EXPERIMENTAL AND CLINICAL STUDIES ON CRYOSURGERY IN DOGS

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CERTIFICATE

This is to certify that this thesis entitled "**EXPERIMENTAL AND CLINICAL STUDIES ON CRYOSURGERY IN DOGS**" submitted in part fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **VETERINARY SURGERY** to the Tamil Nadu Veterinary and Animal Sciences University, Madras - 600 007, is a record of bonafide research work carried out by **Sri.K.B.P.RAGHAVENDER** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma or fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazines.

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ABSTRACT

EXPERIMENTAL AND CLINICAL STUDIES ON CRYOSURGERY IN DOGS

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The present experimental and clinical study on cryosurgery in dogs was conducted on 60 experimental dogs and 50 clinical cases brought for treatment to the out-patient section of the Madras Veterinary College Clinics.

The study was carried out in three parts, under the following layout:

PART		NUMBER OF DOGS
PART A Evaluation of the cryosurgical units		6
PART B		
Group I	Local effects of freezing	12
Group II	Effects of freezing femoral artery and cephalic vein	12
Group III	Systemic effects of freezing	12
Group IV	Effects of freezing cornea and iris during extracapsular cryoextraction of lens	18
PART C Clinical study		50 Clinical cases

The results of the Part-A indicated that Frigitronics CS-76 cryosurgical unit produced minimal spray cone and probe tip temperatures of -195°C and -160°C, and minimal sub-cutaneous temperatures of -170°C and -130°C with the use of spray cone and closed end probe respectively. The Cryosuper VE-4 cryosurgical unit produced minimal probe tip and sub-cutaneous temperatures of -55°C and -40°C respectively, both with the use of nitrous oxide and carbon dioxide. The Cryosuper VE-1 cryosurgical unit was found to have resulted in probe tip temperatures of -80°C and -70°C with the use of nitrous oxide and carbon dioxide respectively. It was concluded that Frigitronics CS-76 was suitable for treatment of large and deeply infiltrated lesions and Cryosuper VE-4 for superficial and small lesions. Cryosuper VE-1 was considered suitable for ophthalmic cryosurgery in dogs.

The results of the part-B indicated that complete necrosis of skin was achieved at temperatures of -30°C and below and necrosis of gingiva at temperatures of -10°C and below.

Cryosurgery resulted in degenerative changes in the femoral artery of 12 dogs and thrombosis in 2 of them. Cryosurgery of the cephalic vein caused complete occlusive thrombosis and necrosis, accompanied with a well developed network of newly formed capillaries and venules.

When 2.5 and 5 percent of the body surface area of 6 dogs each was frozen to a sub-cutaneous temperature of -50°C, a raise in the level of AST, total leucocyte, neutrophil and monocyte counts was observed, which came back to normal by the 21st post-operative day. No changes were observed in total RBC count, haemoglobin content and packed cell volume percent.

While it was found that cornea was comparatively resistant to cryosurgery, iris was severely affected with changes like anterior synechia, presence of free melanin pigment in iridial muscles and intense cellular infiltration.

In the Part-C, a cure rate of 80.85% of the 47 clinical cases with various neoplastic and non-neoplastic lesions was achieved. Three cases of cataract where extracapsular cryoextraction of lens was performed, regained vision. The outcome of the treatment of these 50 clinical cases was discussed.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

CHAPTER I

INTRODUCTION

Cryosurgery has been defined as the selective, controlled destruction of tissues by use of extreme cold. The coolant or the refrigerant used for freezing the tissues is designated as the cryogen. Although several cryogens can be used for cryosurgery, the most commonly used ones are liquid nitrogen, nitrous oxide and carbon dioxide (Goldstein and Hess, 1976; Bojrab, 1978).

Cryosurgery is neither a spectacular cure-all nor a method that has been newly developed. The use of cold in the treatment of diseases was reported as early as 2500 BC (Podkonjak, 1982[a]). However, the modern era of cryosurgery began in 1961 with the development of a practical, controllable system for the use of extreme cold as a physiological and surgical tool by Cooper and Lee. Eversince, the advances in cryosurgery were made in leaps and bounds and of late, reports of monitoring the ice ball and control of cryogenic destruction of tissues using methods as advanced as nuclear magnetic resonance imaging (Isoda, 1989), computed tomography (Reiser ***et al.***, 1983; Isoda, 1989) and ultrasound scanning (Onik ***et al.***, 1991) are not uncommon.

Cryosurgery was introduced into veterinary practice by Borthwick in the year 1971. Since then, various neoplastic and non-neoplastic diseases of the domestic animals have been successfully treated (Goldstein, 1977; Podkonjak, 1982[a]&[b]; Routh, 1992).

Despite the wide availability of liquid nitrogen even in rural veterinary dispensaries and the availability of moderately priced cryosurgical units, there have been very few published reports on cryosurgery in India.

The present study was mainly intended to systematically investigate certain observations made through the literature published. For instance, different workers have reported differently on the local effect of freezing tissues (Gill ***et al.,*** 1971; Chambers and Slatter, 1984; Seim and Hoyt, 1985). Conflicting reports have been published on the outcome of freezing of large blood vessels that either run through or lie in close proximity to the lesions under treatment (Borthwick, 1972; Seim, 1980; Fretz and Holmberg, 1980; Rekhter and Mironov, 1990). The systemic effects of freezing large areas of the dog's body, as might be necessitated during treatment of large neoplasia, have been seldom investigated. Finally, although the technique of cryoextraction of lens has been in practice, reports on the effects of accidental freezing of cornea and iris have not been forthcoming.

In order to systematically investigate the above mentioned aspects of cryosurgical practice, the present experimental and clinical study in dogs was undertaken with the following objectives:

1. To evaluate the efficacy and utility of the cryogens and cryosurgical equipment used in the present study,

2. To study the local effects of freezing,

3. To study the outcome of freezing femoral artery and cephalic vein,

4. To study the systemic changes due to surface freezing of large areas of the body,

5. To study the effects of freezing cornea and iris during cryoextraction of lens and

6. To treat various neoplastic and non-neoplastic diseases of dogs by cryosurgery.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

2.1 History of Cryosurgery

Goldstein and Hess (1976) stated that the first reports of cryosurgery in veterinary medicine came from England in the early 1970s. They reported that Borthwick introduced cryosurgery in veterinary practice in the year 1971.

Baxter (1977[a]) reported that way back in 1851, Arnott used a combination of salt and crushed ice to achieve considerable benefit to his patients suffering from various proliferative lesions. The temperature of about -10°C achieved by his technique, however, was hardly considered to be within the true cryogenic range.

Cryotherapy was first mentioned in the literature thousands of years before Christ. As early as 2500 BC, Edwin Smith Surgical Papyrus referred to the use of cold compresses for treatment of compound skull fractures and infected wounds of the chest. Homer (900 B.C.) and Hippocrates (400 B.C.) reported beneficial effects of local cold to control haemorrhage and reduce swelling in treating chest wounds. Cold as a local anaesthetic was noted in the literature in 1050 A.D. and hypothermia anaesthesia was known to Italian physicians of the 16th and 17th centuries. In 1661, Nepoleon's surgeon general, Baron Dominique Jean Larrey, performed painless amputations on soldiers who had lain in the snow for some time. Refrigeration anaesthesia was pioneered by James Arnott, who also pursued the therapeutic effects of local cooling. Using a mixture of salt and ice at about -10°C, Arnott treated a variety of nonspecific conditions such as neuralgia, pruritus and headaches. In 1845, he pioneered the application of such methods in treating advanced breast cancer and cervical carcinomas. Freezing was forgotten by the turn of the century, although in 1899, White described the dermatologic use of recently developed liquid air in the treatment of lupus, epitheliomas, superficial skin lesions and chanchroids. Nearly four decades later, between 1936 and 1940, 124 selected patients with greatly advanced carcinoma, glioblastoma and Hodgkin's disease were subjected to local or generalised refrigeration. Although the instrumentation was crude with teaspoons welded face to face to become curved capsules to slip under craniotomy incisions, and steinmann pins, converted by tube attachments, used to carry chilled fluids to and from the involved sites, only 11.2% mortality was noticed. Among these 124 patients, 95.7% were relieved of pain. Eight patients with metastatic carcinoma survived more than five years and one was alive 20 years later. With the advent of World War II, events that ensued delayed the progress of cryosurgery. The Nazi's use of hypothermia without anaesthesia on prisoners in concentration camps, became associated with Nazi's other atrocities. Therefore, for several years, any research in cryosurgery was considered deplorable. However, investigation slowly renewed in the field of cryosurgery and Lougheed (1955) and Bartrell (1956) investigated the possible use of its application in neurosurgery. Modern cryosurgery began in 1961 when Cooper and Lee developed a closed cryogenic system with liquid nitrogen as refrigerant. (Podkonjak, 1982[a]).

Henk (1985) reported that although it was Robert Boyle, who in 1683 first demonstrated that cells were destroyed by freezing, modern cryosurgery did not begin until 1961, when Cooper and Lee described an insulated probe, the tip of which was cooled by liquid nitrogen.

While reporting on the history of cryosurgery, Seim and Hoyt (1985) stated that Egyptians used cold for treating traumatised patients in 2500 B.C. They stated that Hippocrates reported its use. James Arnott was credited with being the first to use cold compresses for the management of human breast and skin cancer in 1851. The real expansion, they reported, began in 1961 when Cooper and Lee developed a liquid nitrogen cryosurgical system. Practical methodology for veterinary use was first developed by British veterinarians in the early seventies.

Gage (1989) stated that although the interest in cryosurgery developed quickly after modern cryosurgical apparatus became available in early 1960s, of late, the progress in the development of cryosurgical techniques and apparatus has slowed. To infuse some vitality in this speciality, he suggested collaborative interaction of cryobiologists with cryoengineers.

2.2 Cryogens Used

Goldstein and Hess (1976) reported that although freon has been used for cataract removal, the three most commonly used cryogens in veterinary practice are carbon dioxide, nitrous oxide and liquid nitrogen. It was also stated that the last of these cryogens was most commonly used in tumour therapy.

Bojrab (1978) reported that the most commonly used cryogens are freon, carbon dioxide, nitrous oxide and liquid nitrogen. It was also stated that freon was especially used for cataract removal. While carbon dioxide and nitrous oxide lacked ability to penetrate, it was stated that liquid nitrogen produced freezing of tissues upto a depth of 5 cm.

Crane (1978) stated that for treatment of small lesions on the eyelids, small skin tumours and for cataract cryoadhesion, closed carbon dioxide or nitrous oxide systems are suitable. While diffuse epithelial neoplasia of the oral cavity, perirectal fistulae, and mammary tumours could be frozen by nitrous oxide or liquid nitrogen, it was opined that large skin lesions in vascular areas and high volume tumours of any type required liquid nitrogen as the refrigerant.

Bryne (1980) reported that while liquid nitrogen, nitrous oxide and carbon dioxide are the commonly used cryogens, freons, liquid oxygen, liquid propane and liquid air are also occasionally used for cryotherapy. He, however, discouraged the use of liquid oxygen and liquified propane because of the dangers of combustion-supporting capabilities of the former and the flammability of the latter. The use of freons was also discouraged due to its potential for damaging the environment.

2.2.1 Physical Properties of Cryogens

Bryne (1980) stated that liquid nitrogen produced cooling of tissues by direct extraction of heat when sprayed over the target area. Precooled liquid nitrogen probes chill tissues by direct absorption of heat from the target tissues. Gases like nitrous oxide and carbon dioxide produce cooling of the cryoprobes due to Joule-

Thomson effect. Joule-Thomson effect refers to cooling of the tip of the orifice when a gas under pressure is made to expand through a small orifice. This cooling effect is used to cool cryoprobes which are then applied to the target tissues.

Podkonjak (1982[a]) listed out the boiling points of the commonly used cryogens. It was reported that while ethyl chloride boiled at +12.2°C, freon-12 boiled at -29.4°C, freon-22 at -40.5°C, a mixture of dry ice and alcohol at -78°C, nitrous oxide at -89.5°C and liquid nitrogen at -195.6°C. The sublimation temperature of carbon dioxide was stated to be -78.5°C.

2.3 Cryosurgical Instrumentation

Farris ***et al.*** (1975) described the fabrication of a simple cryosurgical unit. The system consisted of a stainless steel Dewar flask, a No.7 stainless steel tubing, a blood pressure apparatus bulb and tubing. The blood pressure apparatus bulb was used to pressurise the liquid nitrogen in the flask, which then could be delivered as a spray.

Baxter (1977[a]) stated that cryosurgical instruments that use liquid nitrogen as the cryogen, cost more because their design and manufacture involved high vacuum technology. Cryosurgical units using carbon dioxide or nitrous oxide, although less expensive, were considered not powerful enough.

Goldstein (1977) observed that nitrous oxide cryosurgical units produced predictable ice ball formation which provided great precision to the operator. The predictable nature of nitrous oxide ice balls was thought to be due to the fact that they reach equilibrium with the warmth of the surrounding healthy tissue in a fairly constant period of time. This is in direct contrast to liquid nitrogen which has a low start up freeze time. After reaching maximum cooling, liquid nitrogen penetrates rapidly and tends to over freeze lesions that require precise control. The ice ball formed by liquid nitrogen was considered unpredictable and not dependent on time. This increased the chance of damage to healthy tissues, particularly when liquid nitrogen was poured or sprayed into an area. The author further stated that nitrous oxide units are useful because nitrous oxide is available in most veterinary hospitals and also because it does not evaporate when not in use. In comparison, it was stated that liquid nitrogen may not be available in all areas, requires special storage tanks and evaporates constantly. However, it was also stated that no single cryosurgical unit performed the surgery required in every case presented. The development of well designed nitrous oxide cryosurgery units added another dimension for greater accuracy in veterinary cryosurgery. Such units were considered economically feasible for the veterinary practitioner.

Crane (1978) opined that every cryosurgical instrument should be evaluated for its individual suitability. Also, the choice of the cryogen to be used was considered difficult to make.

Podkonjak (1982*) described the several options that should be considered while purchasing a cryosurgical unit in veterinary practice. He stated that in order to be financially acceptable to both the doctor and the client, the cost of equipment and therefore the treatment must be much less than the cost of cryosurgical equipments used in human medicine. The unit should also be capable of performing a wide variety of tasks in many clinical situations. For instance, it was stated that the unit should be capable of freezing delicate corneal tissue, small orifices like canine anal sacs and large lesions like sarcoids in equines. Further, the equipment should be portable to meet the demand of the practicing veterinarian.

Divine and Anderson (1983) opined that nitrous oxide cryosurgical units are more convenient for ocular cryosurgery than liquid nitrogen units.

Vestre and Brightman (1983[a]) compared the efficacy of three nitrous oxide cryosurgical units on the basis of the ability of each unit to freeze the ciliary body of enucleated normal canine eyes. The cryosurgical units used were cryovet cryosurgical unit (Spembly Inc.) Keeler-Amoils cryosurgical unit (Keeler Optical Products) and Frigitronics cryosurgical unit (Frigitronics Inc.). The coolest average temperatures reached by use of these units were -13.4°C, -4.3°C and -17.6°C respectively.

Giannone (1984[c]) found that a spray-tip liquid nitrogen applicator was useful for extensive superficial lesions of ear pinna because it allowed uniform freezing. A pointed-tip cryoprobe was found suitable for lesions around the eyes because it allowed a well controlled circumferential freezing of superficial lesions. On the other hand, a nitrous oxide cryogun with quick defrost capability was found ideal in preventing iatrogenic damage when used on animals that might move during cryotherapy, as in cattle being treated for eye lesions.

Roberts ***et al.*** (1984[b]) observed that liquid nitrogen cryotherapy controlled the pressure in 75% of the 181 glaucomatous eyes that received a single course of therapy. Subsequent retreatments on those eyes increased the control rate to 91.7%. Nitrous oxide cryotherapy controlled the pressure in 66.7% of the eyes treated regardless of any subsequent repeat procedures. The liquid nitrogen system was found to be superior to the nitrous oxide system (P=0.029). Cryogun, a liquid nitrogen cryosurgical system and Kry Med, a nitrous oxide cryosurgical system were used in the study.

Seim and Hoyt (1985) listed out the different types of cryosurgical equipment available and stated that it was their experience that, any portable, handheld, gun type of cryosurgical device using liquid nitrogen, with various interchangeable probes and spray orifices, was the most versatile and practical instrument for veterinary use.

Routh (1992) found that the nitrous oxide cryosurgical units freeze small areas of tissues to -40°C while liquid nitrogen cryosurgical units produce tissue temperature as low as -185°C.

2.3.1 Tissue Temperature Measurement

Goldstein (1977) while attempting to answer the universally asked question about cryosurgery whether tissue thermometry is necessary, stated that the unpredictable nature of liquid nitrogen necessitated the use of thermocouples at most of the times. This was particularly found to be true whenever liquid nitrogen was sprayed or poured. Conversely, the predictable behavior of ice ball formed by nitrous oxide obviated the need for thermocouples. However, it was felt that thermocouples were needed when cryosurgery using liquid nitrogen involved treatment of malignancies or lesions of eye lid or oral cavity.

Equipments used for monitoring tissue temperature, as stated by Bryne (1980), are pyrometers that indicate current flow caused by temperature change sensed at the non-insulated junction point of two dissimilar metallic leads. Digital read-out pyrometers and electrical impedance devices are also available for tissue temperature measurement. The latter device is useful only for monitoring superficial cryosurgical procedures.

Withrow (1980[b]) opined that by far the best means of assessing depth and extent of freezing was with the use of thermocouples. These are needles that could be placed either in or on the margin of the lesion being frozen. He stated that the indirect method of temperature monitoring, on the other hand, has been variously described as subjective monitoring, an art, clinical experience or simply witchcraft. Palpation, visualisation and fixation of the ice ball to the underlying structures were the criteria for indirect method of temperature monitoring. Although thermocouples were considered indispensable, assessment of degree of freezing was found possible by the indirect method.

Vestre and Brightman (1983[a]) during experimental cyclocryotherapy in the clinically normal dog, used a 24-gauge thermocouple needle for monitoring the ciliary body temperature. The needle was passed into the anterior chamber through the cornea 1-2 mm from the limbus. The needle was then directed into the ciliary body through the pupil. The tip of the needle was palpated through the conjunctiva and sclera to ascertain its position.

Seim and Hoyt (1985) stated that freezing could be monitored by visualisation and palpation of the ice ball formed or by direct measurement of the tissue temperature by the use of thermocouples.

2.4 The Cryolesion

2.4.1 Cryolesion and Ice Ball Formation

Borthwick (1972) stated that the cryolesion produced at the time of freezing is called an "ice ball", and it represented the approximate volume of tissues which will die. The size of the ice ball can be appreciated visibly or by palpation.

Joyce (1976) observed that within one to two hours after the cryosurgical insult, considerable edema developed due to vascular damage. The lesion appeared dark and haemorrhagic due to stagnation and diapedesis of the red blood cells. The destroyed tissue began to shrink and sloughed in 7-10 days. Occasionally, it remained in place acting as a bandage. Purulent exudation was noticed. Healing took place by second intention but with minimal scar formation.

