBER 1 = 54.7
OBSERVATION NUMBER 2 = 21.6
OBSERVATION NUMBER 3 = 9.3
OBSERVATION NUMBER 4 = 29.3
OBSERVATION NUMBER 5 = 36
OBSERVATION NUMBER 6 = 36
OBSERVATION NUMBER 7 = 28.7
OBSERVATION NUMBER 8 = 34

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 31.2

GEOMETRIC MEAN = 28.2638927

HARMONIC MEAN = 24.5364953

MEDIAN = 31.65

QUADRATIC MEAN (ROOT MEAN SQUARE) = 33.4949998

ASCENDING ORDER SORT

OBSERVATION NO. 3 = 9.3
OBSERVATION NO. 2 = 21.6
OBSERVATION NO. 7 = 28.7
OBSERVATION NO. 4 = 29.3
OBSERVATION NO. 8 = 34
OBSERVATION NO. 5 = 36
OBSERVATION NO. 6 = 36
OBSERVATION NO. 1 = 54.7

MEASURES OF DISPERSION

VARIANCE = 148.475
STANDARD DEVIATION = 12.1850318
MEAN DEVIATION = 8.975
SEMI-INTERQUARTILE RANGE = 5.425
COEFFICIENT OF VARIATION = 39.0545891%
MAXIMUM VALUE = 54.7
MINIMUM VALUE = 9.3
RANGE OF THE DATA = 45.4

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 25.15
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 31.65
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 36
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 5.425

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS = -.110791667

QUARTILE COEFFICIENT OF SKEWNESS = -.198156681

MOMENT COEFFICIENT OF SKEWNESS = .125080103

MOMENT COEFFICIENT OF KURTOSIS = 3.08844881

THE DISTRIBUTION IS LEPTOKURTIC

Appendix 3.3b (continued)

Day 3

DATA VERIFICATION

OBSERVATION NUMBER 1 = 69.4
OBSERVATION NUMBER 2 = 32
OBSERVATION NUMBER 3 = 12.3
OBSERVATION NUMBER 4 = 29.6
OBSERVATION NUMBER 5 = 37.3
OBSERVATION NUMBER 6 = 42.3
OBSERVATION NUMBER 7 = 27.7
OBSERVATION NUMBER 8 = 29.3

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 34.9875
GEOMETRIC MEAN = 31.7605386
HARMONIC MEAN = 28.426766
MEDIAN = 30.8
QUADRATIC MEAN (ROOT MEAN SQUARE) = 38.2884091

ASCENDING ORDER SORT

OBSERVATION NO. 3 = 12.3
OBSERVATION NO. 7 = 27.7
OBSERVATION NO. 8 = 29.3
OBSERVATION NO. 4 = 29.6
OBSERVATION NO. 2 = 32
OBSERVATION NO. 5 = 37.3
OBSERVATION NO. 6 = 42.3
OBSERVATION NO. 1 = 69.4

MEASURES OF DISPERSION

VARIANCE	= 235.146094
STANDARD DEVIATION	= 15.334474
MEAN DEVIATION	= 11.809375
SEMI-INTERQUARTILE RANGE	= 5.65
COEFFICIENT OF VARIATION	= 43.8284365%
MAXIMUM VALUE	= 69.4
MINIMUM VALUE	= 12.3
RANGE OF THE DATA	= 57.1

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 29.5
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 30.8
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 39.8
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 5.65

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS	= .819232533
QUARTILE COEFFICIENT OF SKEWNESS	= .592920353
MOMENT COEFFICIENT OF SKEWNESS	= .995729894
MOMENT COEFFICIENT OF KURTOSIS	= 3.78658434

THE DISTRIBUTION IS LEPTOKURTIC

Appendix 3.3b (continued)

Day 4

DATA VERIFICATION

OBSERVATION NUMBER 1 = 59
OBSERVATION NUMBER 2 = 16.3
OBSERVATION NUMBER 3 = 23
OBSERVATION NUMBER 4 = 35.6
OBSERVATION NUMBER 5 = 37.6
OBSERVATION NUMBER 6 = 27.3
OBSERVATION NUMBER 7 = 31.3
OBSERVATION NUMBER 8 = 9.7

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 29.975
GEOMETRIC MEAN = 26.5298932
HARMONIC MEAN = 22.9796282
MEDIAN = 29.8
QUADRATIC MEAN (ROOT MEAN SQUARE) = 33.1207639

ASCENDING ORDER SORT

OBSERVATION NO. 8 = 9.7
OBSERVATION NO. 2 = 16.3
OBSERVATION NO. 3 = 23
OBSERVATION NO. 6 = 27.3
OBSERVATION NO. 7 = 31.3
OBSERVATION NO. 4 = 35.6
OBSERVATION NO. 5 = 37.6
OBSERVATION NO. 1 = 59

MEASURES OF DISPERSION

VARIANCE = 198.484375
STANDARD DEVIATION = 14.0884483
MEAN DEVIATION = 10.9
SEMI-INTERQUARTILE RANGE = 8.475
COEFFICIENT OF VARIATION = 47.0006615%
MAXIMUM VALUE = 59
MINIMUM VALUE = 9.7
RANGE OF THE DATA = 49.3

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 19.65
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 29.3
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 36.6
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 8.475

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS = .143734781
QUARTILE COEFFICIENT OF SKEWNESS = -.138643868
MOMENT COEFFICIENT OF SKEWNESS = .618014629
MOMENT COEFFICIENT OF KURTOSIS = 2.92059745

THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3b (continued)

Day 5

DATA VERIFICATION

OBSERVATION NUMBER 1 = 54.7
OBSERVATION NUMBER 2 = 26.6
OBSERVATION NUMBER 3 = 24
OBSERVATION NUMBER 4 = 33.6
OBSERVATION NUMBER 5 = 23.6
OBSERVATION NUMBER 6 = 32
OBSERVATION NUMBER 7 = 30.7
OBSERVATION NUMBER 8 = 14.7

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 29.9875
GEOMETRIC MEAN = 28.199548
HARMONIC MEAN = 26.531579
MEDIAN = 28.65
QUADRATIC MEAN (ROOT MEAN SQUARE) = 31.9064061

ASCENDING ORDER SORT

OBSERVATION NO. 8 = 14.7
OBSERVATION NO. 5 = 23.6
OBSERVATION NO. 3 = 24
OBSERVATION NO. 2 = 26.6
OBSERVATION NO. 7 = 30.7
OBSERVATION NO. 6 = 32
OBSERVATION NO. 4 = 33.6
OBSERVATION NO. 1 = 54.7

MEASURES OF DISPERSION

VARIANCE = 118.768394
STANDARD DEVIATION = 10.8981803
MEAN DEVIATION = 7.7625
SEMI-INTERQUARTILE RANGE = 4.5
COEFFICIENT OF VARIATION = 36.34214413
MAXIMUM VALUE = 54.7
MINIMUM VALUE = 14.7
RANGE OF THE DATA = 40

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 23.8
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 28.65
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 32.8
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 4.5