Reiser ***et al.*** (1983) opined that one of the main problems encountered in cryosurgery was precise control of tissue destruction. Therefore, the "ice ball" formed during cryosurgery in a pig was scanned by computed tomography (CT). The speed of "ice ball" propagation and its diameter were visualised as areas of decreased density on the CT scanner. The authors felt that the use of CT as well as cryoprobes of a very small diameter could facilitate the percutaneous treatment of lesions within the body.

Isoda (1989) investigated and found that magnetic resonance imaging (MRI) and computed tomography (CT) were useful for monitoring the ice ball formed during the process of cryosurgery of experimentally induced tumours in rats.

Onik ***et al.*** (1991) used transrectal ultrasound guidance to guide a cryoprobe percutaneously, using a transperineal approach, into the canine prostate. The extent of freezing was then monitored by assessment of ice ball formation using ultrasound, taking care not to freeze the urethra or the rectum. Six dogs had the procedure without any complications.

2.4.2 Lethal Temperature of Tissues

Gill ***et al.*** (1971) stated that for all practical purposes, all tissue frozen in situ dies and the eventual lesion resembled an infarct.

Borthwick (1972) stated that temperature lethal to living tissues are generally accepted as -20°C and below.

Goldstein and Hess (1976) stated that to ensure complete tissue necrosis, tissues must be frozen to at least -25°C.

Joyce (1976) stated that optimal cryonecrosis of malignant cells occurred between -20 to -40°C, when a double freeze-thaw cycle is used. The maximum percentage of cells were killed when the freezing was rapid and thawing was slow.

Bojrab (1978) stated that minimal temperature required to produce cryonecrosis was -20°C to -30°C with marked increase in cell destruction occurring at temperatures of -40°C to -60°C. Cryonecrosis also depended on the type of cryogen used: the lower the cryogens boiling point, the greater the thermal injury.

Seim (1980) stated that atleast two applications of temperatures ranging from -15°C to -20°C ensured adequate cellular destruction and tissue necrosis.

Chambers and Slatter (1984) noted that a temperature of -20°C destroys the hair follicle without necrosis or scarring of the eye lid because hair follicles are more susceptible to freezing.

Seim and Hoyt (1985) reported that two applications of cryogen with tissue temperatures ranging from -20°C to -30°C usually are adequate to cause necrosis of target tissue.

2.4.3 Mechanism of Cell Death in Cryosurgery

Szyszkowska ***et al.*** (1983) employing histochemical methods, examined the reaction of selected hydrolytic enzymes in the rabbit tongue mucosa and skin after cryoapplication depending on the time of freezing. The results demonstrated the reaction of hydrolases during cryodestruction and regeneration and the dynamics of inflammatory process. The tongue mucosa was found to be more susceptible to cryodestruction than skin. The study confirmed the participation of hydrolytic cellular enzymes in cryonecrosis.

Roberts ***et al.*** (1984[a]), while discussing the mode of action of cryotherapy in experimental cyclocryotherapy, stated that the initial effects produced by cryotherapy are due to tissue dehydration. This was considered a result of increased electrolyte concentration and cell membrane lipoprotein denaturation as extracellular and intracellular water crystallisation occurred. Several hours following cryotherapy, thermal shock and microcirculation thrombosis ensued which resulted in ischaemic necrosis of the involved tissue. The ice crystals formed extracellularly initially and pulled free water from the cells. If the cryotherapy reduced the temperature slowly, the progressive cellular dehydration depressed the freezing point of the cytoplasm below the normal value of -2.2°C. Therefore, intracellular ice crystallisation occurred less frequently and the lethal changes in the cells were delayed. It was suggested that this problem could be solved by rapid freezing and slow thawing of the tissues.

Seim and Hoyt (1985) categorised the three major events at the cellular level during cryonecrosis as the immediate phase, the delayed phase and the late phase. During the immediate phase which occurred during freeze-thaw cycle, extracellular and intracellular fluids froze to form ice crystals. If the thaw is slow, the ice crystals merge to form large crystals that damage the cell wall and the cells die. At the same time, it resulted in withdrawal of water from the cells, leading to toxic concentration of electrolytes in the cells. The proteins that constitute the cellular membrane as well as the membranes of various cell organelles are denatured. Thermal shock due to rapid change of temperature also causes cell death. During the delayed phase, which occurred within a few hours of the freeze-thaw cycle, cellular destruction was due to vascular stasis. During the late phase, possibly, an immunological response is produced in the animals against the tumour cells.

2.4.4 Histopathology of Cryolesion

Fraser and Gill (1967) studied the histological changes produced by ultra freezing of rat liver to -190°C for four minutes. The frozen tissues did not bleed significantly and following thawing, avascular enucleation of the frozen central core of the tissue from the surrounding tissue was achieved. The necrotic area was replaced by a small fibrous scar by the 4th week and by the 6th week, the site of freezing was difficult to find.

Bushby ***et al.*** (1978) reported on the microscopic alterations of the canine skin following freezing for three minutes or to a temperature of -20°C using liquid nitrogen spray. At one hour after freezing, severe edema was noticed that was still evident at 6 hours. The epidermis appeared necrotic by one day and diffuse necrosis was observed by one week. After tow weeks, no recognisable necrotic tissue remained. Healing was complete by one month with regeneration of 4-5 cell layers thick epidermis.

Merideth and Gelatt (1980) observed that following freezing of iris, the musculature and the pigmented cells of iris like stromal melanocytes and posterior epithelium were destroyed. Free melanin was found to be dispersed throughout the iridial stroma, anterior chamber and iridocorneal angle. Small uveal blood vessels were thrombosed. Inflammatory cells, initially neutrophils and latter lymphocytes in moderate numbers invaded the cryosite. The regeneration was incomplete and the atrophied iridial stroma contained reduced numbers of melanocytes and the iris became clinically light brown to white in colour.

Chambers and Slatter (1984) observed maximal conjunctival chemosis at four hours after freezing the eye lids in experimental dogs, which slowly decreased during the next 24 hours. Depigmentation of the conjunctiva and the eye lid margin was observed at 72 hours and dissolution of melanocytes was noticed. Healing was complete by 30 days.

Schneider ***et al.*** (1985) experimentally evaluated the histopathological changes resulting from a controlled freeze of bilateral palmar and plantar digital nerves in five adult horses. Following percutaneous freezing of the nerves or freezing by direct surgical exposure, the horses were euthanised, the nerves were collected and subjected to histological examination. It was found that cryosurgery resulted in neuromas-in-continuity in 4 nerves and nerve fibre depopulation was noticed in three nerves. The consistent finding was nerve fibre degeneration followed by regeneration by the end of the three week observation period.

Burge ***et al.*** (1986) investigated the changes in pigmentation and melanocyte distribution in human skin after standardised freeze injury, and reported that hypopigmentation with a peripheral ring of hyperpigmentation was a consistent finding. Another consistent finding was the absence of melanosomes but the presence of melanocytes. It was concluded that hypopigmentation after the cryosurgical treatment of malignant melanocyte tumours might not equate with cure.

Kramek ***et al.*** (1986) evaluated in 17 dogs, the effects of transepiphyseal freezing of the canine distal femur on its growth and closure. A specially designed cryoprobe was used for the purpose, which was cooled with liquid nitrogen. The left distal femoral physes were sham operated and served as controls. The dogs were radiographed weekly and euthanised from two days to eight weeks following surgery. Histological studies on the distal femoral physes revealed that immediately after freezing, there was extensive haemorrhage in the epiphysis and metaphysis with disruption of the physis. Inflammation and death of the physeal cartilage followed. Blood vessels invaded the physis and eventually bone was formed between the epiphysis and metaphysis, resulting in complete and premature physeal closure. It was concluded that cryoapplication as a clinically acceptable method of epiphysiodesis warranted further research.

Natiella ***et al.*** (1987) subjected the skin of the Rhesus monkey to one or two cycle freezing with liquid nitrogen spray. Temperaturesof -40 to -60°C were used and the sites were evaluated from 7-182 days. Overall healing was considered to be excellent. Long term specimens showed a consistent increase in epidermal thickness at the site of the freeze. This was quantified by the use of computer assisted image analysis. In addition, the elastic component of the skin, seldom studied in repair following skin surgery, did not regenerate.

Matanyi and Kerenyi (1989) detected vacuolisation of the cytoplasm and partial fragmentation of the mitochondria at two hours of freezing of non-malignant ectocervical region of fertile women. Severe irreversible nuclear impairments in these cells was also documented.

Burge and Dawber (1990) investigated the histological changes in hair follicles in guinea pig skin after cryosurgery injuries. They noted a permanent damage to the hair follicles, destruction of dermal connective tissue and dermal scarring. The necrotic dermis sloughed, taking with it the dead follicles and morphologically normal elastic tissue.

2.5 Indications for Cryosurgery

Borthwick (1971) treated two cases of multiple perianal sinuses in German Shepard dogs and stated that the results of uneventful healing at the unhygienic site where one might expect sepsis, were encouraging. Almost complete healing of frozen sites was observed by eight weeks.

Neel ***et al.*** (1973[a]) designed and executed experimental studies in 11 dogs to assess the pathophysiological effects of complete circumferential cryonecrosis of tracheal segments and to study the feasibility of performing cryosurgery within the distal tracheobronchial tree through a bronchoscope. All the 11 dogs survived the procedures and resumed normal activity within 12 hours of operation. Necrosis was localised, reproducible and followed by prompt regeneration of mucosa. The gross architecture of the trachea and bronchus was not permanently altered, and there was no local or regional infection. Tracheal stricture observed in one dog was attributed to persistent infection around a permanent suture placed at operation for the endoscopic identification of the target area. The study indicated that trachea and bronchus could be safely frozen by application of a cryoprobe either to the external surface or to the mucosal surface.

Lane (1974) listed out some of the conditions and procedures where cryosurgery was considered useful. They were: anal furunculosis, anal adenoma, benign skin tumours, biopsy of malignant neoplasms, granulation tissue, inter and supra digital cysts, malignant skin tumours, lendectomy, oral tumours, oral ulceration, tonsillectomy including tonsillar carcinomata and tumours of the eye lids.

Goldstein and Hess (1976) opined that cryosurgery is indicated in papillomas, small histiocytomas, benign and malignant skin tumours, fibrosarcomas, cutaneous malignant melanomas, squamous cell carcinomas, mast cell sarcomas, oral tumours of various kinds, anal and perianal tumours, rectal carcinomas and polyps, nasal cavity tumours and mammary tumours.

Krahwinkel ***et al.*** (1976) used cryosurgery for the treatment of a variety of cancerous and noncancerous diseases in dogs, horses and cats. The lesions treated included anal fistulas, perianal adenoma, adamantinoma, papilloma, melanoma, chondrosarcoma, eosinophilic granuloma etc. It was also reported that the treatment of invasive neoplasms of the oral and nasal cavities was not successful.

Crane (1978) opined that while some small lesions may be handled more quickly with conventional excisional surgery, freezing will provide much faster operating times in patients with several different types of difficult lesions. Cryosurgery may be the only practical way of solving major surgical problems in other patients. Cryosurgery was thought to be useful in geriatric patients with neoplasms since it required minimal anaesthesia.

Neoplasms of the skin and subcutaneous tissues represent the most common tumours seen in dogs (Krahwinkel, 1980). Cryosurgery was considered by him as a good means of treatment in such cases and it was useful even in cases where other treatment modalities have failed.

Liska (1980) found cryosurgery to be an acceptable form of treatment in several anorectal and perianal problems. These included perianal adenomas, perianal carcinomas, perianal fistulae and rectal polyps. The cryosurgery technique was regarded as a reliable, efficient, effective and inexpensive method to cure numerous anorectal diseases.

Giannone (1984[b]) observed that cryotherapy was too often viewed only as an alternative between unsuccessful conventional resection and euthanasia. In a series of articles on cryotherapy in small animals, the author showed that cryotherapy could well be the primary mode of treatment, rather than a last resort.

Giannone (1984[c]) opined that cryosurgery was ideal for removal of nasal polyps and tumours of eye and ear because it resulted in little haemorrhage and rapid healing.

2.6 Indications in Veterinary Ophthalmology

Fraunfelder *et al.* (1973) successfully treated 35 cases of cancer of eye in cattle by cryosurgery using a liquid nitrogen spray unit. The lesions were totally frozen including a margin of 2-3 mm of surrounding normal tissue. A double freeze thaw cycle was adopted. The lesions completely regressed as a result of one or more treatments, depending on their size. The results were considered to be encouraging.

Joyce (1976) at the end of a follow-up study of one year, reported that 13 ocular squamous cell carcinomas were cured by one cryosurgical treatment in cattle, while one required a second treatment. In another instance, one of the tumors regressed greatly, but recurred with rapid growth, necessitating ablation of the eye and adnexa. The tumours that were successfully treated varied in size from 1 cm plaques to 5x3x3 cm masses.

Barrie ***et al.*** (1980) stated that cryosurgery in human and veterinary ophthalmology has been used for the treatment of eyelid and conjunctival neoplasms, iris prolapse, glaucoma, retinal detachment, vitreocorneal adhesion and intraocular neoplasms. They further stated that cryotherapy might be a useful therapeutic modality for cataract lens extraction in the dog. They also stated that the control of pupil size after surgical procedure was a major problem requiring intensive mydriatic therapy and contributed directly to the success or failure of cataract surgery.

Holmberg (1980) stated that during surgical excision of eyelid tumours, maintenance of adequate function of the lid without skin grafting was difficult. Minimising the size of the surgical lesion helped, but often resulted in incomplete removal of the tumour. The normal canine eyelid has been shown to have some resistance to damage by cryonecrosis, which made vigorous cryosurgery useful for treatment of eyelid tumours.

Merideth and Gelatt (1980) observed that cryosurgery was useful in the treatment of various intraocular disorders like retinal detachments, iris prolapse, glaucoma and cataract (lens extractions). Cryosurgery offered two unique features of cryodestruction and cryoadhesion between the various ocular tissues and between ocular tissues and cryosurgical probes.

Brightman **et al.** (1982) described the indications for and a technique of cryosurgery in the treatment of canine glaucoma. Freezing of four to eight spots over the ciliary body in each of the forty four cryosurgical procedures in 20 dogs reduced the intraocular pressures from 39.10 ± 19.90 mm Hg pre-operatively to 22.54 ± 1.84 mm Hg postoperatively. The mean follow up time was 147.57 ± 2.10 weeks.

Wheeler and Severin (1983) achieved excellent results using cryoepilation over a three year period in 12 cases of distichiasis. A nitrous oxide unit, used for a double freeze - thaw cycle to -20°C, destroyed the cilia bearing follicles without damage to adjacent lid tissues. The technique was easy to perform and the results were cosmetically pleasing to the practitioner and the owner.

Chambers and Slatter (1984) successfully treated trichiasis and distichiasis by cryosurgery in eight dogs. Treatment was accomplished by freezing the conjunctival surface approximately 2 mm from the lid margin. The areas over the offending follicles were frozen with a 3 mm glaucoma probe to -89°C. The authors concluded that cryosurgery is an effective treatment for distichiasis and trichiasis in the dog.

Vestre (1984) used cyclocryotherapy to control intraocular pressure in five cases of glaucoma of which, tow were due to trauma, two due to narrowed drainage angles and one was secondary to lens extraction. In all cases, the intraocular pressure decreased, with the usual result being a cosmetic and painless but blind eye.

Holmberg **et al.** (1986) cryosurgically treated nine clinical cases of pigmentary keratitis in dogs. Reduction in corneal pigmentation and improvement of vision occurred in all cases. It was concluded that although cryosurgery was only a means of decreasing corneal pigmentation, it was found to be an effective and repeatable method of restoring vision in dogs with pigmentary keratitis.

2.7 Selection of Patients for Cryosurgery

The five major reasons for selecting patients for cryosurgery, as enunciated by Goldstein and Hess (1976 and 1977) are (a) chronically recurring tumours, (b) surgically difficult cases, (c) tumours in tissues not damaged by freezing, e.g. tumours in bones, tumours around nerve trunks and large blood vessels (d) patients with poor anaesthetic risk and (e) patients where immunotherapy is to be given.

Stuart ***et al.*** (1982) assessed the reasons for failures of cryosurgical treatment in cervical intraepithelial neoplasia. They reported an overall recurrence rate of 10.8% among the 166 women treated by cryosurgery. Treatment failure was considered to be generally due to inadequate assessment, improper patient selection technical difficulties in freezing and poor patient follow-up. It was reported that a majority of failures occurred within the first 12 months and probably represented persistent rather than recurrent disease.

2.8 Anaesthesia for Cryosurgery

Roberts ***et al.*** (1984[a]) premedicated the dogs with 0.04 mg/kg of atropine sulphate and 0.5 mg/kg body weight of morphine sulphate, while performing cyclocryotherapy in experimental dogs. Anaesthesia was induced and maintained with pentobarbital sodium which was satisfactory for the procedure.

Bryson ***et al.*** (1985) cryosurgically treated cervical intraepithelial neoplasia in 453 women over a period of 11 years and stated that in most cases, cryosurgery was performed as an outpatient procedure and no analgesia or anaesthesia was necessary in most patients.

Matsunaga ***et al.*** (1987) observed while treating 51 women with genital condylomata that cryosurgery could be performed on an outpatient basis, with no general anaesthesia or analgesia. Cryosurgery in all the pregnant women was repeated every two weeks until resolution, without the use of anaesthesia.

Rao (1990) treated 28 dogs with different kinds of lesions by cryosurgery. He opined that unless the location of the lesion so demanded, anaesthesia was often unnecessary for cryosurgery.

2.9 Cryosurgical Techniques

Joyce (1976) enlisted the various methods of application of liquid nitrogen. Cotton tipped applicators or solid copper discs of various sizes, precooled by dipping in liquid nitrogen could be applied directly to small superficial lesions. Cryotherapy, it was stated, could also be performed by use of contact freezing by probes chilled by continuous circulation of liquid nitrogen. It was also stated that direct spray of liquid nitrogen onto the lesion allowed treatment of large growths.

Baxter (1977[b]) suggested that digital pressure, tourniquets or even drugs acting on the vascular system could be used to reduce the body's ability to 'reheat' an area and thus produce a more rapid freeze and a slower thaw. He also stated that decision whether to use the spray or close probe method to treat a given condition is important. When the spray method is used, the surrounding normal tissue should be well protected.

Goldstein (1977) described the cryosurgical techniques that could be adopted with the use of nitrous oxide cryosurgical units. The overlapping probe method involved production of multiple overlapping ice balls to form a larger ice ball to increase the total area of necrosis. The base method involved removal of the bulk of a large lesion by conventional surgery and freezing the remaining base. The punch method, on the other hand, involved placement of the cryoprobe into the hole left by punch biopsy.

Farris (1980) compared the results of a single and double freeze-thaw cycle in treatment of bovine ocular squamous cell carcinoma. The results obtained from a single freeze (66% cure) were not as satisfactory as those from a double freeze-thaw cycle to -25°C (97% cure). During the double freeze-thaw regimen, as thawing occurred to 5°C, all lesions were rapidly refrozen.

Merideth and Gelatt (1980) preferred standard corneo-scleral approach for lens extraction in dogs. The cryoprobe was made to contact the lens surface at ambient temperature, and the cryogun was activated. For extracapsular lens extraction, the cryoprobe's contact was limited to 1-2 seconds bonding to only the anterior lens capsule. The probe was slightly raised to prevent freezing of the underlying anterior cortex and rotated, usually tearing a 7-10 mm section. The remaining cataract was slid from the patella fossa. For intracapsular extraction, however, the cryoprobe was allowed 4-6 seconds contact and the lens was lifted and rotated for zonulolysis.

Seim (1980) stated that the factors affecting the degree of cryonecrosis were probe tip temperature, size of the probe tip, size of the spray orifice, duration of freeze, rate of freeze-thaw, number of applications, tissue temperature, and tissue density. In order to achieve predictable results, all the above factors should be taken into consideration.

Podkonjak (1982[a]) categorised the techniques of cryogenic application broadly into two types. The first involved the direct application of the cryogen to the target tissue, e.g. spraying of ethyl chloride, freon, nitrous oxide or liquid nitrogen, while the second type involved the application of prechilled cryoprobes either by hand dipping the probe into the cryogen or by means of elaborate closed-system circulating refrigerant units.

Withrow (1982) while treating nasal tumours in dogs by cryosurgery with liquid nitrogen spray, observed that for better visualisation of the frozen area, the surgeon had to remove his mask and simply blow away the vapour that obscured him view. Although this was done during open nasal surgery, no incidence of infection was reported by the author.

Singh and Chandna (1983) reviewed the different methods of freezing tissues. Base method was defined as one where a bulk of the lesion was removed with a scalpel and the base frozen to ensure death of the remaining tumour tissue. While contact freezing method constituted placement of cryoprobe over the lesion, penetrating or stab freezing method involved introduction of the cryoprobe into the lesion to treat deeper parts of the lesion. On the other hand, spray freezing method or pouring liquid nitrogen onto the lesion comprised of bringing the cryogen in direct contact with the target tissues.

Giannone (1984[a] & 1984[b]) stated that cryotherapy of oral lesions using liquid nitrogen or nitrous oxide was nearly a bloodless procedure and usually required general anaesthesia. It was recommended that the temperature of the surrounding normal tissue be monitored by thermocouples to prevent freezing and that of the lesion to ensure adequate freezing. Use of a double freeze-thaw cycle was recommended to ensure total necrosis of the lesion. Large tumours could either be debulked by partial resection before cryotherapy or could be treated by several overlapping freezes. Frozen tissues should be allowed to thaw naturally without application of warm water to obtain adequate necrosis.

Seim and Hoyt (1985) stated that systemic body temperature and the vascularity of the affected area modify the extent of local freezing. Since highly vascular tissues take longer to freeze and thaw more rapidly, the cryonecrosis of such tissues may be suboptimal. It was stated that tissue vascularity during cryosurgery could be decreased in several ways: when probe tips are used, added pressure on probe and lesion resulted in blanching of the tissues; intralesional epinephrine injections caused local vasoconstriction. In rare instances, major vessels could be ligated or a tourniquet could be used for lesions involving an extremity.

Jones and Darville (1989) studied the possibility of transmission of virus particles from patient to patient by cryotherapy and multi-use caustic pencils. The results showed that transmission by caustic pencils was unlikely, but that virus particles could be transferred into liquid nitrogen cans, if cotton swabs were used for cryosurgery. It was suggested that swabs should not be dipped repeatedly into the flask of liquid nitrogen. Instead, a small quantity of liquid nitrogen should be decanted into a clean vessel and a new swab used for each patient.

Homasson ***et al.*** (1992) studied the combined effect of cryotherapy and chemotherapy in 12 patients with bronchial carcinoma. When i/v injection of radiolabelled Bleomycin was followed by cryotherapy, an increase in the radiolabelled Bleomycin content was found in the tumour area after cryotherapy. This resulted due to tapping of the anti-cancer drug in the tumour and immediately surrounding area due to vascular stasis. It was concluded that chemotherapy might be more effective when combined with cryotherapy.

2.10 Effects of Cryosurgery on Blood Vessels

Borthwick (1972) reported that large vessels are protected by severe cryoinjury by the warming effect of circulating blood. They also contained in their walls white and elastic connective tissue fibers which are unaffected by cryosurgery. It was also reported that cryosurgery could be useful for treatment of tumours in and around bones, nerve trunks and large blood vessels.

Whitaker (1972) discovered using carbon labelling techniques that following cryosurgery operations, the normal blood flow was restored within few minutes but blood vessel permeability became marked within 30 minutes. He reported that stasis became increasingly severe from 2 hours post-operatively and finally only arteries remained patent, although they showed greatly increased permeability.

Fretz and Holmberg (1980) stated that occasionally, a large vessel traversing the lesion will be frozen with the tumour and subsequently traumatised at the time of slough, and could lead to serious haemorrhage. Therefore, it was suggested that prophylactically, these major vessels should be ligated proximal and distal to the lesion.

Seim (1980) stated that the walls of large arteries are resistant to the destructive effects of cryosurgery. It has been shown, he reported, that after a vessel has been frozen solid, it will recanalise and remain viable. He stated that a possible advantage of this phenomenon was the ability to freeze tumours that are growing around large vessels.

Anderson ***et al.*** (1983) for the first time assessed the effects of cryosurgery on foetal ductus arteriosus in 5 foetal lambs. After birth, all the cryosurgically treated lambs had patent ductus arteriosus whereas all the control lambs had a closed ductus arteriosus. The cryosurgically treated group had calcific deposits, necrosis, focal ganglion cell necrosis and loss of muscle and elastic fibre.

Holman ***et al.*** (1983) subjected the left anterior descending coronary artery of 10 dogs to cryosurgery. The arteries were frozen till blood flow ceased to occur, which took 20 ± 4 seconds of cryothermic exposure. Blood supply resumed within 29 ± 8 seconds after termination of cryothermia. Although coronary arteriography at 6 months showed all the arteries to the patent, microscopic examination revealed intimal hyperplasia.

Marsland *et al.* (1983) studied the effects of cryogenic injury on femoral blood vessels in 28 rats. The femoral vessels were dissected out and subjected to cryogenic injury. The rats were sacrificed at intervals upto 2 weeks. Damage to the vessels included necrosis and thrombus formation. The results indicated that significant vascular damage could occur during cryosurgery and the possible complications of this should be considered.

Misaki *et al.* (1983) recommended cryosurgery as a safe and permanent technique for the surgical treatment of arrhythmias in man cryosurgery was considered safe even in the proximity to the coronary arteries, since it caused minimal changes in the vessels. The recommendation was based on experimental studies in sheep followed by clinical application in man.

The abdominal aorta of 27 rats were subjected to freezing in an attempt to study the reparative process by Rekhter and Mironov (1990). An increase in the proliferative activity of the endothelial cells, heteromorphism of endothelial monolayer and myointimal thickening were observed.

2.11 Systemic Effects of Cryosurgery

Balthasar (1957) studied a series of lesions produced by cold in the cerebral cortex of cats. He concluded that cold was the most physiologically acceptable method to produce local necrosis, because it was not complicated by haemorrhage.

Fraser and Gill (1967) observed that the cryolesions depicted haemostatic qualities, invocation of excellent reactive repair process and minimal scar formation. It was also stated that the cryolesion remained biologically inert.

Neel ***et al.*** (1973[b]) demonstrated that tumour-specific transplantation immunity was consistently greater after cryosurgery or ligation-release infarction than after complete excision in experimentally induced mammary adenocarcinoma in mice.

Weyer ***et al.*** (1987) demonstrated a post-operative increase in the total and helper T cells and HLA - DR positive cells in 8 malignant melanoma patients treated by cryosurgery. In 8 patients treated with conventional surgery, these parameters decreased slightly or remained the same. The differences were highly significant (P=0.001) to significant (P=0.01).

2.12 Effects of Cryosurgery on Ocular Structures

Sudarsky ***et al.*** (1965) reported that the use of both -40°C and -190°C probes for transscleral iridocryothermy in rabbits resulted in predominant destruction of the iridic pigmental cells and endothelium. Full thickness holes developed frequently in irides frozen at their bases by the -190°C cryoprobes. The vascular and connective tissues of the iris degenerated more slowly.

Farris ***et al.*** (1977) froze the cornea of cattle and horses for 30 seconds with a direct spray of liquid nitrogen. The 1 mm ice ball formed produced oedema of the cornea which cleared within two weeks with no visible damage.

Barrie *et al.* (1980) exposed the base and pupillary zones of the iris in dogs for 10 seconds to a -79°C cryoprobe (iridocryothermy). The resultant iritis lasted 7-10 days as evidenced by aqueous flare, hypotony and miosis. At 21 days, the iris was depigmented and had an irregular pupil. The initial histopathologic changes after iridocryothermy were iridic intrastromal haemorrhage, exudation and neutrophil infiltration. The iridic pigmented epithelium was lysed, and the melanin granules were liberated into the iridic stroma, posterior and anterior chambers. Irides frozen at the pupillary zone exhibited sphincter muscle degeneration. By 21 days after iridocryothermy, the irides were depigmented and atrophic.

Roberts *et al.* (1984[a]) performed cyclocryotherapy using a liquid nitrogen system in 10 dogs, to determine the temperatures achieved at the ciliary body and ciliary processes. A brass probe measuring 2.5 x 6.5 x 58 mm was used. The probe was centered 5 mm posterior to the limbus. Cryotherapy was performed either until the anterior edge of the ice ball extended 1 mm across the limbus into clear cornea (1 mm rule) or for 30 seconds (30 second rule). Thawing occurred without the aid of external warming and the probe attachment was maintained until spontaneous release occurred. By allowing the ice ball to extend 1 mm into clear cornea, an acceptable temperature range at the ciliary body was consistently obtained (-10.42 ± 1.52°C). The same technique carried out for 30 seconds resulted in excessively cold temperatures (-23.23 ± 3.16°C). Following cryotherapy, the average probe detachment time was 140.6 and 157.5 seconds for the 1 mm and 30 second rules respectively.

Holmberg ***et al.*** (1986) exposed 40 normal dogs to double freezes of a 1 cm diameter area of their central corneas. Liquid nitrogen spray method was used for the purpose. After producing a discrete layer of frost on the cornea, the freeze was maintained for 15 seconds. The time/temperature combination lowered the temperature of the corneal epithelium to -40°C. Postoperative sequelae and final histopathologic results were evaluated. All eyes suffered epithelial damage and developed corneal oedema. Three weeks later, the corneas returned to a normal or near normal condition. Erosion of the corneal epithelium was noticed for the first seven postoperative days, as confirmed by fluorescein dye technique.

Chan ***et al.*** (1990) performed transcorneal freezing of the central area of the rabbit cornea with a 1 mm diameter probe at -60°C for 15 seconds. Enface sections of cornea showed a cell-free circular area in the stroma 1-2 days after wounding that persisted upto 6 days. By 7th day, the stroma and keratocyte population appeared normal. One day after cryodamage, the destroyed epithelium in the wounded area was replaced, possibly by migrating cells from the wound margin. Some polymorphonuclear leucocyte like cells were present at the basal epithelial region. The damaged portion of the cornea was close to the normal by 21 days.

2.13 Clinical Reports on Cryosurgery

Lane and Burch (1975) reported that 35 of the 40 dogs treated for anal furunculosis by cryosurgery healed completely with single or multiple treatments. Five cases were not completely resolved and one of them had to be euthanised because of unsatisfactory response to cryosurgery.

Joyce (1976), while treating equine sarcoids of varying sizes noted that the size of the lesion was not as important as its anatomic location. Even masses as large as 10x5x5 cm were treated successfully by cryosurgery.

Hilbert ***et al.*** (1977) treated squamous cell carcinoma around the eyes of three horses with liquid nitrogen, using cryotherapy probes as the method of application. In two cases, there was complete regression of the tumour while in the third case, remission and relief of discomfort were temporary. It was opined that liquid nitrogen probes were suitable for treating tumours in the skin and around the eyes.

Lane (1977) achieved total cure from sarcoids in 33 of the 50 horses treated. The majority of the remaining horses were rendered suitable for work following cryosurgery with liquid nitrogen spray. It was concluded that cryosurgery could be regarded as one of the treatments of choice for equine sarcoids and that best results were obtained from early treatment.

Fretz and Barber (1980) cryosurgically treated 50 horses with 204 sarcoids. They reported a success rate of 63% and 70% depending on the number of horses freed from sarcoids and the number of lesions regressed, respectively. Over caution resulting in inadequate freezing and necrosis was stated to be one of the causes of recurrence.

Richart ***et al.*** (1980) retrospectively analysed 2839 human patients who had been treated by cryotherapy for cervical intraepithelial neoplasia in nine different hospitals. The cumulative risk of developing cervical intraepithelial neoplasia after successful cryotherapeutic management was estimated to be 0.41% at 5 years, 0.44%

at 10 years and 0.44% at the 14th year. There was no significant difference in risk between patients originally treated for the three grades, i.e. grades 1,2 and 3 of cervical intraepithelial neoplasia in women.

Budsberg ***et al.*** (1981) performed cryosurgery in 31 dogs to treat perianal fistulas. One to three treatments were found necessary. Most owners (92%) were satisfied with the improvement following cryotherapy. These results were compared with a series treated surgically, in which the success rate was 25%.

Hemmingsson ***et al.*** (1981) cryosurgically treated 181 women of different age groups with cervical intraepithelial neoplasia and reported a failure rate of 16%. Liquid nitrogen cryosurgical unit was used for therapy.

Giannone (1984[a]) successfully treated a case of multiple cutaneous lymphosarcoma lesions in a 10-year old spayed overweight German Shepard. The dog was presented with numerous non-pruritic, ulcerated, welt-like lesions extending from the left axilla distal to the elbow and caudal to the left flank. Following cryotherapy by a 4 mm closed-tip nitrous oxide probe, the lesions healed completely within 5 weeks. Some untreated lesions also regressed probably due to an immune response induced by cryosurgery.

Idowu (1985) obtained regression of canine transmissible venereal tumours (TVT) in 14 of the 15 dogs treated by cryosurgery. One dog developed a tumour in the inguinal lymph node 7 months later. Cryosurgery was considered effective in the treatment of canine TVT.

Omara-Opyene **et al.** (1985) achieved a cure rate of 88.7% in 62 cattle treated for squamous cell carcinoma of the vulva by cryosurgery. It was observed that smaller lesions responded well. Larger lesions could not be frozen satisfactorily using the cryosurgical unit available. It was concluded that, if instituted early in the clinical course of the disease, cryotherapy of bovine vulval carcinoma was superior to surgical excision, but the cost of the equipment might limit its application in the field.

Sloss **et al.** (1986) examined the eyes of 737 Hereford cattle in 3 herds and recorded ocular squamous cell carcinoma in 90 of them. The lesions were less than 5 mm in diameter and were cryosurgically treated by application of prechilled copper probes dipped in liquid nitrogen. Seventy one percent of the lesions regressed after one or two treatments.

Lanigan and Robinson (1987) used liquid nitrogen spray to cryosurgically treat 27 human patients with 35 dermatofibromas. The results were described as good or excellent in over 90% of the cases. Cryosurgery resulted in cosmetically acceptable scar formation.

Matsunaga **et al.** (1987) cryosurgically treated fifty one pregnant women, 16 in the second and 35 in the third trimester, for genital condylomata acuminata. Nineteen of them had cervical cryotherapy in the second and third trimesters of pregnancy, for cervical involvement. Atleast two cryosurgical treatment sessions were necessary for complete resolution of the lesions in all the women. All condylomata resolved during pregnancy and no patient had residual disease at six weeks postpartum. The newborn infants were also free from condylomata. Labor and delivery were unaffected even by cervical cryotherapy.

Lommatzsch ***et al.*** (1990) treated eighty one cases of conjunctival melanomas in man between 1960 and 1988. The therapeutic procedures adopted were local excision or local excision followed by brachytherapy with Sr-90/Y-90 or local excision followed by cryotherapy using liquid nitrogen or local excision followed by beam irradiation. It was concluded that local excision followed either by beta ray irradiation (Sr-90/Y-90) or cryotherapy were the procedures of choice.

Venkataswamy and Ramakrishna (1990) treated 10 cattle with ocular squamous cell carcinoma using cryosurgery. In five of them, cryosurgery was the only treatment modality adapted while in the other five, surgical excision preceded cryosurgery of the base. All but one of the lesions treated regressed completely. One lesion that recurred 45 days later also regressed following a second cryosurgical treatment.

Handley ***et al.*** (1991) compared the efficacy of interferon α2a combined with cryotherapy and cryotherapy alone in the treatment of anogenital warts in 60 human patients. Both the groups provided satisfactory results and the addition of interferon α2a did not provide any advantage over the use of cryotherapy alone.

Kristiansen ***et al.*** (1991) treated 96 women with intraepithelial cervical neoplasia by cryotherapy. They were monitored for 10-14 years with an attendance rate of 98%. Treatment resulted in cure in 92%. Nine patients had persisting lesions and three patients had recurrent lesions. The last recurrence was diagnosed 6.7 years after cryosurgery. It was concluded that cryosurgery was an economical and a gentle procedure.

Angra (1992) reported that cryotherapy, followed by oral administration of aspirin for 6 weeks led to recovery from vernal catarrh of the palpebral conjunctiva in 15 human patients. Recurrence at the end of one year follow-up was found to be low (3.3 %).

Routh (1992) successfully treated a case of anal furunculosis in a Terrier cross, a case of squamous cell carcinoma in a Boxer and a case a pedunculated mass on tarsus in an Irish setter by using cryosurgical technique. The recovery was uneventful in all the three cases and the cosmetic effect was reported to be good.

Vasantha and Lakshmipathi (1993) successfully treated a case of congenital dermoid cyst in the left eye covering cornea and sclera in a 20 day old calf by cryosurgery. The blunt end of a scalpel pre-cooled by dipping in liquid nitrogen was used for freezing the tissue. Complete resolution was attained after three episodes of cryosurgery, performed on alternate days.

2.14 Advantages of Cryosurgery

Farris ***et al.*** (1975) listed out the advantages of cryosurgery over conventional methods of squamous cell carcinoma therapy. The advantages reported were : (a) simplicity and rapid application, (b) minimal or no haemorrhage, (c) analgesia of the frozen tissue both during the procedure and the post-operative period, (d) unlike radiation therapy, cryosurgery could be safely repeated without adverse cumulative effects on the patient or the operator and (e) cryosurgery required no pre-operative or post-operative medication.

Goldstein and Hess (1976) enumerated the advantages of cryosurgery. They stated that cryosurgery required minimal anaesthesia, caused minimal haemorrhage, enabled treatment of tumours difficult to excise while preserving normal surrounding tissues, provided palliative effect in animals with grave prognosis and probably elicited an immune response to tumours. Further, it was stated that cryosurgery could be readily used with adjunctive therapies like chemotherapy or immunotherapy.

Joyce (1976) listed out the advantages of cryosurgery . He stated that minimal pain was associated with freezing, little haemorrhage occurred, scarring was minimal, the instruments were easy to use, tumours could often be treated under local analgesia, tumour cells did not spread (as they might with a scalpel) and circulating antibodies to certain types of tumours might be stimulated.