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS = .368183429
QUARTILE COEFFICIENT OF SKEWNESS = -.077777779
MOMENT COEFFICIENT OF SKEWNESS = 1.0681819
MOMENT COEFFICIENT OF KURTOSIS = 3.81798782
THE DISTRIBUTION IS LEPTOKURTIC

Appendix 3.3b (continued)

Day 6

DATA VERIFICATION

OBSERVATION NUMBER 1 = 47.7
OBSERVATION NUMBER 2 = 21.6
OBSERVATION NUMBER 3 = 29
OBSERVATION NUMBER 4 = 34.6
OBSERVATION NUMBER 5 = 23.3
OBSERVATION NUMBER 6 = 29.3
OBSERVATION NUMBER 7 = 36
OBSERVATION NUMBER 8 = 35

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 32.0625
GEOMETRIC MEAN = 31.1623384
HARMONIC MEAN = 30.2981523
MEDIAN = 31.95
QUADRATIC MEAN = 32.9817942
(ROOT MEAN SQUARE)

ASCENDING ORDER SORT

OBSERVATION NO. 2 = 21.6
OBSERVATION NO. 5 = 23.3
OBSERVATION NO. 3 = 29
OBSERVATION NO. 6 = 29.3
OBSERVATION NO. 4 = 34.6
OBSERVATION NO. 8 = 35
OBSERVATION NO. 7 = 36
OBSERVATION NO. 1 = 47.7

MEASURES OF DISPERSION

VARIANCE = 59.7548438
STANDARD DEVIATION = 7.73271258
MEAN DEVIATION = 6.2625
SEMI-INTERQUARTILE RANGE = 4.675
COEFFICIENT OF VARIATION = 24.1176221%
MAXIMUM VALUE = 47.7
MINIMUM VALUE = 21.6
RANGE OF THE DATA = 26.1

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 26.15
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 31.95
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 35.5
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 4.675

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT
OF SKEWNESS = .0436457466

QUARTILE COEFFICIENT
OF SKEWNESS =-.24064171

MOMENT COEFFICIENT
OF SKEWNESS = .356360911

MOMENT COEFFICIENT
OF KURTOSIS = 2.73389701

THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3b (continued)

Day 7

DATA VERIFICATION

OBSERVATION NUMBER 1 = 51
OBSERVATION NUMBER 2 = 19.3
OBSERVATION NUMBER 3 = 18.3
OBSERVATION NUMBER 4 = 29.3
OBSERVATION NUMBER 5 = 9.6
OBSERVATION NUMBER 6 = 29
OBSERVATION NUMBER 7 = 32.3
OBSERVATION NUMBER 8 = 33.7

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 27.8125
GEOMETRIC MEAN = 25.1474227
HARMONIC MEAN = 22.2544551
MEDIAN = 29.15
QUADRATIC MEAN = 30.1541551
(ROOT MEAN SQUARE)

ASCENDING ORDER SORT

OBSERVATION NO. 5 = 9.6
OBSERVATION NO. 3 = 18.3
OBSERVATION NO. 2 = 19.3
OBSERVATION NO. 6 = 29
OBSERVATION NO. 4 = 29.3
OBSERVATION NO. 7 = 32.3
OBSERVATION NO. 8 = 33.7
OBSERVATION NO. 1 = 51

MEASURES OF DISPERSION

VARIANCE = 136.341094
STANDARD DEVIATION = 11.6765189
MEAN DEVIATION = 9.859375
SEMI-INTERQUARTILE RANGE = 7.1
COEFFICIENT OF VARIATION = 41.98298931
MAXIMUM VALUE = 51
MINIMUM VALUE = 9.6
RANGE OF THE DATA = 41.4

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 18.8
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 29.15
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 33
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 7.1

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT
OF SKEWNESS =-.343638378

QUARTILE COEFFICIENT
OF SKEWNESS =-.457746479

MOMENT COEFFICIENT
OF SKEWNESS = .412847717

MOMENT COEFFICIENT
OF KURTOSIS = 2.7849484

THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3b (continued)

Day 10

DATA VERIFICATION

OBSERVATION NUMBER 1 = 46.7
OBSERVATION NUMBER 2 = 17
OBSERVATION NUMBER 3 = 18.6
OBSERVATION NUMBER 4 = 27.3
OBSERVATION NUMBER 5 = 15
OBSERVATION NUMBER 6 = 5.6
OBSERVATION NUMBER 7 = 21
OBSERVATION NUMBER 8 = 13.3

ASCENDING ORDER SORT

OBSERVATION NO. 6 = 5.6
OBSERVATION NO. 8 = 13.3
OBSERVATION NO. 5 = 15
OBSERVATION NO. 2 = 17
OBSERVATION NO. 3 = 18.6
OBSERVATION NO. 7 = 21
OBSERVATION NO. 4 = 27.3
OBSERVATION NO. 1 = 46.7

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 14.15
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 17.8
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 24.15
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 5

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 20.5625

GEOMETRIC MEAN = 17.659319

HARMONIC MEAN = 14.8512445

MEDIAN = 17.8

QUADRATIC MEAN = 23.5462046
(ROOT MEAN SQUARE)

MEASURES OF DISPERSION

VARIANCE = 131.607344
STANDARD DEVIATION = 11.4720244
MEAN DEVIATION = 8.328125
SEMI-INTERQUARTILE RANGE = 5
COEFFICIENT OF VARIATION = 55.7910001%
MAXIMUM VALUE = 46.7
MINIMUM VALUE = 5.6
RANGE OF THE DATA = 41.1

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT
OF SKEWNESS = .72240955

QUARTILE COEFFICIENT
OF SKEWNESS = .269999999

MOMENT COEFFICIENT
OF SKEWNESS = 1.17602836

MOMENT COEFFICIENT
OF KURTOSIS = 3.7731045

THE DISTRIBUTION IS LEPTOKURTIC

Appendix 3.3b (continued)

Day 14

DATA VERIFICATION

OBSERVATION NUMBER 1 = 36
OBSERVATION NUMBER 2 = 10.6
OBSERVATION NUMBER 3 = 11.6
OBSERVATION NUMBER 4 = 26
OBSERVATION NUMBER 5 = 5.3
OBSERVATION NUMBER 6 = 10.3
OBSERVATION NUMBER 7 = 17
OBSERVATION NUMBER 8 = 20.3

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 17.1375
GEOMETRIC MEAN = 14.6896781
HARMONIC MEAN = 12.4875696
MEDIAN = 14.3
QUADRATIC MEAN (ROOT MEAN SQUARE) = 19.5390315

ASCENDING ORDER SORT

OBSERVATION NO. 5 = 5.3
OBSERVATION NO. 6 = 10.3
OBSERVATION NO. 2 = 10.6
OBSERVATION NO. 3 = 11.6
OBSERVATION NO. 7 = 17
OBSERVATION NO. 8 = 20.3
OBSERVATION NO. 4 = 26
OBSERVATION NO. 1 = 36