Neel (1980) reported that cryosurgery augmented immunity that is specifically directed against the tumour in several murine tumour systems. Both cell mediated and humoral immunity were reported to be involved. The degree of augmentation of immunity following cryosurgery was stated to be greater than that following a period of tumour growth, cold-knife excision and electrocoagulation of tumour.

Podkonjak (1982[a]) summed up the advantages of cryosurgery. He stated that using cryosurgery, cells can be selectively destroyed with little or no damage to adjacent tissues and surgical intervention could be undertaken in areas that otherwise would be impossible to treat. Cryosurgery was reported to be painless, accompanied with slight loss of blood, less time consuming and it allowed painless tissue specimen collection for biopsy. Cryosurgery also enabled treatment of some chronic lesions with good cosmetic outcome. It was postulated that reepithelialisation was hastened after cryosurgery and it probably elicited an immune response to tumours.

Henk (1985) observed that cryosurgery in man was painless, could be often performed without anaesthesia and that the post-operative phase was usually pain-free. He further stated that cryosurgery did not produce haemorrhage and healing progressed to cause minimal scarring.

Orth ***et al.*** (1992) cryosurgically treated 182 rectal cancer patients and stated that the prospects of fixation of artificial anus could be avoided in 80 percent of the cases.

Prusiewiczowa and Lenkiewicz (1992) treated 56 human patients with severe chemical and thermal burns. They reported that cryosurgery hastened the process of regeneration of the corneal epithelium and clearing up of its opacification.

2.15 Adverse Effects and Complications of Cryosurgery

The disadvantages of cryosurgery as perceived by Goldstein and Hess (1976) included emission of foul odour, accidental freezing and damage to the vital normal tissues and excessive scarring due to excessive freezing.

Harvey (1978) reported the occurrence of two cases of fatal air embolism in dogs during cryotherapy of oral neoplasms using liquid nitrogen spray. It was advised that an operator should be cautious of this complication, especially when spray method of liquid nitrogen application is used in highly vascular areas.

Fretz and Barber (1980) reported the occurrence of facial paralysis due to accidental overrun of liquid nitrogen while treating a case of equine facial sarcoids. In another instance, during treatment of 50 cases of equine sarcoids, over freezing of a sarcoid located on the metatarsal-phalangeal joint, resulted in slough of the lateral collateral ligament and an open joint, which subsequently became infected.

Harvey (1980) observed that oral cryosurgery was not without risk. It was stated that soft tissues of the oral cavity responded to trauma with oedema. Post-operative oedema was found to be particularly troublesome after cryosurgery of pharyngeal masses, since swelling might be severe enough to obstruct the airway. Similarly, cryosurgery of the tongue lesions might cause oedema of such severity as to hamper eating and drinking for several days.

Brightman ***et al.*** (1982) while reporting on cryosurgical treatment of glaucoma in dogs, stated that post-operative inflammatory reaction and associated choroidal edema could be a predisposing cause to retinal detachment. It was felt that the use of corticosteroids prevented the occurrence of retinal detachment following cryosurgery.

While discussing the disadvantages of cryosurgery, Podkonjak (1982[a]) stated that it was accompanied by malodour and might cause transient pain in adjacent tissues. Cryotherapy sometimes caused discolouration of hair, could lead to accidental injury of healthy tissues and could cause fatal air embolisation. However, he opined that the advantages of cryosurgery far outweighed the disadvantages, especially because the complications are rare.

Withrow (1982) while treating nasal tumours by cryosurgery, noticed inadvertent loss of a part of hard palate in four dogs with resulting oral-nasal communication. Further occurrence of this complication was prevented in eight dogs by careful monitoring and avoiding over-freezing of the palate. It was concluded that cryosurgery had not appreciably altered the survival of dogs with nasal tumours.

Elton (1983) listed out haemorrhage, severe systemic reactions in cold-sensitive individuals, full-thickness skin necrosis, syncope and sudden death as the possible complications of cutaneous cryosurgery in man.

Vestre and Brightman (1983[b]) reported that the adverse reactions to the cyclocryosurgical procedures included retinal detachments, chemosis, conjunctivitis, transient increased intraocular pressure, uveitis, iris depigmentation and formation of corneal granulation tissue.

Following cryosurgery, it cannot be said with certainty that the margins were clear of tumor. The difficulty in assessing the extent of tissue destruction following cryosurgery was considered a major disadvantage. Although adequate cell destruction could be ensured at the surface and margin of the tumours, active tumour could persist at the depth of the lesion. Secondary haemorrhage was occasionally encountered post-operatively at 7-10 days. As late as 1985, it was considered that cryosurgery had no role in the treatment of oral cancer and premalignant lesions within the mouth in man (Henk, 1985).

Macdonald and Critchlow (1987) carried out experiments on the tumour potentiating effects of cryosurgery in carcinogen treated hamster cheek pouch. The cheek pouches in 11 hamsters were treated with topical application of carcinogen DMBA for 8 weeks. Two episodes of cryosurgery located within the area of previous carcinogen application enhanced the neoplastic process by comparison with matched controls. It was stated that two episodes of cryosurgery may have a greater effect in provoking overt malignancy than a single episode of cryosurgery.

Schlinkert and Chambers (1990) observed a case of near fatal nitrogen embolism during hepatic cryosurgery in man. It was suggested that the use of liquid nitrogen probe which prevent direct contact of liquid nitrogen with the tissue being frozen, prevented this type of injury.

2.16 Sequelae of Cryosurgery

Joyce (1975) while reporting on the treatment of equine sarcoids, stated that cryosurgery could by no means be considered as the total answer; however, it could be another valuable route of therapy. Of the six cases treated, two had recurrence. These two cases, however, also recurred following conventional surgery.

Krahwinkel ***et al.*** (1976) observed that lick granuloma in two dogs recurred 5 months following regression with cryosurgery. It was thought that the regression was due to the destruction of the sensory nerve endings, with recurrence due to subsequent reinnervation. However, the five months regression was considered to be encouraging since these lesions were resistant to other modes of therapy.

Fretz and Holmberg (1980) concluded that infection was not a common complicating factor in cryosurgery. Although the lesions are likely to be superficially infected, discharge and malodour from these wounds are mostly the normal reaction of frozen tissue. In instance where cryolesions did get infected, it was usually related to premature removal of the eschar, which acted as biologic dressing. The other sequelae reported included swelling, damage to vital structures, nitrogen run-off, nitrogen emboli, and pathologic fractures.

Holmberg (1980) observed that the post-operative sequelae of the palpebral tumours treated by cryosurgery were predictable. While haemorrhage, swelling and ocular discharge were transient sequelae, damage to melanocytes, hair follicles and other adnexal structures was permanent.

Withrow (1980[a]) attempted to cryosurgically treat five cases of osteosarcoma in dogs. Survival ranged from 2 weeks to 12 months with a mean survival of 3.9 months. Quality of life was considered poor in one case and good in four cases until pathological fracture occurred in two cases. The goal in treating these five dogs with cryosurgery was to relieve pain, preserve limb function and prolong survival. None of these goals was consistently achieved. It was considered that the failure was a result of poor technique as well as the natural behaviour of the tumour.

Elton (1983) recorded that cutaneous cryosurgery in man has resulted in various sequelae like pseudoepitheliomatous hyperplasia, nerve damage, pigmentary problems, tissue defects, delayed healing, scar formation and the recurrence of benign and malignant lesions.

Kristiansen ***et al.*** (1991), during cryosurgical treatment of intraepithelial cervical neoplasia in women, commented that the efficacy of cryosurgery was dependant on the area of the lesion and not the degree. Overlooked invasive growth at the time of treatment was thought to be the most frequent reason for the apparent development of the cervical cancer after cryotherapy.

Ward ***et al.*** (1992) recorded recurrence of squamous cell carcinoma of the cornea in a 12 year old Shih tzu dog, one year after its cryosurgical treatment.

2.17 Limitations of Cryosurgery

Oral tumours that were incurable when treated by conventional methods of surgery, such as excision or electrocautery, were also found to be incurable when treated by cryosurgery (Harvey, 1980). In addition, it was stated that cryosurgery had no ability to eradicate extensive oral lesions or to significantly alter the course of advanced cancers.

MacCalla ***et al.*** (1992) observed that squamous cell carcinoma of the eyelids in a 5 year old pony did not respond to two episodes of cryosurgery. However, immunotherapy with injections of BCG led to regression of the lesion.

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

The experimental part of the present study was conducted on 60 apparently healthy dogs of either sex, between one year and four years of age. All the dogs, weighing between 8-12 Kg, were obtained from the lethal chamber, Corporation of Madras. The dogs were housed in kennels under identical conditions.

The clinical part of the study was carried out on 50 dogs presented to the Out-patient section of the Madras Veterinary College Clinics, with various neoplastic and non-neoplastic conditions.

3.1 Design of Study

Out of the 60 experimental dogs, 6 were used for evaluating the cryosurgical units.

Twelve dogs each were used for studying the local effects of freezing, effects of freezing blood vessels and the systemic effects of freezing. In the remaining 18 dogs, the effects of freezing cornea and iris during extracapsular cryoextraction of lens were studied. The animals used in the present study were grouped as follows:

PART		NUMBER OF DOGS
PART A		
Evaluation of the cryosurgical units		6
PART B		
Group I	Local effects of freezing	12
Group II	Effects of freezing femoral artery and cephalic vein	12
Group III	Systemic effects of freezing	12
Group IV	Effects of freezing cornea and iris during extracapsular cryoextraction of lens	18
PART C		
Clinical study		50 Clinical cases

3.2 Materials Used

3.2.1 Frigitronics CS-76 Cryosurgical Unit*

The Frigitronics CS-76, a self-pressurizing, portable, non-electric, liquid nitrogen cryosurgical system was employed as one of the cryosurgical units. The unit, along with accessories is presented in plates **1** & **2**. Frigitronics CS-76 requires 2 liters of liquid nitrogen to be poured in its two cylinders. Liquid nitrogen can then be used as a spray by using spray cones or can be used to cool closed end probes that can be applied to the lesion.

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* Frigitronics CS-76 - Frigitronics of Connecticut Inc., USA.

PLATE-1. FRIGITRONICS CS-76 WITH THERMOCOUPLE NEEDLES AND PROBES

PLATE-2. ACCESSORIES FOR FRIGITRONICS CS-76 [From top 25 mm, 5 mm & 2 mm spray cones and closed end probe]

3.2.2 Cryosuper VE-4 Cryosurgical Unit*

Cryosuper VE-4, a self-contained cryosurgical unit, ready for use when attached to a cylinder of nitrous oxide or carbon dioxide was used in the present study. The Joule-Thomson cryosurgical unit, and its accessories are presented in plates **3** & **4**. This unit could be activated by pressing a lever on the cryogun, which led to release and expansion of the gas resulting in cooling of the closed end probe. The probe can then be directly applied to the target tissue.

3.2.3 Cryosuper VE-1 Ophthalmic Cryosurgical Unit**

Cryosuper VE-1, also a Joule-Thomson cryosurgical unit, working on nitrous oxide or carbon dioxide was used for ophthalmic cryosurgery in the present study. The unit is depicted in plate **5**. The closed end probe of this unit could be cooled by pressing a foot switch.

3.2.4 Cryogens Used

Liquid nitrogen was used as the cryogen with Frigitronics CS-76 cryosurgical unit. Two liters of liquid nitrogen, stored in a 30 liters dewar fitted with a withdrawal device, was transferred to the Frigitronics CS-76 cryosurgical unit every time before use.

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* Cryosuper VE-4 - Vignesh Enterprises, Madras, India.

** Cryosuper VE-1 - Vignesh Enterprises, Madras, India.

PLATE-3. CRYOSUPER VE-4

PLATE-4. PROBES FOR CRYOSUPER VE-4

PLATE-5. CRYOSUPER VE -1

With the other two cryosurgical units, either carbon dioxide gas or nitrous oxide gas stored in 31 kg cylinders was used as the cryogen.

3.2.5 Thermocouple Used

Thermocouple needles supplied along with the Frigitronics CS-76 cryosurgical unit, were used for temperature measurement in the present study.

3.3 Part-A: Evaluation of Cryosurgical Units

The three cryosurgical units used in the present study were evaluated by measuring the minimum probe tip temperature and the minimum sub-cutaneous temperature they could produce.

3.3.1 Probe-tip Temperature Measurement

The thermocouple needles were kept in direct contact with the tip of the probes of the three cryosurgical units and at the tip of the spray cone of Frigitronics CS-76 and the units were activated. The minimal temperature produced in each case and the time taken for reaching the minimal temperature were recorded. The exercise was repeated 12 times to study any variations in the probe tip temperature.

3.3.2 Tissue Temperature Measurement

Frigitronics CS-76 and Cryosuper VE-4 were evaluated by measuring the minimal sub-cutaneous temperatures achieved by the use of these two units.

The thermocouple needles were introduced sub-cutaneously. The cryoprobes were then placed in direct contact with the skin over the tip of the thermocouple needles and the cryosurgical units were activated. The minimum temperature attained and the time taken for the same were noted. Six dogs under general anaesthesia were used to carry out this part of the study.

In each dog after induction of general anaesthesia, skin was frozen at different locations selected at random on either sides of the abdomen. The minimal sub-cutaneous temperature produced by the cryosurgical units was recorded. The dogs were euthanised after the study.

3.4 Part-B: Group-I: Freezing Skin and Gingiva to Different Temperatures

In 12 dogs, the skin and gums were frozen with liquid nitrogen using Frigitronics CS-76, employing a 25 mm spray cone and a 5 mm spray cone respectively.

3.4.1 Preparation of the Animals and Anaesthesia Used

The dogs were kept off feed for 12 hours. Water was withheld for 4 hours prior to anaesthesia. The dogs were premedicated with atropine sulphate* (0.04 mg/kg, s/c) and triflupromazine hydrochloride** (1 mg/kg, i/v). Anaesthesia was

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* Atropine Sulphate - Tamilnadu Dhadha Pharmaceuticals, Madras, India.

** Siquil - Sarabhai Chemicals, Baroda, India.

induced and maintained by intravenous administration of thiopentone sodium*** to effect.

3.4.2 Preparation of Site

On the lateral aspect of the abdomen, six 5 x 5 cm areas, three on either side of the abdomen, were prepared by shaving and cleaning thoroughly. The surrounding tissues were smeared with petroleum jelly and covered with thick gauze or lint cloth to prevent freezing of the surrounding tissues due to nitrogen run-off. This protective care was taken whenever liquid nitrogen was used as a spray throughout the present study.

3.4.3 Cryosurgical Technique

Using liquid nitrogen spray with 25 mm spray cone of Frigitronics CS-76, each of the six sites was rapidly frozen to -10°C, -20°C, -30°C, -40°C, -50°C and -60°C respectively. The frozen areas were allowed to thaw naturally. A second freeze-thaw cycle was repeated immediately after first thawing.

Using liquid nitrogen spray with 5 mm spray cone, 6 sites on the gum of the upper jaw on either sides were similarly frozen to the said six temperatures. A second freeze-thaw cycle was adopted immediately after first thawing.

*** Intraval Sodium - May and Baker Ltd., Bombay, India.

3.4.4 Evaluation of the Local Effects of Freezing

3.4.4.1 Gross Pathological Examination

The gross pathological changes at the sites of freezing were noticed from the day of freezing until complete healing of all the lesions.

3.4.4.2 Histopathological Examination

Skin and gingival specimens for histopathology were obtained on the 5th, 7th, 14th, 21st and 28th day after freezing. The tissue specimens were fixed in 10 percent formalin. The tissues were then processed and embedded in paraffin wax. Sections of 3-5μ thick were cut and stained with Haematoxylin-Eosin (H & E) stains and mounted. The sections were then examined under microscope to study the changes produced in the tissues by cryosurgery.

3.5 Group-II: Freezing Femoral Artery and Cephalic Vein

This was carried out on 12 dogs.

3.5.1 Preparation of the Animals and Anaesthesia

The 12 dogs of this group were prepared and anaesthetised as described in Group I.

3.5.2 Freezing Femoral Artery

The operative site on the medial aspect of the left thigh was prepared by clipping, shaving and thorough scrubbing of the area with soap and water.

3.5.2.1 Operative Procedure

The dogs were placed in left lateral recumbency, and the right hind limb was secured away from the operative site. The operative site was adequately draped. A 6 cm long skin incision directly over the region of the left femoral triangle on the medial aspect of the thigh was made. Blunt dissection of fascia was carried out, exposing the femoral vessels. The femoral artery was separated from the surrounding tissues by blunt dissection, and isolated by passing the blades of curved scissors under the artery. The surrounding tissues and underlying femoral vein and nerve were covered with gauze to protect them from freezing. The staved off portion of the femoral artery was then frozen for one minute by liquid nitrogen spray and allowed to thaw naturally. The freeze-thaw cycle was repeated for a second time. The thawing time was recorded and the protective gauze pad was removed from under the femoral artery. The artery was replaced and the skin wound was closed by using 3/0 black braided silk in a simple interrupted pattern. Post-operative care comprised of daily cleaning of the skin wound with povidone iodine.

3.5.3 Evaluation of the Effects of Freezing Femoral Artery

The effects of freezing the femoral artery were studied by noting the clinical changes produced at the site of freezing and by performing aortography by catheterisation of the contralateral femoral artery before euthanising the dogs.

Six dogs of this group were sacrificed on the 7th post-operative day and the rest on the 14th post-operative day. Post-mortem was conducted and the gross and histopathological changes were noted.

3.5.3.1 Aortography

The dogs were anaesthetised as described earlier and positioned in right lateral recumbency. The left hind limb was drawn away and secured. The right femoral artery was exposed through an incision at the femoral triangle under aseptic conditions. Two bull dog clamps were applied to the femoral artery. Between the two bull dog clamps, a small incision sufficient to enable insertion of the catheter was made on the artery. A paediatric angiographic catheter was inserted into the lumen of the femoral artery and pushed proximally towards the aorta after removing the proximal bull dog clamp. The catheter was pushed until the tip was lying just proximal to the aortic quadrification. Survey radiographs were obtained to ascertain the position of the tip of the catheter. The catheter was then anchored to the femoral artery by means of a ligature. The catheter was repeatedly flushed with heparinised saline. The dog was then positioned in such a way that the left femoral artery, at the level of the femur was centered for radiography. Once the dog was positioned properly, a syringe filled with 10 ml of sodium iothalamate* was attached to the free end of the catheter. The contrast media was injected rapidly. Ventro-dorsal radiograph was taken as the intra-aortic injection of the contrast media was half way through. After completion of aortography, the catheter was withdrawn and the femoral artery was ligated. The skin wound was closed.

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* Conray - 420 - May and Baker Ltd., Bombay, India.

3.5.3.2 Gross Pathological Examination

Following aortography procedure, the dogs were euthanised. The medial aspect of the left thigh was dissected to expose the femoral vessels and their surroundings. Gross pathological changes produced due to freezing of the femoral artery were noted.

3.5.3.3 Histopathological Changes

The segment of the femoral artery that was subjected to cryosurgery by double freeze-thaw cycle was severed and fixed in 10 percent formalin. The tissues were then processed and embedded in paraffin wax. Sections of 3-5μ thick were cut and stained by Haematoxylin-Eosin (H & E) stains. The sections were then examined under microscope to note the changes produced in the vessels due to cryosurgery.

3.5.4 Freezing Cephalic Vein

This part of the experiment was conducted on the same 12 dogs where the femoral artery was frozen.

3.5.4.1 Operative Procedure

The segment of the cephalic vein lying about one to two inches distal to the elbow joint was surgically exposed and the vein was isolated by blunt dissection from the surrounding tissue. The tissue surrounding the vein was protected as described for femoral artery. The exposed portion of the cephalic vein was frozen for one minute by direct spray of liquid nitrogen. The vein was allowed to thaw naturally and a second freeze-thaw cycle was repeated. The skin incision was closed in a routine manner after replacing the cephalic vein.

3.5.5 Evaluation of the Effects of Freezing Cephalic Vein

The effects of freezing cephalic vein were studied by noting the clinical changes produced at the site of the freezing and by performing venography of the cephalic vein, on the day of sacrificing the dogs.

3.5.5.1 Venography

Venepuncture of the cephalic vein was performed by using a 20g needle distal to the site of freezing. Lateral radiographs were obtained as sodium iothalamate was being injected intravenously.

3.5.5.2 Gross Pathological Examination

Following euthanasia of the dogs, gross pathological changes at the site of freezing were noted.

3.5.5.3 Histopathological Examination

Tissues collected from the site of freezing were subjected to histopathological examination as described earlier.

3.6 Group-III: Systemic Effects of Freezing

In an attempt to study the systemic changes induced by freezing, 2.5 percent and 5 percent of the body surface area was frozen using liquid nitrogen spray in 6 dogs each.

3.6.1 Preparation of the Animals and Anaesthesia

The dogs were anaesthetised as described earlier. The skin on the left lateral abdominal wall was shaved, washed thoroughly with soap and water and dried.

3.6.2 Cryosurgical Procedure

The body weights of the 12 dogs used in this part of the study were recorded. The body surface areas of these dogs were derived from the body weight-body surface area chart presented by Nelson and Couto (1992). In six dogs, 2.5 percent of the body surface area was calculated and marked on the lateral aspect of the abdomen. Similarly, in the other six dogs, 5 percent of the body surface area was calculated and marked off on the lateral abdomen. Using a 25 mm spray cone, the entire 2.5 percent and 5 percent of the body surface area was frozen to a sub-cutaneous temperature of -50°C measured randomly. The skin was allowed to thaw naturally and refrozen. The depth of freezing was assessed by randomly measuring the sub-cutaneous temperature by thermocouple needles at 6 places within the area frozen. In addition, if-the skin adhered to deeper tissues due to ice ball formation, freezing of this area was discontinued and the surrounding areas within the marked off region were frozen.

No post-operative care of any kind was taken and the dogs were left in the kennel without dressing or administration of antibiotics.

The systemic effects of freezing large areas of the skin were assessed by studying the following parameters:

3.6.3 Gross Pathological Examination

The changes produced at the site of freezing were noted.

3.6.4 Haematological Examination

Venous blood was collected in vials containing sodium citrate as anti-coagulant. Blood samples were collected before cryosurgery and on the 1st, 3rd, 5th, 7th, 14th, 21st and 28th day after cryosurgery. Total red blood cell count (RBC), total leucocyte count (TLC), packed cell volume (PCV) and haemoglobin content were estimated. Blood smears were also made from a drop of blood collected by puncturing the tip of the ear pinna at the above mentioned intervals. Differential leucocyte counts were made from the stained smears. The procedures described by Coles (1982) were used to perform the haematological examination.

3.6.5 Serum Biochemical Examination

Serum samples in these 12 dogs were collected before cryosurgery and on the 1st, 3rd, 5th, 7th, 14th, 21st and 28th day after cryosurgery. The following parameters were assessed :

3.6.5.1 Aspartate Amino Transferase (AST or SGOT)

Aspartate amino transferase (AST or SGOT) was estimated from the serum samples by kinetic method using Ciba-Corning Express plus* auto analyser.

3.6.5.2 Total Serum Protein (TSP)

Total serum protein was estimated by adopting end point method using Ciba-Corning Express plus auto analyser.

3.6.5.3 Serum Potassium

Serum potassium levels were estimated by ion selective electrode method with reference electrode using Ciba-Corning 664 Fast-4* auto analyser.

3.6.6 Statistical Analysis

The data thus obtained were subjected to statistical analysis using analysis of variance techniques by adopting factorial experiment under completely random design to study the effects of cryosurgery on the parameters studied.

3.7 Group-IV: Freezing Cornea and Iris During Extracapsular Cryoextraction of Lens

In this group, extracapsular cryoextraction of lens was done in 6 dogs. In 6 dogs, following cryoextraction of lens, the cornea was frozen in a double freeze-thaw

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* Ciba - Corning Diagnostics Corporation, Medfield, M.A. U.S.A.,

cycle. Similarly, iris was frozen in another 6 dogs to study the effects of accidental freezing of these structures during cryoextraction of lens.

3.7.1 Preparation of Animals and Anaesthesia

The animals were prepared and anaesthetised as described in groups I, II and III. In addition, 1 percent atropine sulphate eye drops* and gentamicin eye drops** were instilled on 3-4 occasions one day prior to surgery. The eye lashes were clipped and the periocular area was prepared by scrubbing, taking care not to allow the solution to come into contact with the eye ball.

3.7.2 Operative Procedures

3.7.2.1 Extracapsular Cryoextraction of Lens

The animal was secured in lateral recumbency with sponge pads under the head to place the eye in an easily accessible position.

Lateral canthotomy was performed and a Castroveijo eye lid speculum was used to keep the globe exposed. The globe was held steady by applying fixation forceps close to the limbus at 12 O' clock position. A stab incision was made at the 12 O' clock position on the limbus, entering the anterior chamber. The incision was extended on either side to about 160 degrees with corneal scissors (**Plate 6**). During this process extreme care was taken to prevent damage of the iris. Over-manipulation

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* Atropine Sulphate 1% - Ophthalmics and Drugs India, Bombay, India.

** Bactigen eye drops - FDC Limited, Bombay, India.

of cornea was avoided to prevent corneal oedema. The anterior capsule of the lens was then exposed. The tip of the cataract probe of the Cryosuper VE-1 cryosurgical unit was placed in direct contact with the anterior capsule of the lens and the unit was activated by pressing the foot switch. This led to cryoadhesion of the probe to the lens capsule. The probe was then slightly raised and rotated till the anterior capsule peeled off (**Plate 7**). The capsule of the lens was wiped off from the probe and the probe was reintroduced and this time, cryoadhesion of the probe was achieved with the lens substance and the lens was removed (**Plate 8**) without disturbing the posterior capsule.

The anterior chamber was then irrigated with sterile normal saline solution. The limbal incision was closed by simple interrupted sutures of 6/0 silk (**Plate 9**). The canthotomy incision was closed in a routine manner.

The six dogs where extracapsular cryoextraction of lens was done served as controls.

3.7.2.2 Freezing of Cornea after Cryoextraction of Lens

Following cryoextraction of lens in 6 dogs as described above, the cryoprobe was brought into contact with the endothelial surface of the cornea and activated. The cornea was frozen for one minute and allowed to thaw. The freeze-thaw cycle was repeated again after the first thawing. The corneal incision was closed as described.

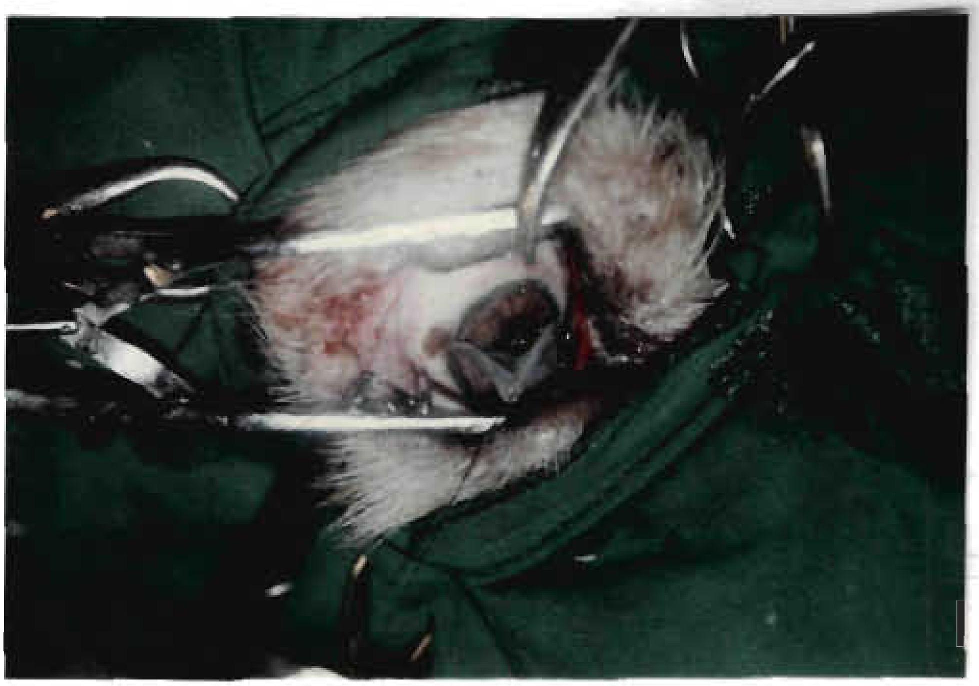

PLATE-6. LIMBAL INCISION

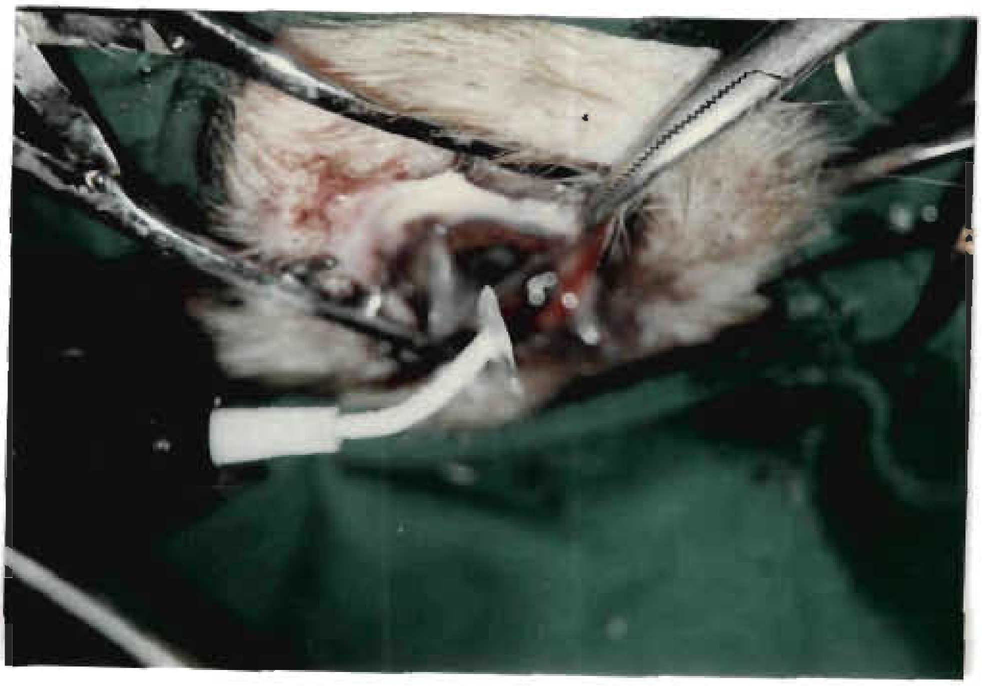

PLATE-7. CRYOEXTRACTION OF ANTERIOR CAPSULE

PLATE-8. CRYOEXTRACTION OF LENS

PLATE-9. LIMBAL INCISION SUTURED.

3.7.2.3 Freezing of Iris after Cryoextraction of Lens

Following cryoextraction of lens in another 6 dogs as described, the iris was subjected to a double freeze-thaw cycle. Each time the iris was frozen for one minute duration. The corneal incision was closed as described.

3.7.3 Post-operative Care

Ampicillin injection* at the dose of 250 mg was intramuscularly administered twice a day for five post-operative days. Gentamicin eye drops were instilled 4 times a day for five post-operative days.

3.7.4 Assessment of the Changes due to Ocular Cryosurgery

The changes produced due to these operative procedures were analysed by studying the following parameters :

3.7.4.1 Clinical Observations

The changes produced by the said operative procedures were recorded for 7 post-operative days.

3.7.4.2 Tonometry

The intraocular tension was recorded before and on the 7th and 14th day of the operation by using a Schiotz's tonometer.

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* Dynacil-vet - Hindustan Antibiotics Ltd., Pune, India.

3.7.4.3 Fluorescein Dye Test

A drop of 2 percent fluorescein sodium* solution was instilled in the operated eye and the dye was immediately washed off with sterile normal saline solution. The eye was then examined under Wood's lamp to note any abrasions or ulcers on the cornea.

3.7.4.4 Gross Pathological Changes

Three dogs each where the cornea and iris were frozen following cryoextraction of lens were sacrificed on the 7th and the 14th post-operative days respectively. The gross pathological changes in the operated eyes were noted.

3.7.4.5 Histopathological Changes

The portions of cornea and iris subjected to double freeze-thaw cycles were dissected out along with a small portion of the surrounding normal tissue on either sides. The tissues were fixed in 10 percent buffered formalin and processed for histopathological examination as described earlier.

3.8. Part-C: Clinical Study

The details of the 50 cases treated by cryosurgery were as follows :

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* Fluorescein Sodium 2% - Ophthalmics and Drugs India, Bombay, India.

Kind of Lesion	No. of dogs treated
Transmissible venereal tumour	18
Fibrosarcoma	5
Hypertrophic otitis externa	5
Papilloma	2
Perianal fistulae	3
Perianal adenoma	3
Lick granuloma	5
Sebaceous adenoma	2
Adamantinoma	1
Fibroma	3
Cataract	3

The clinical outcome of cryosurgery of these cases was recorded.

Results

CHAPTER IV

RESULTS

4.1 Part-A: Evaluation of Cryosurgical Units

4.1.1 Frigitronics CS-76 Cryosurgical Unit

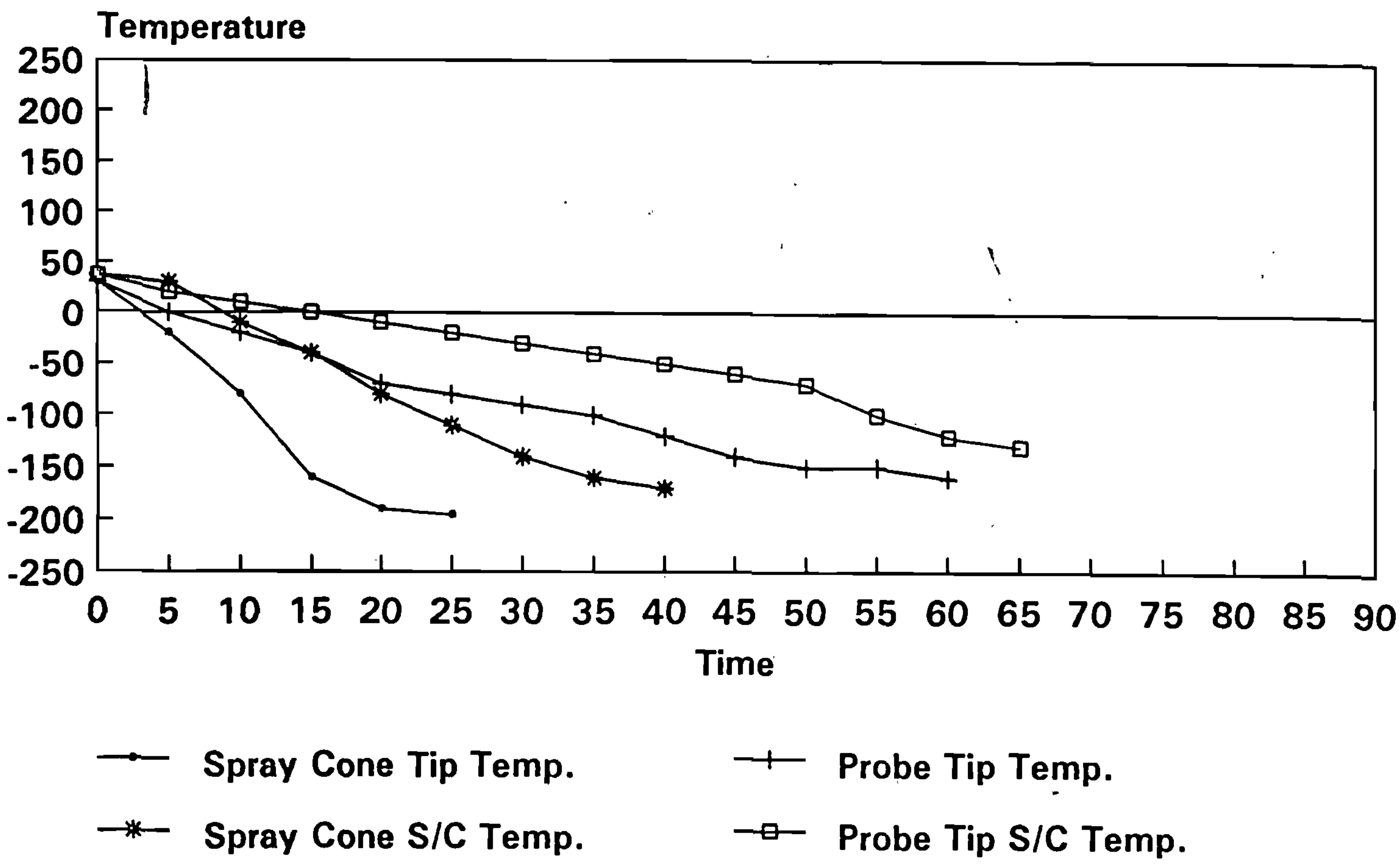
With the use of this cryosurgical unit, when liquid nitrogen was directly sprayed over the thermocouple needle, a temperature of -195°C could be achieved within 25 seconds.

When the closed end probe of the Frigitronics CS-76 cryosurgical unit was kept in direct contact with the thermocouple, a minimum probe tip temperature of - 160°C was obtained in 60 seconds.

It was observed that direct spray of liquid nitrogen produced a minimal sub-cutaneous temperature of -170°C in 40 seconds. On the other hand, the use of closed end liquid nitrogen probe resulted in lowering of the sub-cutaneous temperature to -130°C. The time taken for this reduction in temperature was noticed to be 65 seconds. The temperature changes are depicted in figure **1**.