MEASURES OF DISPERSION

VARIANCE = 88.0798438
STANDARD DEVIATION = 9.38508623
MEAN DEVIATION = 7.721875
SEMI-INTERQUARTILE RANGE = 6.35
COEFFICIENT OF VARIATION = 54.7634%
MAXIMUM VALUE = 36
MINIMUM VALUE = 5.3
RANGE OF THE DATA = 30.7

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 10.45
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 14.3
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 23.15
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 6.35

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS = .987824164
QUARTILE COEFFICIENT OF SKEWNESS = .393700787
MOMENT COEFFICIENT OF SKEWNESS = .737779582
MOMENT COEFFICIENT OF KURTOSIS = 2.53682127
THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3b (continued)

Day 21

DATA VERIFICATION

OBSERVATION NUMBER 1 = 36.7
OBSERVATION NUMBER 2 = 17.3
OBSERVATION NUMBER 3 = 11
OBSERVATION NUMBER 4 = 32
OBSERVATION NUMBER 5 =-1
OBSERVATION NUMBER 6 = 4.5
OBSERVATION NUMBER 7 = 15
OBSERVATION NUMBER 8 = 7

MEASURES OF CENTRAL TENDENCY

ARITHMETIC MEAN = 15.2875

GEOMETRIC MEAN = 0

HARMONIC MEAN NOT COMPUTED SINCE ZERO OBSERVATION WAS ENCOUNTERED

MEDIAN = 13

QUADRATIC MEAN = 19.6362866
(ROOT MEAN SQUARE)

ASCENDING ORDER SORT

OBSERVATION NO. 5 =-1
OBSERVATION NO. 6 = 4.5
OBSERVATION NO. 8 = 7
OBSERVATION NO. 3 = 11
OBSERVATION NO. 7 = 15
OBSERVATION NO. 2 = 17.3
OBSERVATION NO. 4 = 32
OBSERVATION NO. 1 = 36.7

MEASURES OF DISPERSION

VARIANCE	= 151.876094
STANDARD DEVIATION	= 12.3238819
MEAN DEVIATION	= 10.034375
SEMI-INTERQUARTILE RANGE	= 9.5
COEFFICIENT OF VARIATION	= 80.6135858%
MAXIMUM VALUE	= 36.7
MINIMUM VALUE	=-1
RANGE OF THE DATA	= 37.7

MEDIAN AND QUARTILES

1ST QUARTILE VALUE 5.65
OCCURS AT OBSERVATION POINT 2.5

2ND QUARTILE (MEDIAN) VALUE 13
OCCURS AT OBSERVATION POINT 4.5

3RD QUARTILE VALUE 24.65
OCCURS AT OBSERVATION POINT 6.5

SEMI-INTERQUARTILE RANGE = 9.5

MEASURES OF SKEWNESS AND KURTOSIS

PEARSON'S COEFFICIENT OF SKEWNESS	= .536849263
QUARTILE COEFFICIENT OF SKEWNESS	= .226315789
MOMENT COEFFICIENT OF SKEWNESS	= .547519748
MOMENT COEFFICIENT OF KURTOSIS	= 2.04980253