The Frigitronics CS-76 cryosurgical unit did not produce any untoward effect even when used continuously for 5 minutes. However, within 3 minutes of continuous usage, the cryogun (or the handle) became very cold and had to be held with a piece *of cloth to prevent cooling of the operator's hand.*

Figure - 1
CRYOGENIC TEMPERATURES PRODUCED
BY FRIGITRONICS CS - 76



4.1.2 Cryosuper VE-4 Cryosurgical Unit

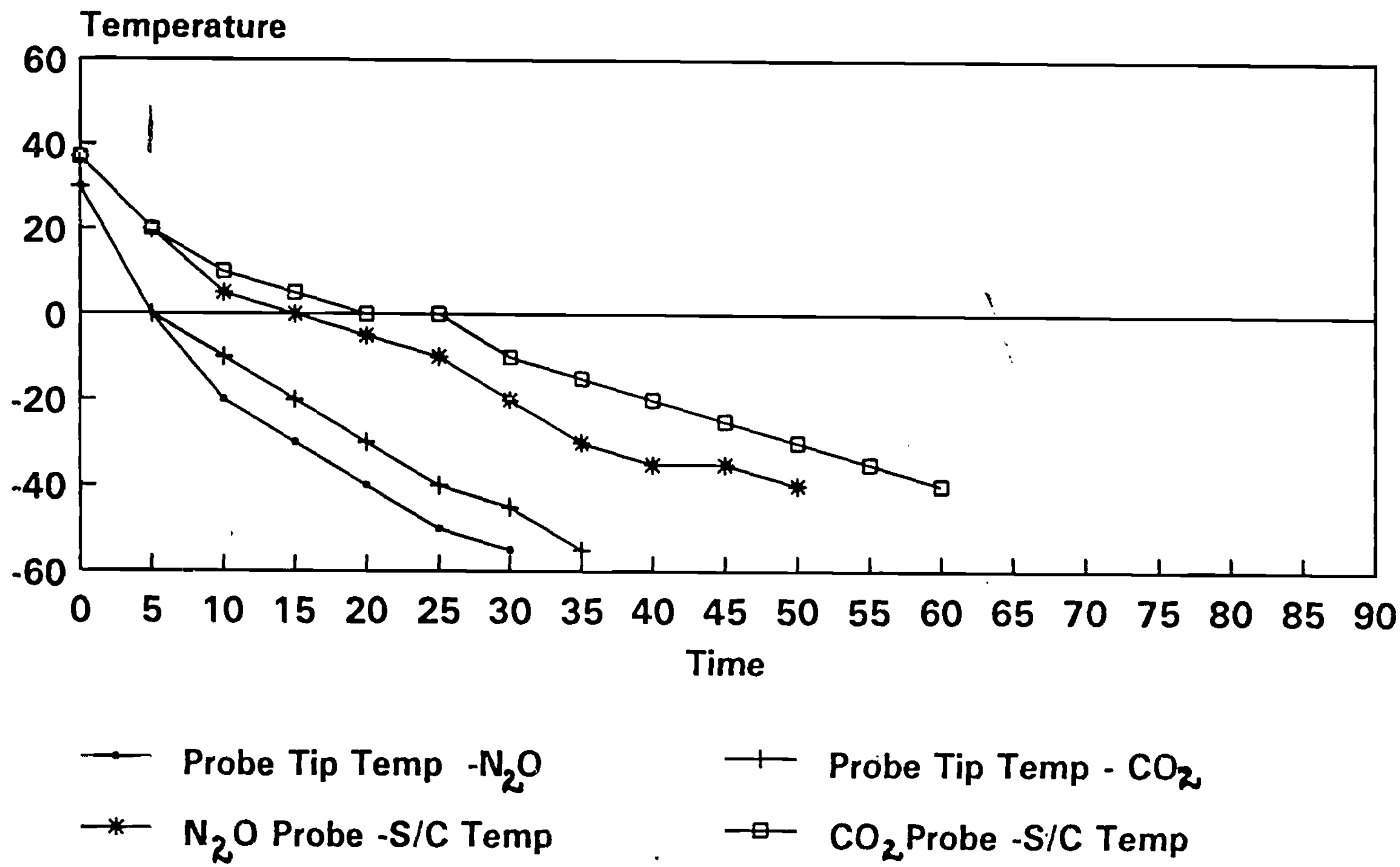
With this cryosurgical unit, when nitrous oxide gas was used as the cryogen, the minimal probe tip temperature that could be achieved was -55°C. The time taken for the same was observed to be 30 seconds.

The use of carbon dioxide gas as the cryogen in the Cryosuper VE-4 cryosurgical unit produced minimal probe tip temperature of -55°C within 35 seconds.

The Cryosuper VE-4 cryosurgical unit was found to have produced a minimum sub-cutaneous temperature of -40°C within 50 seconds when nitrous oxide was used as the cryogen. With the use of carbon dioxide as the refrigerant, the cryosuper VE-4 produced a minimum temperature of -40°C in 60 seconds at the sub-cutaneous level, as indicated by the sub-cutaneously placed thermocouple needle. The changes in the probe tip and sub-cutaneous temperature are represented in figure **2**.

It was observed every time with the use of the Cryosuper VE-4 cryosurgical unit that ice began to form at the control knob of the cryogun after two minutes of continuous operation, thereby jamming all the movements of the metallic parts of the cryogun. During the next 30 seconds, ice also began to form at the exhaust outlet and the cryosurgical unit stopped functioning. The unit, however, started functioning after a lapse of at least 10 minutes during which time, the ice formed at the consoles melted off.

Figure - 2
CRYOGENIC TEMPERATURES PRODUCED
BY CRYOSUPER VE - 4



4.1.3 Cryosuper VE-1 Cryosurgical Unit

The Cryosuper VE-1 cryosurgical unit produced a minimal probe tip temperature of -80°C in 40 seconds when nitrous oxide was used and a minimal probe tip temperature of -70°C in 50 seconds when carbon dioxide was used as the refrigerant (**Figure 3**).

Continuous use of the cryosuper VE-1 cryosurgical unit did not produce any untoward effect in the functioning of the cryosurgical unit.

4.2 Part-B: Group-I: Local Effects of Freezing

The thawing time for all the lesions was found to be ranging from 3-5 minutes

4.2.1 Skin Freezing - Gross Pathological Observations

Irrespective of the degree to which the skin was frozen, the events that ensued were common to all the sites of skin subjected to double freeze-thaw cycle to a temperature of -10,-20,-30,-40, -50 or -60°C. Within about 1-2 hours after freezing, there was considerable oedema of the frozen sites but it was limited to the circular sites of freezing. By six hours all the sites frozen to the said temperatures turned red in colour and this congestion of the site increased in intensity by the first day of freezing. By about the 3rd day, the congested areas of skin turned gradually brownish and by the 5th day of cryosurgery turned black in colour. Sloughing of these lesions started by the 6th day and was found to be complete at all the sites in all the dogs by the 8th day (**Plate 10**).

Figure - 3
CRYOGENIC TEMPERATURES PRODUCED
BY CRYOSUPER VE - 1

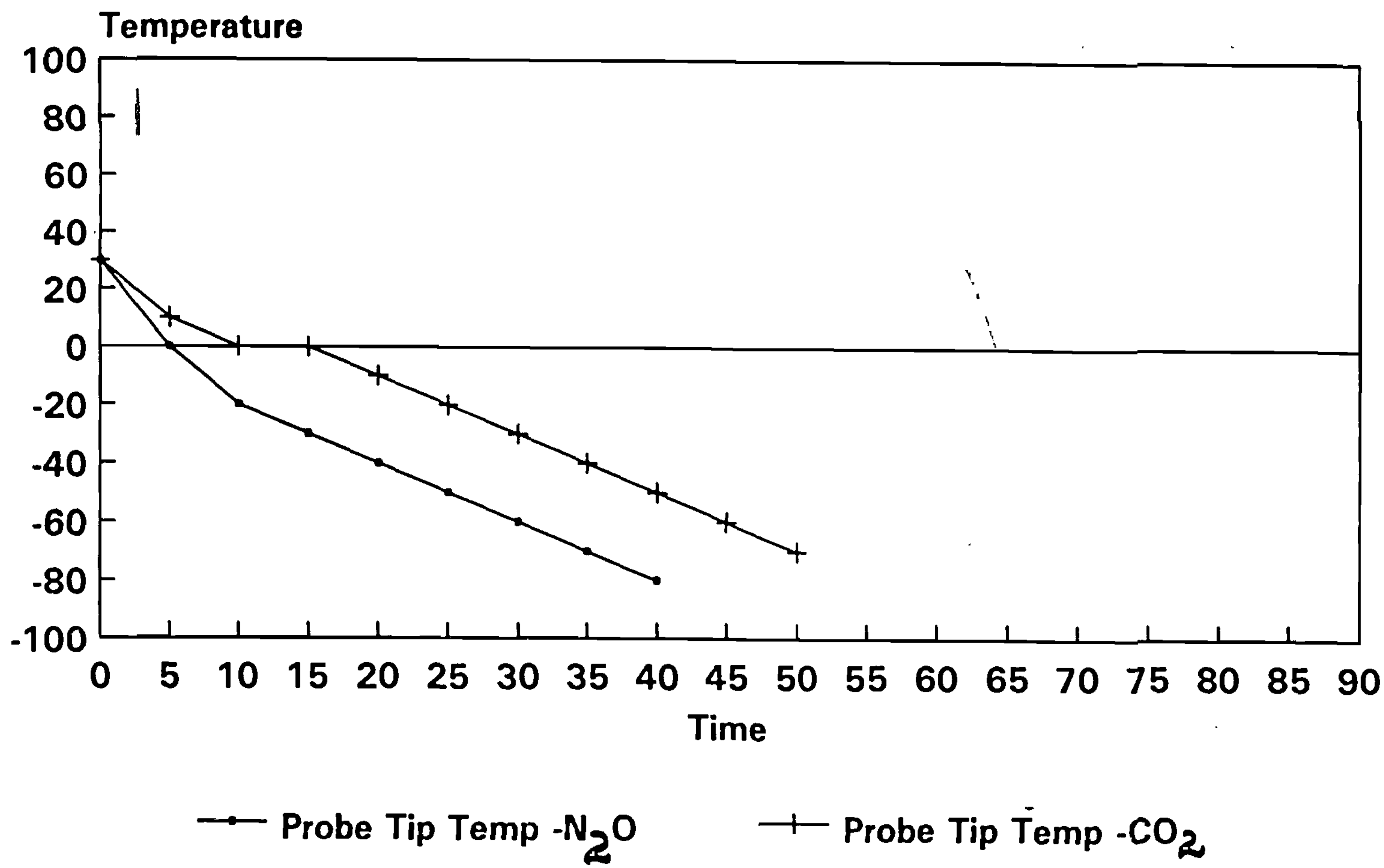


PLATE-10. SLOUGHING OF CRYOLESIONS - VARIOUS STAGES

Following sloughing of the necrosed skin, the cryolesions appeared like fresh granulation tissue. However, some amount of serosanguinous discharge was noticed at each of the lesions in all the dogs. The dogs exhibited a tendency to lick off the exudates and within 2-4 days, the lesions became dry and exhibited a tendency to heal.

By the second week, the lesions exhibited a tendency to heal with normal appearance at the periphery of the lesions.

By the end of the 3rd week, healing was noticed to be progressing adequately and over half of all the lesions, from towards the periphery were covered by normally appearing skin. The sites frozen to -10°C and -20°C healed by this time.

At the end of the 4th week, all the cryolesions frozen to different temperatures were completely healed. All the healed cryolesions appeared lighter in colour when compared to the surrounding skin. The sequence of events are depicted in Plates **11-16**.

On naked eye examination of the cryolesions, no difference could be observed in the lesions produced by subjecting the skin to different temperatures. However, sites frozen to -10°C and -20°C healed a week earlier.

During the entire course of the observation of the lesions produced by subjecting the skin to different temperatures, the cryolesions were found to be sharply demarcated from the surrounding tissues. No oedema or inflammatory reaction could be noticed in the tissue contiguous with the cryolesion.

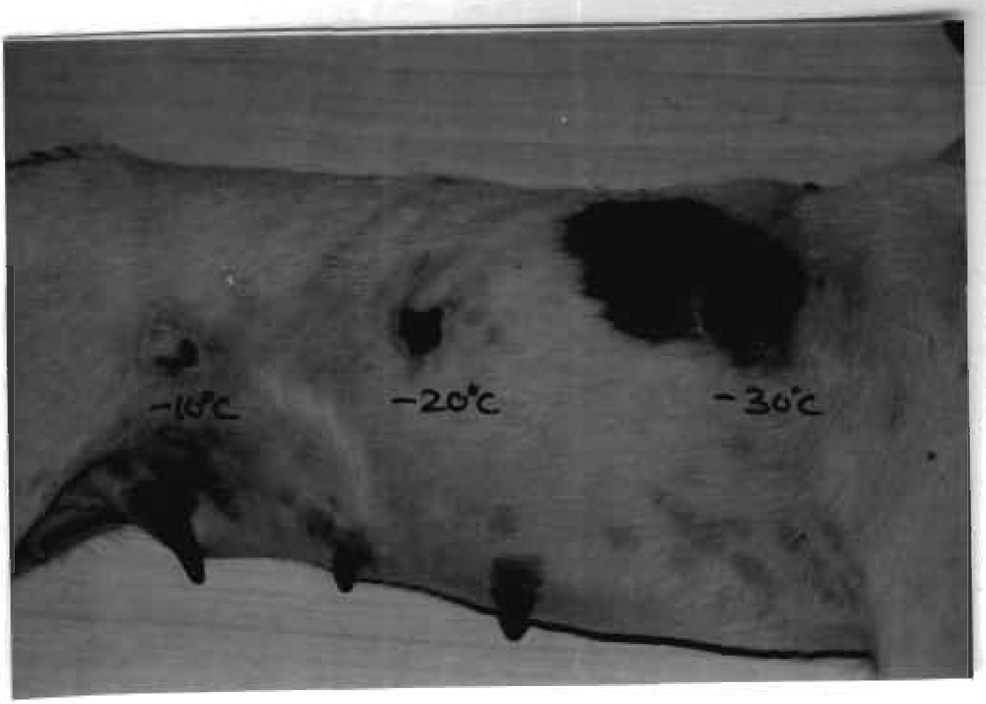


PLATE-11. SKIN CRYOLESIONS - 7th POST-OPERATIVE DAY

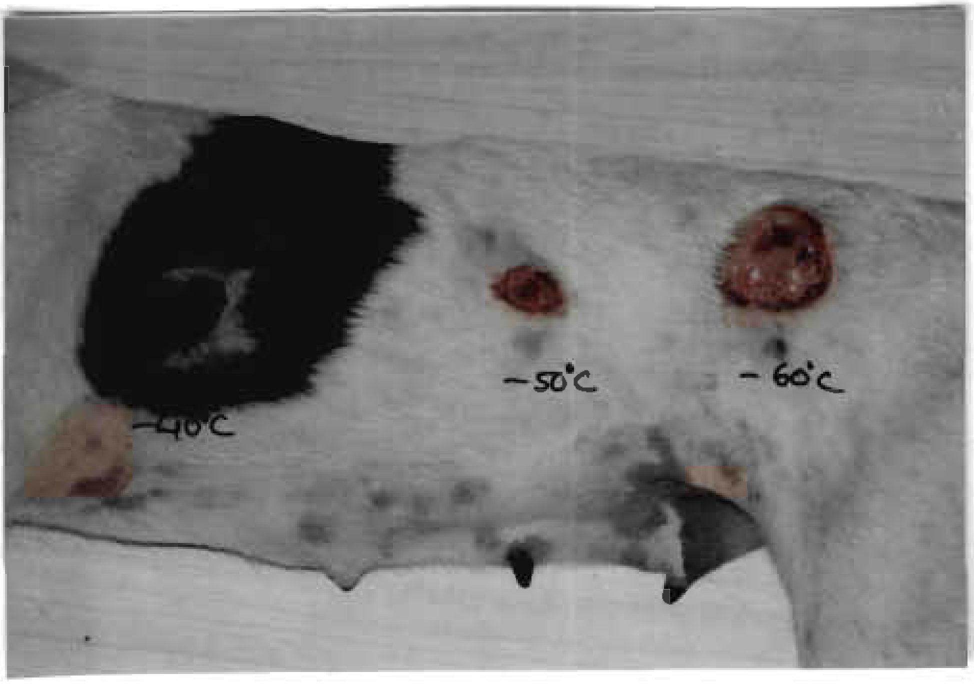


PLATE-12. SKIN CRYOLESIONS-7th POST-OPERATIVE DAY

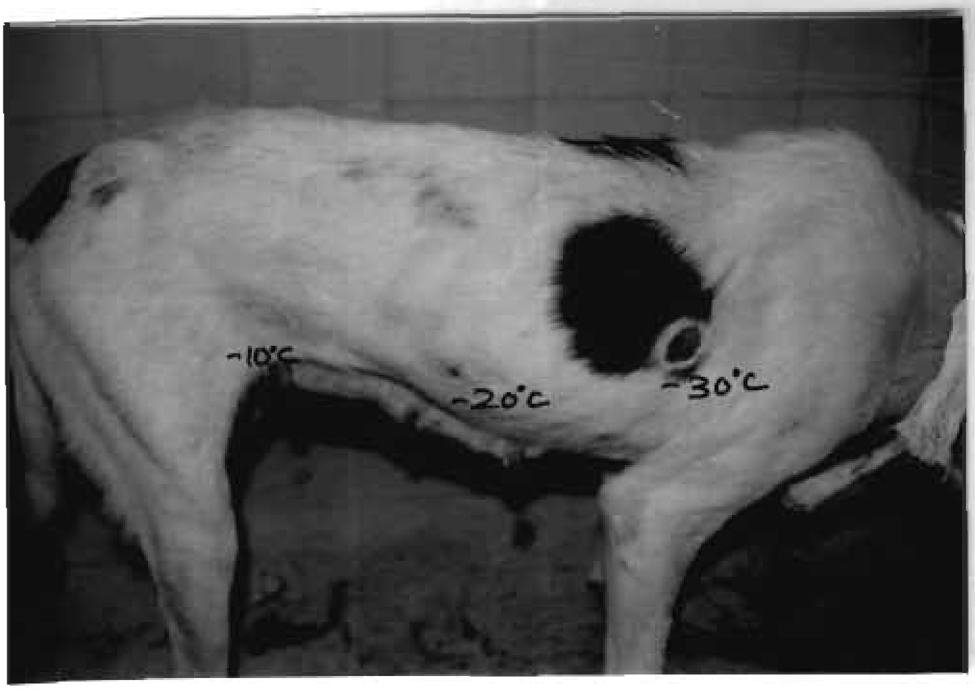


PLATE-13. SKIN CRYOLESIONS - 21st POST-OPERATIVE DAY

PLATE-14. SKIN CRYOLESIONS - 21st POST-OPERATIVE DAY

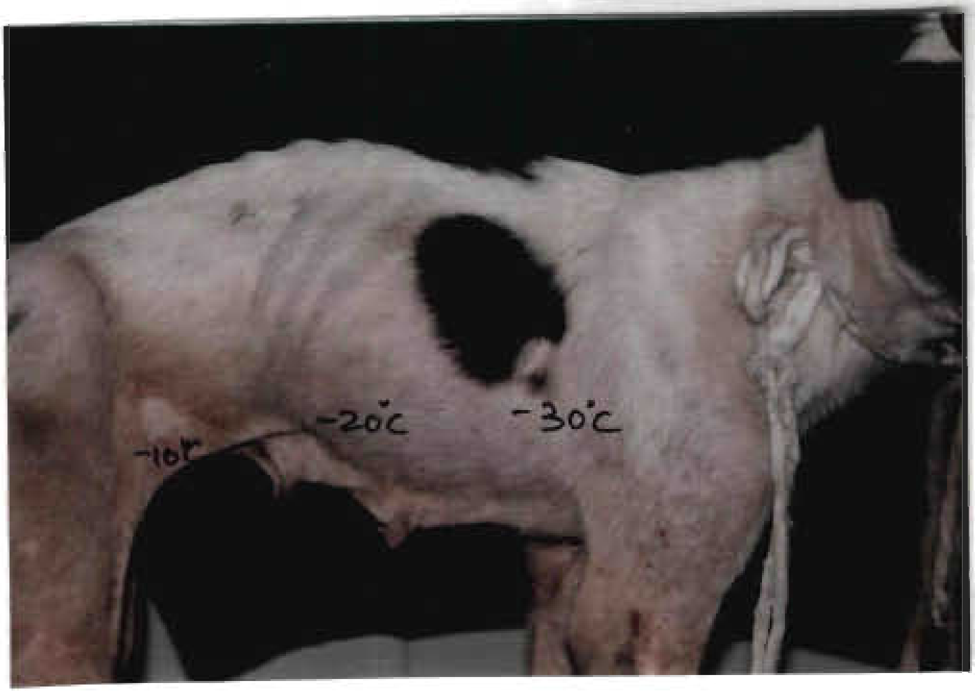


PLATE-15. SKIN CRYOLESIONS - 28th POST-OPERATIVE DAY

PLATE-16. SKIN CRYOLESIONS - 28th POST-OPERATIVE DAY

4.2.2 Histopathological Observations

4.2.2.1 Skin Frozen to -10°C

By the 5th day of cryosurgery, the epidermis appeared to be necrotic and pale staining in many areas. The dermis also had several necrotic areas, interspersed with islands of live tissue, where the cellular and nuclear details were still visible. The dermal collagen was oedematous and appeared to be fragmented near the epidermis. The necrotic changes were more pronounced towards the dermal tract, wherein the fragmentation was more severe. Mild lymphocytic infiltration was noticed.

The biopsy specimens obtained on the 7th day following sloughing of the cryolesions were characterised by the absence of epidermis. The blood vessels appeared congested and haemorrhage was present with cellular infiltration. A few glands and hair follicles were noticed. Uniformly spread fibroblasts were seen along with numerous budding blood vessels. In some areas, plenty of collagen was noticed.

Histopathological sections of the specimens obtained on the 14th day resembled granulation tissue, showing numerous fibroblasts and cellular infiltration. Several newly formed blood vessels were also seen.

By the 21st day the epidermis was fully formed. Dermis was found to have been made up of fibrous tissue. Cellular infiltration still persisted in some areas. The epidermis was found to have regenerated and was multilayered with a thick layer of keratin.

By the 28th day, multilayered epidermis with a thick layer of keratin was noticed. In the dermal region, a few glands with focal lymphocytic infiltration in some places was observed.

4.2.2.2 Skin Frozen to -20°C

By the 5th day, epidermis was found to be necrosed and stained uniformly pink. Dermis was necrosed at places and showed mild oedema (**Plate 17**). Cellular infiltration was found to be severe. Hair follicles in the deeper layers showed degenerative changes.

By the 7th day, the lesion resembled an ulcer. The epidermis was absent. The blood vessels were congested. Numerous fibroblasts and several budding vessels were discernible.

The 14th day sections showed numerous fibroblasts and cellular infiltration.

By the 21st day, the epidermis was almost fully formed. In some areas, haemorrhage and cellular infiltration still persisted.

By the 28th day, the epidermis was fully formed and mild hyperkeratosis was evident. A few glands and hair follicles were noticed (**Plate 18**).

4.2.2.3 Skin Frozen to -30°C to -60°C

On the 5th day, all these sections frozen to -30°C and below to -60°C, showed complete necrosis of the epidermis throughout the sections. The dermis and deeper

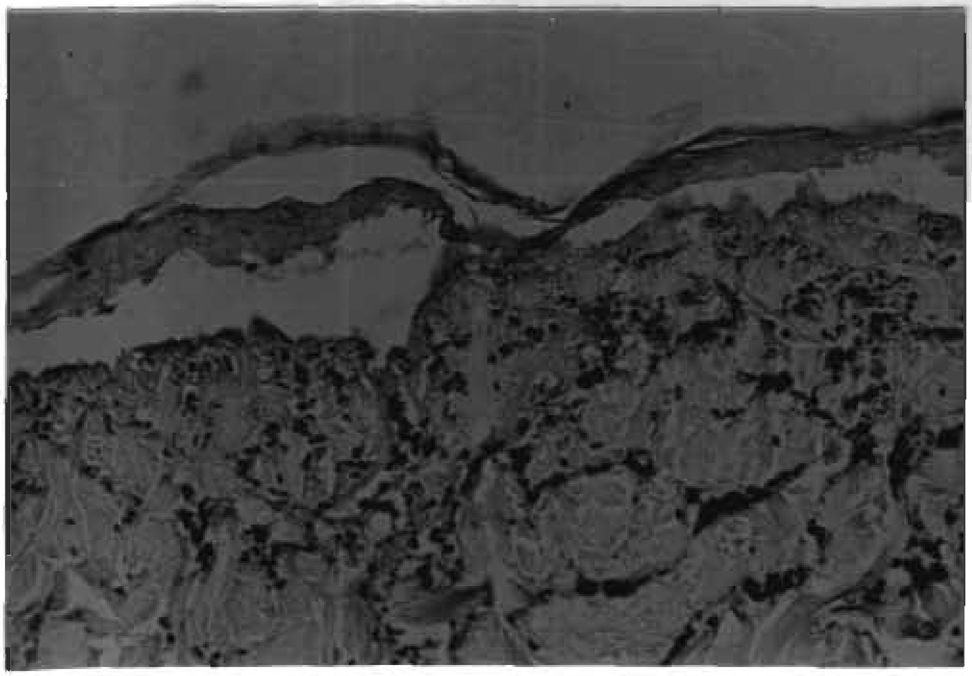


PLATE-17. MICROPHOTOGRAPH SHOWING PARTIAL NECROSIS OF SKIN AT -20°C. 5th POST-OPERATIVE DAY. *H&E X320*

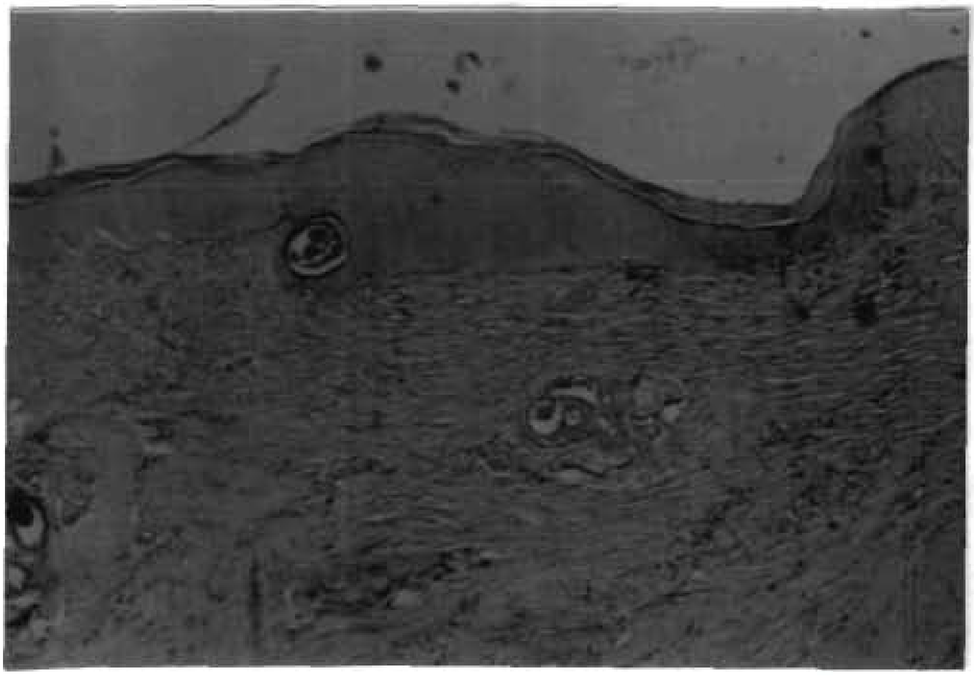


PLATE-18. MICROPHOTOGRAPH SHOWING COMPLETE HEALING OF SKIN AT - 20°C. 28th POST-OPERATIVE DAY. H&E X80 layers also appeared completely necrosed and the cellular details were not discernible (**Plate 19**). Dermal collagen stained pale and appeared fragmented. The sections were completely devoid of all cellular details. The blood vessels were thrombosed. There was slight oedema in the necrosed dermal region. The cellular details in the dermal glands and hair follicles were completely lost and the tissues appeared homogenous. In all the sections, deep dermis showed extensive cellular infiltration.

By the 7th day, following sloughing of the necrosed skin, the lesion depicted congested blood vessels, haemorrhage along with cellular infiltration. Uniformly spread fibroblasts along with numerous budding blood vessels was noticed.

By the 14th day, the lesions resembled granulation tissue with numerous fibroblasts and cellular infiltration. The epidermis had not yet regenerated.

By the 21st day, epidermis of 2-3 layers thick was formed in some places. The dermis was made up of fibrous tissue without any evidence of glands and hair follicles.

By the 28th day, the epidermis had completely regenerated and was multilayered with mild hyperkeratosis noticed all over its surface. There was no evidence of glands and hair follicles (**Plate 20**). In some sections, focal areas of mild lymphocytic infiltration was still discernible in the deeper areas.

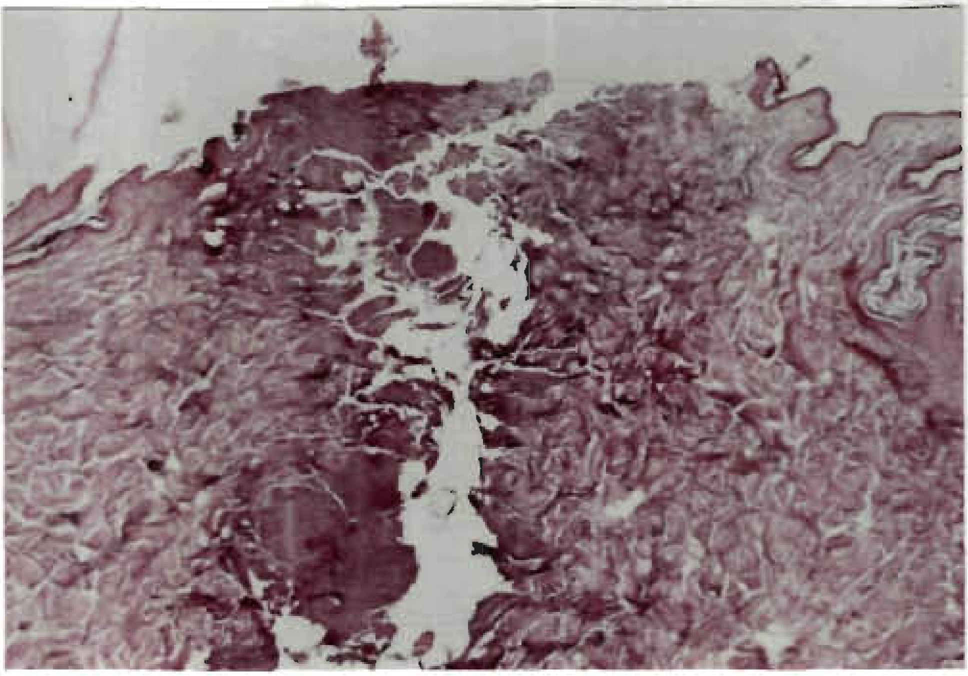


PLATE-19. MICROPHOTOGRAPH SHOWING COMPLETE NECROSIS OF SKIN AT -30°C. 5th POST-OPERATIVE DAY. H&E X80

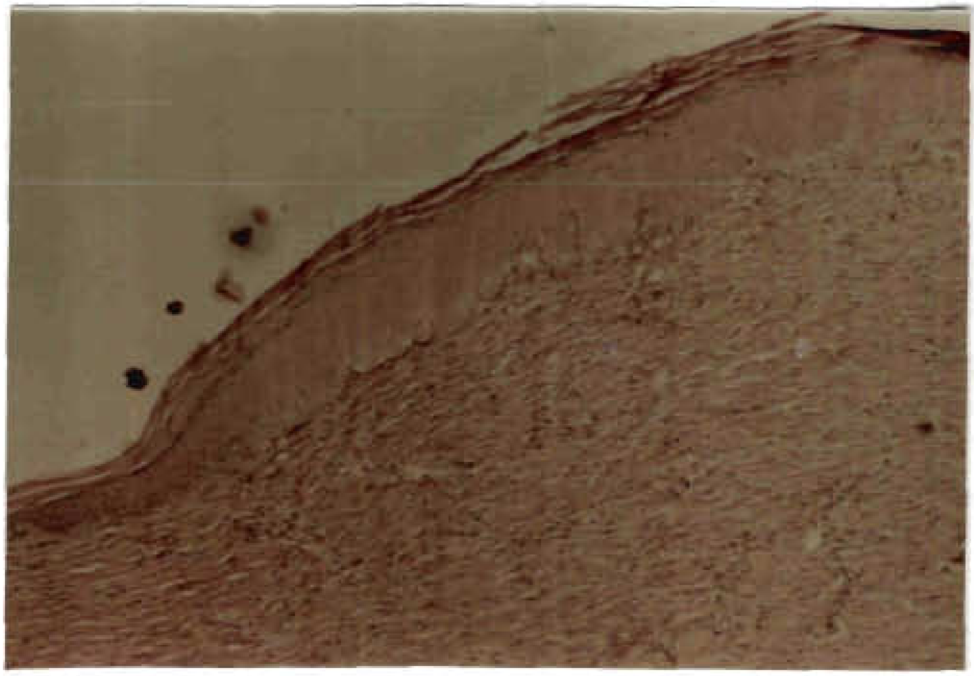


PLATE-20. MICROPHOTOGRAPH SHOWING COMPLETE HEALING OF SKIN AT -30°C. 28th POST-OPERATIVE DAY. H&E X100

4.2.3 Freezing of Gingiva

4.2.3.1 Gross Pathological Observations

When the gums were frozen to the 6 different temperatures of -10°C, -20°C, -30°C, - 40°C, -50°C and -60°C, there was severe congestion of the frozen sites within two hours of freezing. Severe oedema of the gums and the entire upper lips and cheek was noticed; so much so that the entire mouth appeared swollen. There was complete necrosis of all the frozen sites by the 5th day and the necrosed portions appeared pale in colour. Sloughing was complete by the 7th day at all the 6 sites of freezing. The complete thickness of the gingiva had sloughed, exposing the maxillary bone, irrespective of the freezing temperature (**Plate 21**). However, the bone appeared normal and did not depict any changes. The exposed portion of the bone was partially covered by scar tissue by the 14th day and by the 21st post-operative day, healing appeared complete and the frozen sites blended well with the surrounding tissues.

4.2.3.2 Histopathological Observations

Irrespective of the temperature to which the gingiva was frozen, the sections obtained on the 5th day of freezing stained uniformly pale and were devoid of all cellular or architectural details (**Plate 22**).

Since the bone was exposed and the entire thickness of the gingiva had sloughed off by the 7th day, no tissue for histopathological examination could be collected. The presence of a very thin layer of scar tissue on the 14th post-operative day also prevented the collection of samples for histopathological examination.

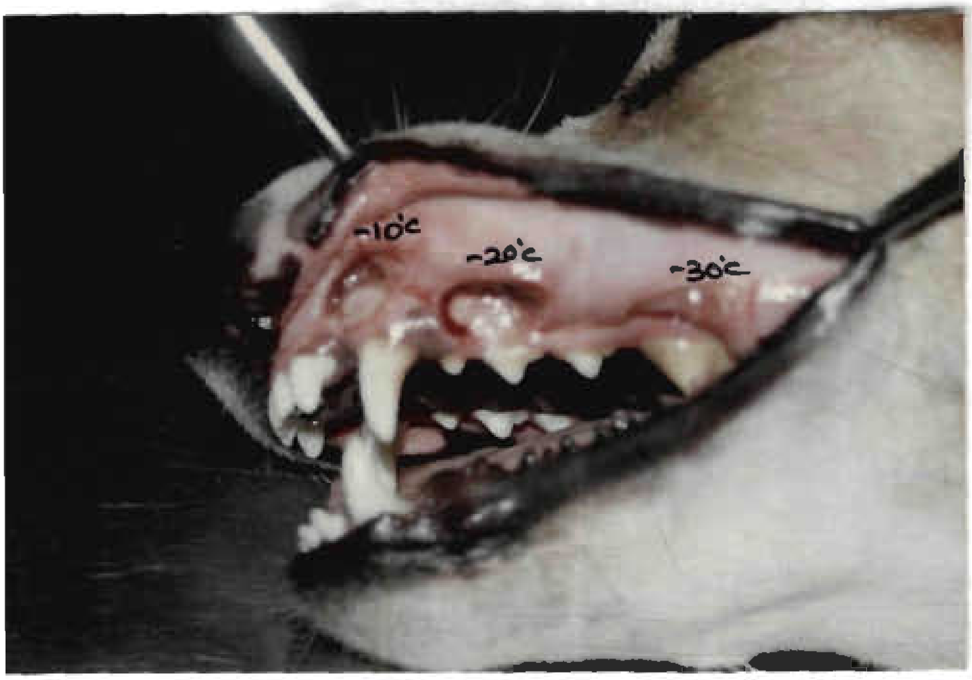


PLATE-21. GINGIVAL CRYOLESIONS EXPOSING MAXILLA.
5th POST-OPERATIVE DAY

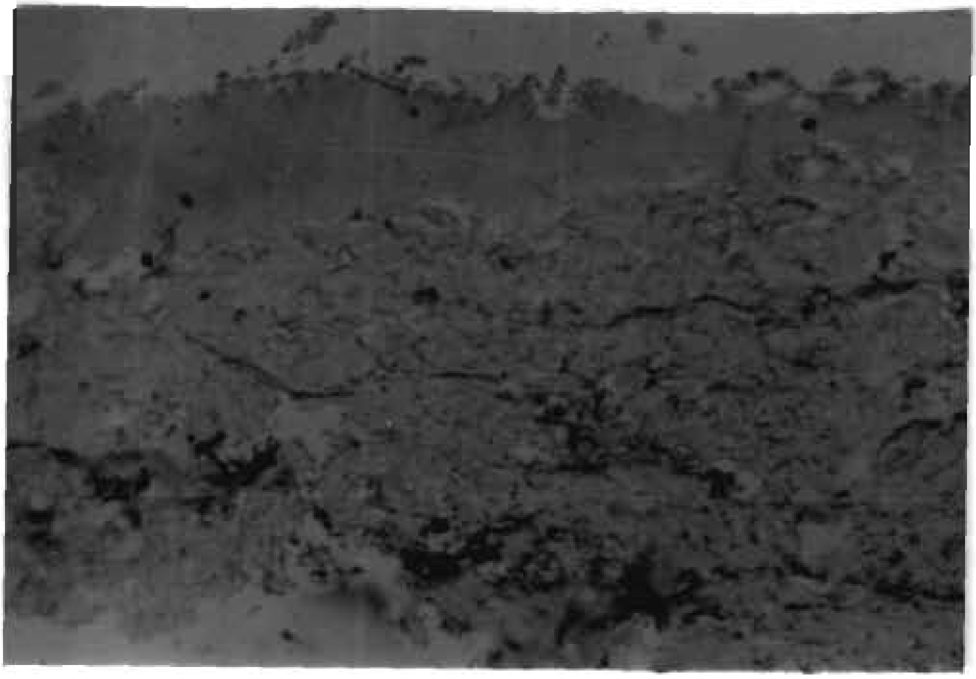


PLATE-22. MICROPHOTOGRAPH SHOWING COMPLETE NECROSIS OF GINGIVA AT -10°C. 5th POST-OPERATIVE DAY. H&E X80

However, the sections obtained by the 21st day of cryosurgery revealed scar tissue formation, characterised by the presence of numerous fibroblasts and mild cellular infiltration. The mucosa had completely regenerated by this time.