THE DISTRIBUTION IS PLATYKURTIC

Appendix 3.3c Paired statistical parametric and non-parametric analyses of the individual post-operative swelling measurements in a crossover trial with a single i.m. injection of betamethasone (Celestone®)tested against placebo in 8 dogs.

```
PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 1
RESULT 1 RESULT 2   DIFF
  22.00   53.10 -31.10
  12.30   31.00 -18.70
   6.70   24.00 -17.30
   5.00   28.00 -23.00
  -5.30   45.00 -50.30
   1.00   20.60 -19.60
  25.00   56.00 -31.00
  27.30   18.70   8.60
MEAN DIFF -22.80  N=  8
VARIANCE 276.53   S.D.  16.63
S.E.   5.88   COEFF.VAR. -72.93
SX -182.4   SS  6094.40001
MEAN R1  11.75  MEAN R2  34.55
T   -3.88

PAIRED T-TEST & SIGNED RANK TEST  BETAMETHASONE VERSUS PLACEBO DAY 1
DIFF     SOURCE   SIGNED RANK
   8.60    8            1
 -17.30    3           -2
 -18.70    2           -3
 -19.60    6           -4
 -23.00    4           -5
 -31.00    7           -6
 -31.10    1           -7
 -50.30    5           -8
TOTAL POSITIVE RANKS 1
TOTAL NEGATIVE RANKS 35
TOTAL RANKS SHOULD BE 36

PAIRED T-TEST & SIGNED RANK TEST  BETAMETHASONE VERSUS PLACEBO DAY 2
RESULT 1 RESULT 2   DIFF
  17.30   54.70 -37.40
  22.00   21.60   0.40
   9.00    9.30  -0.30
   3.70   29.30 -25.60
  -5.00   36.00 -41.00
  14.00   36.00 -22.00
  27.70   28.70  -1.00
  18.60   34.00 -15.40
MEAN DIFF -17.79  N=  8
VARIANCE 275.20   S.D.  16.59
S.E.   5.87   COEFF.VAR. -93.26
SX -142.3   SS  4457.53
MEAN R1  13.41  MEAN R2  31.20
T   -3.03

PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 2
DIFF     SOURCE   SIGNED RANK
  -0.30    3           -1
   0.40    2            2
  -1.00    7           -3
 -15.40    8           -4
 -22.00    6           -5
 -25.60    4           -6
 -37.40    1           -7
 -41.00    5           -8
TOTAL POSITIVE RANKS 2
TOTAL NEGATIVE RANKS 34
TOTAL RANKS SHOULD BE 36
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Appendix 3.3c (continued)

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PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 3
RESULT 1 RESULT 2   DIFF
   25.70   69.40  -43.70
   29.60   32.00   -2.40
   10.40   12.30   -1.90
   12.70   29.60  -16.90
    7.70   37.30  -29.60
   18.00   42.30  -24.30
   26.70   27.70   -1.00
   32.30   29.30    3.00
MEAN DIFF  -14.60  N=  8
VARIANCE  282.29   S.D.   16.80
S.E.     5.94   COEFF.VAR. -115.09
SX -116.8   SS  3681.32
MEAN R1   20.39  MEAN R2   34.99
T    -2.46

PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 3
DIFF     SOURCE   SIGNED RANK
   -1.00   7           -1
   -1.90   3           -2
   -2.40   2           -3
    3.00   8            4
  -16.90   4           -5
  -24.30   6           -6
  -29.60   5           -7
  -43.70   1           -8
TOTAL POSITIVE RANKS 4
TOTAL NEGATIVE RANKS 32
TOTAL RANKS SHOULD BE 36

PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 4
RESULT 1 RESULT 2   DIFF
   29.00   53.00  -31.00
   41.00   16.30   24.70
   12.40   23.00  -10.60
   15.40   35.60  -20.20
    6.00   37.60  -31.60
   41.70   27.30   14.40
   39.30   31.30    7.00
   21.00    9.70   11.30
MEAN DIFF   -4.50  N=  8
VARIANCE  473.16   S.D.   21.75
S.E.     7.69   COEFF.VAR. -483.38
SX -36   SS  3474.1
MEAN R1   25.48  MEAN R2   29.98
T    -0.59

PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 4
DIFF     SOURCE   SIGNED RANK
    7.00   7           1
  -10.60   3          -2
   11.30   8           3
   14.40   6           4
  -20.20   4          -5
   24.70   2           6
  -31.00   1          -7
  -31.60   5          -8
TOTAL POSITIVE RANKS 14
TOTAL NEGATIVE RANKS 22
TOTAL RANKS SHOULD BE 36
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Appendix 3.3c (continued)

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PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 5
RESULT 1 RESULT 2   DIFF
   37.70    54.70  -17.00
   41.60    26.60   15.00
   11.40    24.00  -12.60
   12.70    33.60  -20.90
   37.70    23.60   14.10
   40.30    32.00    8.30
   33.30    30.70    2.60
   19.00    14.70    4.30
MEAN DIFF    -0.78  N=  8
VARIANCE  199.67   S.D.    14.13
S.E.     5.00   COEFF.VAR.=1823.30
SX -6.20000002   SS  1402.52
MEAN R1    29.21  MEAN R2    29.99
T     -0.16

PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 5
DIFF     SOURCE    SIGNED RANK
     2.60    7              1
     4.30    8              2
     8.30    6              3
   -12.60    3             -4
    14.10    5              5
    15.00    2              6
   -17.00    1             -7
   -20.90    4             -8
 TOTAL POSITIVE RANKS 17
 TOTAL NEGATIVE RANKS 19
 TOTAL RANKS SHOULD BE 36

PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 6
RESULT 1 RESULT 2   DIFF
   29.30    47.70  -18.40
   41.60    21.60   20.00
    0.70    29.00  -28.30
    7.00    34.60  -27.60
   15.00    23.30   -8.30
   35.00    29.30    5.70
   33.30    36.00   -2.70
   27.30    35.00   -7.70
MEAN DIFF    -8.41  N=  8
VARIANCE  271.86   S.D.    16.49
S.E.     5.83   COEFF.VAR. -196.00
SX -67.3    SS  2469.17
MEAN R1    23.65  MEAN R2    32.06
T     -1.44

PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 6
DIFF     SOURCE    SIGNED RANK
    -2.70    7             -1
     5.70    6              2
    -7.70    8             -3
    -8.30    5             -4
   -18.40    1             -5
    20.00    2              6
   -27.60    4             -7
   -28.30    3             -8
 TOTAL POSITIVE RANKS 8
 TOTAL NEGATIVE RANKS 28
 TOTAL RANKS SHOULD BE 36
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Appendix 3.3c (continued)

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PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 7
RESULT 1 RESULT 2   DIFF
  25.30  31.00 -25.70
  84.30  19.30  15.00
  -1.00  18.30 -19.30
  13.40  29.30 -15.90
  17.30   9.60   7.70
  91.00  29.00   2.00
  27.30  32.30  -5.00
  22.60  33.70 -11.10
MEAN DIFF  -6.54  N=  8
VARIANCE 197.20   S.D.  14.04
S.E.    4.96   COEFF.VAR.-214.80
SX -52.3   SS  1722.29
MEAN R1  21.28  MEAN R2  27.81
T   -1.32


PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 7
DIFF    SOURCE   SIGNED RANK
   2.00   6            1
  -5.00   7           -2
   7.70   5            3
 -11.10   8           -4
  15.00   2            5
 -15.90   4           -6
 -19.30   3           -7
 -25.70   1           -8
TOTAL POSITIVE RANKS 9
TOTAL NEGATIVE RANKS 27
TOTAL RANKS SHOULD BE 36


PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 10
RESULT 1 RESULT 2   DIFF
   22.70   46.70  -24.00
   33.60   17.00   16.60
    0.40   18.60  -18.20
   10.40   27.30  -16.90
    6.30   15.00   -8.70
   32.70    5.60   27.10
   15.00   21.00   -5.00
   20.60   13.30    7.30
MEAN DIFF   -2.73  N=  8
VARIANCE  328.20   S.D.   18.12
S.E.    6.41   COEFF.VAR. -664.82
SX -21.8   SS  2356.8
MEAN R1   17.84  MEAN R2   20.56
T    -0.43


PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 10
DIFF    SOURCE   SIGNED RANK
   -5.00   7           -1
    7.30   8            2
   -9.70   5           -3
   16.60   2            4
  -16.90   4           -5
  -19.20   3           -6
  -24.00   1           -7
   27.10   6            8
TOTAL POSITIVE RANKS 14
TOTAL NEGATIVE RANKS 22
TOTAL RANKS SHOULD BE 36
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Appendix 3.3c (continued)

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PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERBUS PLACEBO DAY 14
RESULT 1 RESULT 2   DIFF
   11.00    36.00  -25.00
   34.60    10.60   24.00
    1.40    11.60  -10.20
    2.40    26.00  -23.60
    7.70     5.30    2.40
   24.00    10.30   13.70
   26.30    17.00    9.30
   14.60    20.30   -5.70
MEAN DIFF    -1.89  N=  8
VARIANCE  306.56   S.D.    17.51
S.E.     6.19   COEFF.VAR. -927.62
SX -15.1   SS  2174.43
MEAN R1    15.25  MEAN R2    17.14
T     -0.30

PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERBUS PLACEBO DAY 14
DIFF     SOURCE   SIGNED RANK
    2.40    5            1
   -5.70    8           -2
    9.30    7            3
  -10.20    3           -4
   13.70    6            5
  -23.60    4           -5
   24.00    2            7
  -25.00    1           -8
 TOTAL POSITIVE RANKS 16
 TOTAL NEGATIVE RANKS 20
 TOTAL RANKS SHOULD BE 36


 PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 21
 RESULT 1 RESULT 2   DIFF
    22.00    36.70  -14.70
    38.30    17.30   21.00
     5.00    11.00   -6.00
     6.00    32.00  -26.00
     3.30    -1.00    4.30
    27.30     4.30   23.00
    22.00    15.00    7.00
    16.30     7.00    9.30
 MEAN DIFF     2.24  N=  8
 VARIANCE  287.43   S.D.    16.95
 S.E.     5.99   COEFF.VAR.   757.71
 SX  17.9   SS  2052.87
 MEAN R1    17.53  MEAN R2    15.29
 T      0.37


 PAIRED T-TEST & SIGNED RANK TEST BETAMETHASONE VERSUS PLACEBO DAY 21
 DIFF     SOURCE   SIGNED RANK
     4.30    5            1
    -6.00    3           -2
     7.00    7            3
     9.30    8            4
   -14.70    1           -5
    21.00    2            6
    23.00    6            7
   -26.00    4           -8
 TOTAL POSITIVE RANKS 21
 TOTAL NEGATIVE RANKS 15
 TOTAL RANKS SHOULD BE 36
```

Appendix 3.4 Individual and mean values for post-operative swelling in ml measured by limb volumetry in 8 dogs injected i.m. with 3 mg betamethasone (B) and placebo (P) before surgery in a crossover trial.

Dog	Day 1		Day 2		Day 3		Day 4		Day 5	
no.	B	P	B	P	B	P	B	P	B	P
1	22	54	17	55	26	69	28	59	38	55
2	12	31	22	22	30	32	41	16	42	37
3	7	24	9	9	10	12	12	23	11	24
4	5	28	4	29	13	30	15	36	13	34
5	-5	45	-5	36	8	37	6	38	38	24
6	1	21	14	36	18	42	42	27	40	32
7	25	56	28	29	27	28	38	31	33	31
8	27	19	19	34	32	29	21	10	19	15
Mean	11.8	34.7	13.5	31.3	20.5	34.9	25.4	30.0	29.3	30.3

Dog	Day 6		Day 7		Day 10		Day 14		Day 21	
no.	B	P	B	P	B	P	B	P	B	P
1	29	48	25	51	23	47	11	36	22	37
2	42	22	34	19	34	17	35	11	38	17
3	1	29	-1	18	0	19	1	12	5	11
4	7	35	13	29	10	27	2	26	6	32
5	15	23	17	10	6	15	8	5	3	1
6	35	29	31	29	33	6	24	10	27	4
7	33	36	27	32	16	21	26	17	22	15
8	27	35	23	34	21	13	15	20	16	7
Mean	23.6	32.1	21.1	27.8	17.9	20.6	15.3	17.1	17.4	15.3

Appendix 3.5 Individual and mean values for post-operative pain in mm assessed by a VAS in a crossover trial with a single i.m. injection of 3 mg betamethasone (B) tested against placebo (P) in 8 dogs.

Dog	Day 1		Day 2		Day 3		Day 4		Day 5	
no.	B	P	B	P	B	P	B	P	B	P
1	20	2	10	0	45	3	10	4	18	9
2	2	0	3	8	3	0	3	17	3	36
3	2	0	1	0	1	15	1	16	3	16
4	7	2	0	1	0	0	5	1	6	3
5	20	3	15	3	14	12	15	1	9	11
6	1	22	2	18	3	44	3	60	3	7
7	9	0	14	0	1	4	1	0	8	13
8	1	0	3	21	3	15	1	21	5	6
Mean	7.8	3.6	6.0	6.4	8.8	11.6	4.9	15.0	6.9	12.6

Dog	Day 6		Day 7		Day 10		Day 14		Day 21	
no.	B	P	B	P	B	P	B	P	B	P
1	28	5	8	5	8	2	0	2	6	2
2	19	12	19	18	13	21	6	0	7	0
3	3	8	0	17	0	19	0	0	0	0
4	0	3	7	4	7	3	0	0	0	4
5	14	22	14	10	14	10	0	4	0	0
6	2	6	4	5	8	10	6	5	0	0
7	0	5	0	5	0	14	0	4	0	4
8	5	12	1	17	17	4	5	15	5	0
Mean	8.9	9.1	6	10.1	8.4	10.4	2.1	3.8	2.3	1.3

Appendix 3.6 Individual and mean values for post-operative limping in mm assessed by a VAS in a crossover trial with a single i.m. injection of 3 mg betamethasone (B) tested against placebo (P) in 8 dogs.

Dog	Day 1		Day 2		Day 3		Day 4		Day 5	
no.	B	P	B	P	B	P	B	P	B	P
1	46	9	24	14	52	14	25	15	12	11
2	20	68	4	24	3	100	0	17	10	17
3	7	79	6	10	6	6	11	6	6	6
4	47	11	11	11	11	19	10	19	1	34
5	7	4	7	24	0	50	3	0	11	13
6	0	8	0	0	11	7	4	19	9	20
7	11	-	0	-	7	-	7	-	0	-
8	0	6	0	14	0	7	0	0	14	0
Mean	17.3	26.4	6.5	13.9	11.3	25.4	7.5	10.9	7.9	14.4

Dog	Day 6		Day 7		Day 10		Day 14		Day 21	
no.	B	P	B	P	B	P	B	P	B	P
1	5	4	13	4	7	1	7	0	0	0
2	5	6	5	7	4	16	0	1	0	0
3	5	5	12	5	9	6	3	1	0	0
4	11	21	8	21	7	19	14	12	14	6
5	7	6	7	9	7	19	11	7	0	0
6	8	18	8	17	14	5	7	0	7	0
7	0	-	0	-	2	-	0	-	0	-
8	7	0	7	0	7	0	6	0	0	0
Mean	6.0	8.6	7.5	9.0	7.1	9.4	6.0	3.0	2.6	0.9

Appendix 3.7 Individual readings of rectal temperatures (°C) pre-operatively and after orthopaedic surgery in a crossover trial with a single i.m. injection of 3 mg betamethasone (B) tested against placebo (P) in 8 dogs.

Dog	Pre-operatively		Day 1		Day 2		Day 3		Day 4		Day 5	
no.	B	P	B	P	B	P	B	P	B	P	B	P
1	37.8	-	39.0	38.0	38.6	38.5	39.4	37.9	38.3	38.7	37.9	38.5
2	38.0	37.7	39.6	40.2	39.4	38.8	39.3	38.2	39.1	39.9	39.8	38.5
3	37.8	37.5	39.0	39.9	39.1	38.8	39.0	39.2	38.0	39.5	39.1	39.0
4	38.0	37.5	38.8	39.2	38.3	39.1	39.1	38.4	38.2	37.6	38.4	38.7
5	38.5	38.0	38.3	38.8	38.6	38.8	38.5	37.9	38.2	38.7	38.5	39.0
6	38.2	38.5	39.0	38.5	38.0	38.7	37.8	39.0	39.6	38.3	39.0	38.0
7	38.0	38.0	38.4	39.2	38.8	38.5	38.6	38.4	38.8	38.5	38.8	38.7
8	38.3	37.8	39.0	38.7	38.0	38.5	38.4	38.4	38.9	39.0	39.0	38.7
Mean	38.1	37.9	38.9	39.1	38.6	38.7	38.8	38.4	38.6	38.8	38.8	38.6

Dog	Day 6		Day 7		Day 10		Day 14		Day 21	
no.	B	P	B	P	B	P	B	P	B	P
1	37.8	38.0	38.1	38.2	38.5	38.0	37.8	38.0	37.7	37.7
2	38.8	39.1	39.4	37.9	39.3	38.1	39.0	38.7	37.6	38.8
3	39.4	39.1	38.9	38.7	38.5	38.5	38.3	39.1	38.1	38.2
4	38.8	38.8	38.7	37.8	38.7	37.9	38.2	38.0	38.0	38.0
5	38.2	38.5	38.2	38.3	37.2	37.9	37.0	38.0	37.3	38.3
6	38.5	38.2	38.5	38.0	38.0	37.8	38.0	37.2	38.5	37.0
7	38.6	38.3	38.8	38.1	38.1	38.2	38.7	38.0	37.6	38.4
8	38.0	38.2	38.6	38.0	38.5	38.5	38.4	38.7	38.6	37.9
Mean	38.5	38.5	38.7	38.1	38.4	38.1	38.2	38.2	37.9	38.0

Appendix 3.8 Individual and mean values of endogenous cortisol levels (nmol/l) before and after orthopaedic surgery in a crossover trial with a single i.m. injection of 3 mg betamethasone (B) tested against placebo (P) in 8 dogs (am = morning values, pm = afternoon values, - = not assessed)

	Pre-operatively												Day of operation	
	Day 4		Day 3				Day 2				Day 1			
Dog no.	am		am		pm		am		pm		am		pm	
	B	P	B	P	B	P	B	P	B	P	B	P	B	P
1	-	34	15	34	14	25	23	35	40	36	52	24	1	33
2	53	13	47	35	45	60	-	47	55	34	24	34	19	-
3	-	50	-	30	67	56	41	43	56	34	51	45	16	76
4	28	59	32	32	30	21	32	30	30	66	28	32	24	58
5	23	55	28	39	22	27	-	37	45	67	68	46	3	70
6	24	18	27	13	18	13	38	14	63	22	45	41	-	21
7	37	31	49	39	37	32	74	87	35	44	45	60	13	60
8	29	33	37	42	48	17	32	23	24	14	51	52	84	22
Mean	32	37	34	33	35	31	40	40	44	40	46	42	23	49

	Post-operatively													
	Day 1				Day 2				Day 3		Day 4		Day 5	
Dog no.	am		pm		am		pm		am		am		am	
	B	P	B	P	B	P	B	P	B	P	B	P	B	P
1	0.1	28	3	15	9	13	6	25	3	24	26	30	24	48
2	9	48	8	31	9	72	6	42	6	28	6	24	35	19
3	11	40	14	39	11	43	8	56	11	28	28	30	41	38
4	12	66	7	46	9	45	6	37	9	-	4	13	23	71
5	17	-	3	38	3	45	3	39	2	-	39	82	-	-
6	10	-	6	52	5	28	5	17	-	13	-	27	-	-
7	9	62	10	-	5	51	2	42	6	32	45	79	-	-
8	73	38	-	-	24	44	31	42	24	26	25	70	-	-
Mean	18	47	9	37	9	43	8	38	9	25	25	44	31	44

Appendix 4.1 Details of patients, anaesthetic and surgical procedures in a crossover trial with oral administration of 300 mg phenylbutazone (PB) twice daily tested against placebo (P) in 8 dogs.

Dog no	Sex	Pre-operative weight (kg)		Induction to incision (min)		Incision to last suture (min)		Induction to swallowing reflex (min)	
		PB	P	PB	P	PB	P	PB	P
1	F	15	14	14	6	32	32	55	40
2	F	13	16	6	10	38	32	46	33
3	M	14	15	7	8	36	37	58	50
4	F	15	15	9	5	37	28	53	43
5	F	13	12	8	7	28	38	38	47
6	F	12	13	9	6	31	32	43	49
7	M	17	17	7	11	34	35	42	56
8	M	17	18	12	6	31	35	60	43
Mean		14.5	15	9	7.4	33.4	33.6	49.4	54.3

Appendix 4.2 Details of patients, anaesthetic and surgical procedures in a crossover trial with oral administration of 25 mg indomethacin or 5 mg indomethacin (I) twice daily tested against placebo (P) in 7 dogs and 8 dogs respectively.

Dog no.	Sex	Pre-operative weight (kg)		Induction to incision (min)		Incision to last suture (min)		Induction to swallowing reflex (min)	
25 mg Indomethacin		I	P	I	P	I	P	I	P
1	F	16	16	7	13	32	29	50	56
2	M	16	16	3	6	36	26	58	34
3	F	20	20	6	8	39	32	54	43
5	M	15	16	6	7	27	29	34	47
6	M	17	21	7	5	25	33	33	41
7	F	16	15	6	4	23	21	37	30
8	M	21	19	8	6	28	26	45	35
Mean		17.3	17.6	6.1	7.0	30.3	28.0	44.4	40.9
5 mg Indomethacin									
1	M	18	19	7	8	30	31	47	51
2	F	16	17	4	9	24	23	41	40
3	F	16	18	5	8	28	25	67	45
4	M	20	18	2	7	27	26	37	44
5	F	16	16	8	7	33	30	43	54
6	F	17	18	7	9	29	23	42	34
7	F	21	19	5	6	29	28	47	43
8	F	20	21	9	1	30	30	66	49
Mean		18.0	18.3	5.9	6.9	28.8	27.0	48.8	45.0

Appendix 4.3 Individual and mean values for post-operative swelling in ml measured by a water displacement method in 8 dogs given placebo (P) and 300 mg phenylbutazone (PB) orally twice daily for 8 days in a crossover trial.

Dog	Day 1		Day 2		Day 3		Day 4		Day 5	
no	PB	P	PB	P	PB	P	PB	P	PB	P
1	17	25	21	28	26	24	28	28	30	35
2	25	14	35	21	32	19	39	28	37	36
3	22	48	24	43	26	48	29	41	31	43
4	19	50	23	34	21	30	20	24	24	28
5	13	4	9	0	6	-6	18	2	26	6
6	20	22	14	31	13	23	31	23	32	18
7	33	25	36	29	37	29	32	30	50	26
8	40	17	38	22	26	34	42	51	42	61
Mean	23.6	25.6	25.0	26.0	23.4	25.1	29.9	28.4	34.0	31.6

Dog	Day 6		Day 7		Day 10		Day 14		Day 21	
no.	PB	P	PB	P	PB	P	PB	P	PB	P
1	29	31	23	30	6	26	21	24	8	24
2	36	33	35	36	34	35	34	31	20	12
3	31	34	23	28	36	25	25	28	16	10
4	31	28	15	27	16	24	18	23	2	19
5	25	3	29	-4	31	-6	30	-10	21	-15
6	32	18	40	17	23	29	28	22	32	15
7	48	19	37	15	42	12	39	18	37	18
8	40	88	36	87	30	41	34	45	22	36
Mean	34.0	31.8	29.8	29.5	27.3	23.3	28.6	22.6	19.8	14.9

Appendix 4.4 Individual and mean values for post-operative swelling in ml measured by a water displacement method in 7 dogs given placebo (P) and 25 mg indomethacin (I) orally twice daily for 2 1/2 days in a crossover trial.

Dog	Day 1		Day 2		Day 3		Day 4		Day 5	
no	I	P	I	P	I	P	I	P	I	P
1	55	33	47	27	32	29	37	22	33	14
2	18	33	13	29	16	17	19	21	13	16
3	31	36	39	40	52	36	51	27	49	33
5	37	38	29	28	17	28	29	22	36	23
6	21	47	32	40	30	35	41	20	46	42
7	25	43	15	29	11	27	11	31	15	31
8	38	64	28	52	40	39	61	46	69	43
Mean	31.1	42.0	29.0	35.0	28.3	30.1	35.6	27.0	37.3	28.9

Dog	Day 6		Day 7		Day 10		Day 14		Day 21	
no.	I	P	I	P	I	P	I	P	I	P
1	33	12	31	8	28	13	35	12	29	9
2	17	16	20	16	14	23	17	16	15	8
3	38	37	39	29	34	22	28	26	26	30
5	32	19	23	11	23	10	18	17	12	13
6	40	48	39	49	34	41	30	33	16	33
7	23	28	14	24	18	25	18	19	10	23
8	71	46	75	43	62	46	53	43	4	23
Mean	36.3	29.4	34.4	25.7	30.4	25.7	28.4	23.7	16.0	19.9

Appendix 4.5 Individual and mean values for post-operative swelling in ml measured by a water displacement method in 8 dogs given placebo (P) and 5 mg indomethacin (I) orally twice daily for 8 days in a crossover trial.

Dog	Day 1		Day 2		Day 3		Day 4		Day 5	
no	I	P	I	P	I	P	I	P	I	P
1	30	52	33	52	23	47	29	44	24	40
2	41	50	45	23	29	27	18	30	16	21
3	23	36	22	35	24	33	17	33	13	-4
4	29	35	32	32	30	20	34	18	27	15
5	49	28	37	15	35	15	27	3	30	4
6	25	43	11	39	9	29	14	30	15	31
7	16	33	23	30	21	31	22	30	17	32
8	38	39	33	37	40	49	33	31	26	41
Mean	31.4	39.5	29.5	32.9	26.4	31.4	24.3	27.4	21.0	22.5

Dog	Day 6		Day 7		Day 10		Day 14		Day 21	
no.	I	P	I	P	I	P	I	P	I	P
1	24	41	25	49	30	42	28	34	29	32
2	10	12	13	24	14	9	13	12	17	18
3	18	28	5	26	26	41	8	31	20	21
4	15	24	14	23	19	20	14	21	8	18
5	22	2	18	8	12	19	12	13	18	13
6	12	32	11	21	25	24	22	24	18	17
7	12	15	8	22	13	44	6	38	4	34
8	22	40	18	45	32	36	30	30	26	26
Mean	16.9	24.3	14.0	27.3	21.4	29.4	16.6	25.4	17.1	22.4

Appendix 4.6 Individual and mean values for post-operative pain in mm assessed by a VAS in a crossover trial with 300 mg phenylbutazone (PB) administered orally twice daily tested against placebo (P) in 8 dogs.

Dog	Day 1		Day 2		Day 3		Day 4		Day 5	
no.	PB	P	PB	P	PB	P	PB	P	PB	P
1	6	44	6	14	12	14	5	8	8	22
2	31	7	8	7	4	12	4	22	3	19
3	23	32	3	13	0	5	0	15	11	7
4	2	99	2	67	1	41	2	0	2	20
5	4	16	1	15	1	15	2	15	6	9
6	33	48	22	29	15	26	36	16	13	4
7	0	24	4	14	0	13	24	13	22	7
8	45	2	18	1	14	3	12	13	11	6
Mean	18.0	34.0	8.0	20.0	5.9	16.1	10.6	12.8	9.5	11.8

Dog	Day 6		Day 7		Day 10		Day 14		Day 21	
no.	PB	P	PB	P	PB	P	PB	P	PB	P
1	32	16	9	14	14	5	10	4	2	7
2	2	38	3	16	4	4	4	3	9	0
3	0	8	5	2	0	0	0	1	0	0
4	0	0	0	4	3	0	0	0	0	0
5	13	14	12	8	5	16	2	6	4	3
6	17	8	26	15	15	10	17	5	14	2
7	5	6	13	4	4	15	11	6	4	1
8	5	1	4	35	4	15	4	6	0	5
Mean	9.3	11.4	9.0	12.3	6.1	8.1	6.0	3.9	4.1	2.3

Appendix 4.7 Individual and mean values for post-operative pain in mm assessed by a VAS in a crossover trial with 25 mg indomethacin (I) administered orally twice daily tested against placebo (P) in 7 dogs.

Dog	Day 1		Day 2		Day 3		Day 4		Day 5	
no.	I	P	I	P	I	P	I	P	I	P
1	26	65	16	96	7	48	58	64	19	57
2	0	21	0	13	0	18	0	46	0	11
3	0	21	0	0	0	8	17	5	0	0
5	23	49	16	36	0	47	6	46	17	47
6	0	0	0	17	0	-	0	-	0	7
7	0	12	0	12	0	13	0	7	0	6
8	0	100	7	96	7	95	0	100	100	75
Mean	7.0	38.3	5.6	38.6	2.0	38.2	11.6	44.7	19.4	29.0

Dog	Day 6		Day 7		Day 10		Day 14		Day 21	
no	I	P	I	P	I	P	I	P	I	P
1	19	87	29	66	29	67	6	49	14	26
2	7	19	12	18	23	19	23	13	7	4
3	0	7	7	7	4	7	4	0	12	7
5	7	0	0	19	16	10	0	5	0	0
6	0	0	0	0	0	0	0	0	0	5
7	0	6	0	4	8	5	7	0	0	0
8	20	100	20	100	20	100	19	64	19	13
Mean	7.6	31.3	9.7	30.6	14.3	29.7	8.4	18.7	7.4	7.9

Appendix 4.8 Individual and mean values for post-operative pain in mm assessed by a VAS in a crossover trial with 5 mg indomethacin (I) administered orally twice daily tested against placebo (P) in 8 dogs.

Dog	Day 1		Day 2		Day 3		Day 4		Day 5	
no.	I	P	I	P	I	P	I	P	I	P
1	8	0	15	27	8	40	0	10	0	25
2	48	8	48	7	20	0	9	0	8	14
3	26	0	59	44	49	8	26	29	40	0
4	7	51	19	70	0	37	0	38	0	61
5	50	83	50	41	49	28	70	28	0	19
6	10	8	8	0	0	0	7	0	0	0
7	100	34	0	26	6	10	0	24	0	0
8	27	0	19	8	19	8	18	0	8	0
Mean	34.5	23.0	27.3	27.9	18.9	16.4	16.3	16.1	7.0	14.9

Dog	Day 6		Day 7		Day 10		Day 14		Day 21	
no.	I	P	I	P	I	P	I	P	I	P
1	0	0	0	0	10	0	0	0	0	0
2	7	0	0	0	0	0	10	0	0	0
3	19	8	18	100	84	8	65	23	27	24
4	0	78	0	100	7	38	0	72	0	19
5	36	0	49	9	10	8	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0
7	0	8	0	0	0	0	0	22	0	0
8	8	20	0	0	21	8	0	0	0	0
Mean	8.8	14.3	8.4	26.1	16.5	7.8	9.4	14.6	3.4	5.4

Appendix 4.9 Individual readings of rectal temperatures (°C) pre-operatively and after orthopaedic surgery in a crossover trial with 300 mg phenylbutazone (PB) administered orally twice daily tested against placebo (P) in 8 dogs.

Dog	Pre-operatively		Day 1		Day 2		Day 3		Day 4		Day 5	
no.	PB	P	PB	P	PB	P	PB	P	PB	P	PB	P
1	37.8	37.8	38.3	39.2	39.5	39.4	40.2	38.8	39.3	38.8	38.5	38.2
2	37.5	37.6	38.8	39.0	38.5	38.7	38.3	39.0	39.1	38.8	38.2	38.5
3	37.0	37.6	38.8	38.5	39.4	38.7	38.6	38.6	38.2	37.8	37.5	38.3
4	38.0	37.4	38.2	39.0	39.3	38.5	38.7	38.0	37.5	38.4	38.4	38.0
5	38.4	38.2	39.7	39.1	39.7	38.8	39.6	39.4	39.8	39.7	39.6	39.3
6	38.6	38.4	38.7	39.5	39.0	39.2	38.8	39.2	39.0	39.2	39.2	38.4
7	38.5	39.8	38.8	41.0	38.7	39.4	38.2	39.4	37.9	39.8	38.2	38.0
8	39.2	38.0	39.5	39.4	39.0	39.2	39.0	39.1	39.6	39.5	39.1	39.6
Mean	38.1	38.1	38.9	39.3	39.1	39.0	38.9	38.9	38.8	38.9	38.6	38.5

Dog	Day 6		Day 7		Day 10		Day 14		Day 21	
no.	PB	P	PB	P	PB	P	PB	P	PB	P
1	38.5	38.8	38.6	39.0	39.2	37.8	38.9	38.0	38.2	37.8
2	38.0	38.3	38.3	38.4	38.6	38.4	38.5	39.5	38.5	37.9
3	38.0	38.3	38.6	37.8	38.0	37.4	38.0	38.8	38.0	37.5
4	38.1	38.2	37.8	38.5	38.0	38.0	39.2	37.9	37.6	37.8
5	39.5	39.0	39.0	38.5	39.0	38.5	39.0	38.3	38.2	38.0
6	40.0	38.2	38.5	39.5	38.4	37.2	38.6	38.6	38.2	37.2
7	39.5	37.6	37.5	39.5	37.5	38.1	37.7	37.9	37.4	37.2
8	39.6	39.5	38.0	40.0	38.5	38.8	38.9	38.4	38.0	37.2
Mean	38.9	38.5	38.3	38.9	38.4	38.0	38.6	38.4	38.0	37.6

Appendix 4.10 Individual readings of rectal temperatures (°C) pre-operatively and after orthopaedic surgery in a crossover trial with 25 mg indomethacin (I) administered orally twice daily tested against placebo (P) in 7 dogs.

Dog	Pre-operatively		Day 1		Day 2		Day 3		Day 4		Day 5	
no.	I	P	I	P	I	P	I	P	I	P	I	P
1	39.1	37.9	38.4	38.4	38.2	38.5	39.0	38.2	38.3	38.2	37.9	38.2
2	37.0	37.8	38.1	38.5	38.4	38.5	38.5	38.5	38.2	38.6	38.2	37.8
3	37.8	37.8	38.7	38.5	38.5	38.4	38.7	38.3	38.8	38.2	37.5	38.0
5	38.2	38.0	39.0	39.3	39.2	39.0	38.2	38.2	37.8	38.5	38.5	39.0
6	39.2	37.8	38.6	38.4	38.3	38.3	38.4	38.1	38.1	37.9	37.5	38.6
7	38.2	38.0	39.0	39.4	38.8	39.6	39.0	39.4	39.3	39.3	39.3	38.9
8	38.0	37.4	38.8	38.9	38.7	39.6	38.7	38.3	38.8	37.9	38.2	38.2
Mean	38.0	37.8	38.6	38.8	38.6	38.8	38.6	38.4	38.4	38.4	38.2	38.4

Dog	Day 6		Day 7		Day 10		Day 14		Day 21	
no.	I	P	I	P	I	P	I	P	I	P
1	38.0	37.7	38.5	38.5	38.7	38.4	38.5	39.1	38.2	38.0
2	38.2	37.8	38.7	38.6	38.2	38.2	38.3	38.3	38.5	38.2
3	37.8	38.2	38.0	38.4	37.7	38.0	38.8	38.2	38.1	38.0
5	38.6	39.2	39.0	38.5	38.2	38.2	38.5	38.5	38.3	38.8
6	37.5	38.8	37.4	38.6	37.8	38.1	38.2	38.3	38.2	37.6
7	39.3	38.8	39.3	39.0	39.4	39.0	39.2	39.2	39.4	39.4
8	38.5	38.4	38.4	38.2	38.5	39.0	39.2	37.5	37.8	38.0
Mean	38.3	38.4	38.5	38.5	38.4	38.4	38.7	38.4	38.4	38.3

Appendix 4.11 Individual readings of rectal temperatures (°C) pre-operatively and after orthopaedic surgery in a crossover trial with 5 mg indomethacin (I) administered orally twice daily tested against placebo (P) in 8 dogs.

Dog	Pre-operatively		Day 1		Day 2		Day 3		Day 4		Day 5	
no.	I	P	I	P	I	P	I	P	I	P	I	P
1	38.0	39.2	38.4	38.3	38.1	38.0	37.8	37.7	37.8	37.8	39.8	37.7
2	37.6	37.0	38.3	38.7	38.0	37.8	37.3	37.1	37.6	36.8	37.6	36.6
3	37.9	37.0	38.5	39.6	37.9	38.4	38.4	37.2	37.5	37.2	37.6	37.7
4	37.0	37.5	38.5	38.4	37.8	38.4	37.9	38.3	37.9	37.8	37.9	37.7
5	38.2	38.4	37.8	38.3	38.4	38.2	38.4	38.4	38.6	38.0	38.5	37.6
6	37.5	37.5	38.2	38.4	38.0	38.7	37.8	38.5	38.2	37.8	38.2	38.5
7	38.2	37.5	38.1	37.8	38.2	37.5	38.2	38.0	37.7	37.7	37.7	37.5
8	37.3	37.5	38.4	39.2	38.5	37.9	38.4	38.9	39.3	38.4	38.2	38.4
Mean	37.7	37.7	38.4	38.6	38.1	38.1	38.0	38.0	38.1	37.7	38.2	37.7

Dog	Day 6		Day 7		Day 10		Day 14		Day 21	
no.	I	P	I	P	I	P	I	P	I	P
1	37.8	38.3	38.0	38.2	38.4	38.8	36.8	39.0	37.1	38.0
2	37.6	36.8	37.2	36.0	38.4	36.8	38.1	36.0	36.8	37.2
3	37.8	37.7	37.3	36.8	37.4	36.8	38.2	36.6	36.5	36.6
4	38.1	38.3	38.0	37.9	38.6	37.7	37.7	38.8	37.7	36.8
5	38.5	39.0	38.4	38.0	37.8	38.8	37.8	39.2	38.0	38.0
6	39.0	38.8	38.3	37.8	38.3	38.6	38.8	38.6	36.7	37.2
7	38.4	37.9	37.8	38.0	36.8	37.8	37.1	38.4	37.1	37.1
8	37.8	39.4	37.6	38.1	38.0	38.4	38.3	38.4	37.0	38.1
Mean	38.1	38.3	37.8	37.6	38.0	38.0	37.9	38.1	37.1	37.4