4.3 Group-II: Effects of Freezing Femoral Artery and Cephalic Vein

4.3.1 Freezing Femoral Artery

4.3.1.1 Clinical Observations

During freezing of femoral artery, it was observed that spraying of liquid nitrogen directly over the exposed artery resulted in complete cessation of blood flow through the artery. The artery appeared stone hard on palpation. However, blood flow resumed within 10 seconds due to thawing and the artery appeared normal and began pulsating. The same sequence of events was also noticed during the second freeze-thaw cycle. Healing of the skin wound occurred uneventfully within 8 days in all the dogs.

Upto four days after freezing, there was oedema of a small portion of skin around the site of freezing of femoral artery. However, pulse could be palpated immediately after the double freeze-thaw cycle of the femoral artery.

4.3.1.2 Radiographic Observations

The abdominal aortography performed to note the changes in the femoral artery at the site of double freeze-thawing did not depict any abnormalities in the radiographs obtained in four of the six dogs sacrificed on the 7th post-operative day and in all the six dogs sacrificed on the 14th post-operative day. The contrast radiograph in one dog on the 7th post-operative day depicted femoral arterial occlusion (**Plate 23**) and in another dog sacrificed on the same day, partial occlusion of the femoral artery was noticed in the radiograph (**Plate 24**). There was no evidence of discontinuity in the arterial lumen, stenosis or rupture of the artery in the other ten dogs subjected to cryosurgery (**Plate 25**).

4.3.1.3 Gross Pathological Observations

In the animals sacrificed on the 7th day after subjecting the femoral artery to double freeze-thaw cycle, in 5 of the 6 dogs, mild diffuse haemorrhage was observed around the frozen site (**Plate 26**). Adhesion of the femoral artery with the surrounding muscles was a constant finding. In one of the dogs, extensive extravasation was observed all over the medial aspect of the thigh (**Plate 27**).

By the 14th day, a small ecchymotic spot could still be observed at the site of freezing of the femoral artery (**Plate 28**). Adhesions of the position of the femoral artery subjected to cryosurgery with the surrounding tissues was present in all the cases.

4.3.1.4. Histopathological Observations

On the 7th day of freezing the femoral artery, the histopathological sections in four of the six dogs showed oedema of the arterial structure and the periarterial area. In one dog, a small thrombus was found attached to the arterial endothelium (**Plate 29**). There was haemorrhage around the arterial area and intense infiltration of macrophages and neutrophils in the periarterial fat (**Plate 30**). However, in all

PLATE-23. AORTOGRAM SHOWING FEMORAL ARTERIAL OCCLUSION. 7th POST-OPERATIVE DAY

PLATE-24. AORTOGRAM SHOWING PARTIAL FEMORAL ARTERIAL OCCLUSION. 7th POST-OPERATIVE DAY

PLATE-25. AORTOGRAM SHOWING NO CHANGES IN FEMORAL ARTERY. 14th POST-OPERATIVE DAY

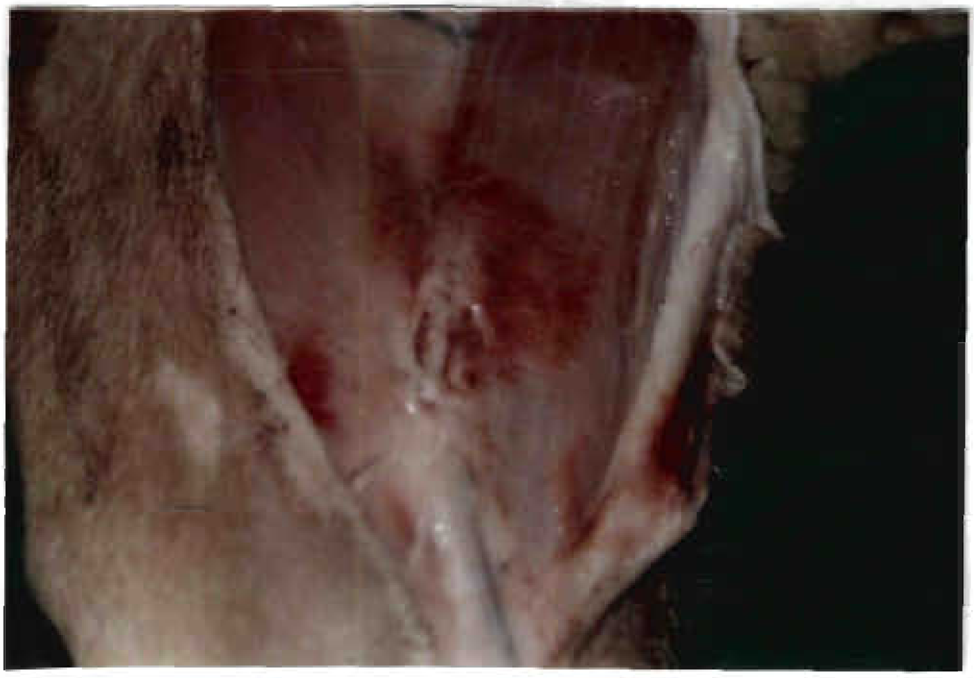


PLATE-26. MILD HAEMORRHAGE AROUND FEMORAL ARTERY. 7th POST-OPERATIVE DAY

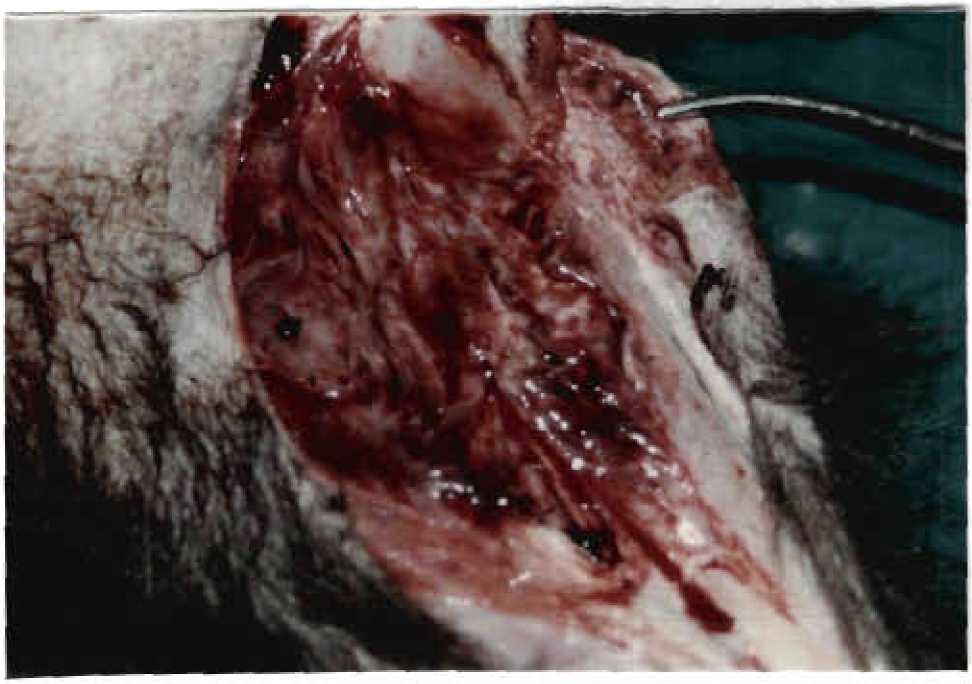


PLATE-27. EXTRAVASATION AROUND FEMORAL ARTERY.
7th POST-OPERATIVE DAY

PLATE-28. ECCHYMOTIC SPOT AROUND FEMORAL ARTERY.
14th POST-OPERATIVE DAY

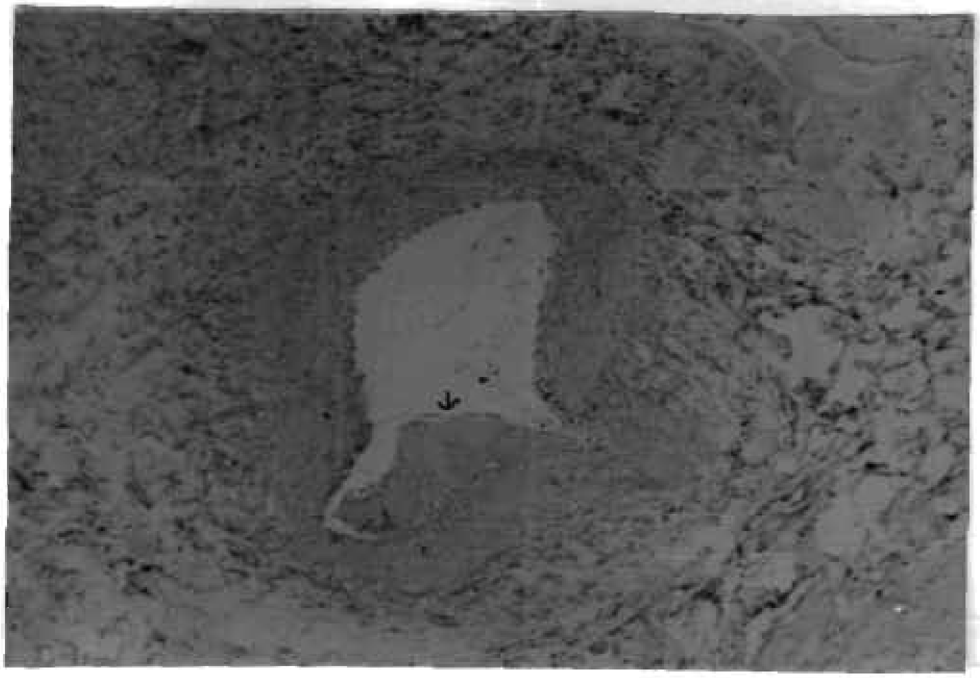


PLATE-29. MICROPHOTOGRAPH SHOWING FEMORAL ARTERIAL THROMBOSIS. H&E X80

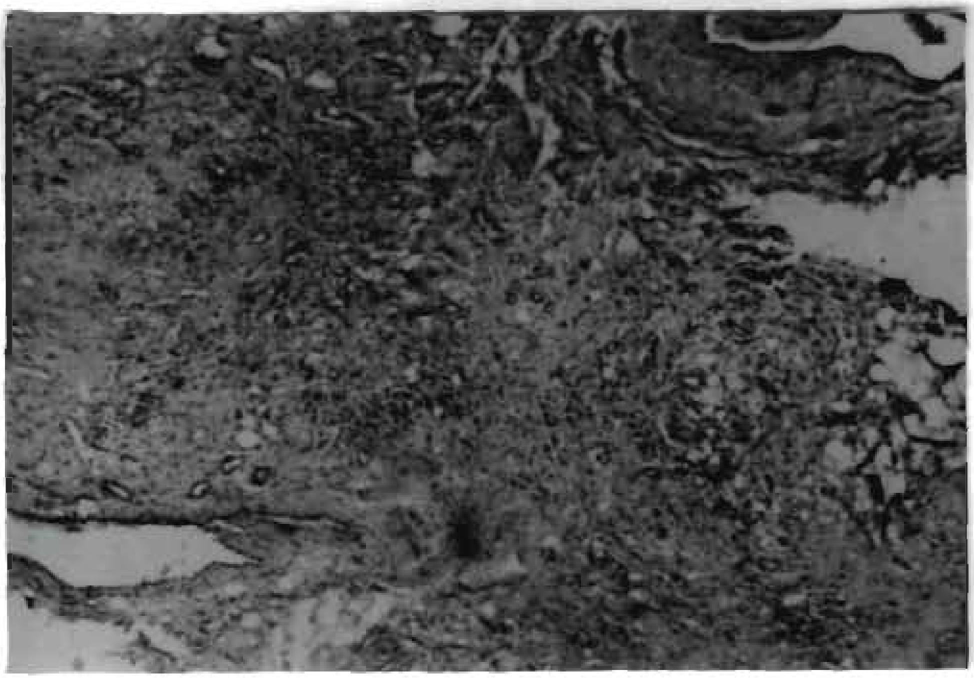


PLATE-30. MICROPHOTOGRAPH SHOWING PERI-ARTERIAL INFILTRATION. H&E X80 these 5 dogs the arterial endothelium was intact. In the 6th dog sacrificed on the 7th post-operative day, a thrombus completely filling-up the artery was seen. Degenerative changes within the artery were evident. Everything in this section appeared as a homogenous mass.

By the 14th day, the arteries appeared nearly normal, although slight oedema and infiltration of monocytes and neutrophils still persisted. In some areas, slight haemorrhage in the periarterial region was still noticed. Mild degenerative changes in the arterial wall were seen in all cases.

4.3.2 Freezing Cephalic Vein

4.3.2.1 Clinical Observations

During freezing of the cephalic vein, it took about one to two minutes for the solid frozen cephalic vein to thaw during the two freezes in all the animals. By the first day, there was considerable oedema of the region directly overlying the frozen part of the vein, which persisted for 4-5 days in all the dogs. By the 7th day, all the dogs were free from oedema. The skin incision healed uneventfully in all the 12 dogs.

4.3.2.2 Radiographic Observations

Contrast radiography of the cephalic vein obtained on the 7th day of all the dogs depicted complete obliteration of the frozen portion of the cephalic vein in all the dogs. However, in the radiographs obtained on the 7th day of freezing the cephalic vein, an extensive network of newly formed venules and capillaries was found to have formed, connecting the distal and proximal portions of the frozen part of the cephalic vein (**Plate 31**).

The radiographs obtained on the 14th day of cryosurgery also depicted complete obliteration of the frozen portion of the cephalic vein. However, the number of the newly formed venules and capillaries was reduced by this time, but the thickness of the vessels had increased and they were fewer in number than those seen on the 7th day of cryoinjury. The network of vessels formed was also seen to have communicated the distal and proximal ends of the portion of the cephalic vein subjected to cryosurgery (**Plate 32**).

4.3.2.3 Gross Pathological Observations

In the dogs sacrificed on the 7th day, slight congestion around the frozen area of the cephalic vein was noticed. The portion of the cephalic vein that was frozen was found to be shrunken in diameter. A few drops of clear colourless fluid was discharged on incision of the area overlying in frozen portion of cephalic vein.

By the 14th day of cryosurgery, neither congestion nor oedema was observed at the site of freezing of the cephalic vein. The frozen part of the cephalic vein was noticed to have further shrunken and it appeared as a thin black strand of venous tissue.

PLATE-31. VENOGRAM SHOWING CEPHALIC VENOUS OCCLUSION-
7th POST-OPERATIVE DAY

PLATE-32. VENOGRAM SHOWING CEPHALIC VENOUS OCCLUSION-
14th POST-OPERATIVE DAY

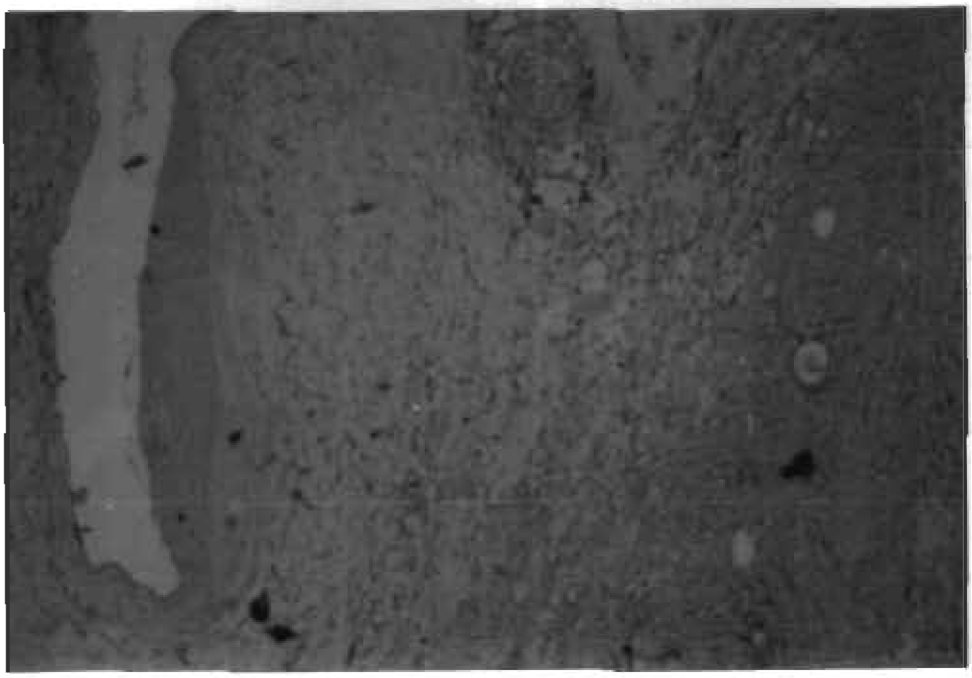


PLATE-33. MICROPHOTOGRAPH SHOWING CEPHALIC VENOUS THROMBOSIS AND PERIVENOUS FIBROVASCULAR *TISSUE. H&E X80*

PLATE-34. MICROPHOTOGRAPH SHOWING MARKED PROLIFERATION OF NEW BLOOD VESSELS IN THE PERIVENOUS TISSUE. H&E X80

PLATE-35. CRYOLESION SHOWING ERYTHEMA. 6th POST-OPERATIVE HOUR

PLATE-36. PARTIAL SLOUGHING OF CRYOLESION. 14th POST-OPERATIVE DAY

PLATE-37. ALMOST COMPLETE HEALING. 35th POST-OPERATIVE DAY

4.4.2 Haematological Examination

4.4.2.1 Total Red Blood Cell Count

The average red blood cell count before freezing 2.5 percent (of the skin) of the body surface area to a temperature of -50°C was 4.87 ± 0.39 millions/cmm. On the first day after freezing 2.5 percent of the body surface area in 6 dogs, the mean red blood cell count was found to be 4.82 ± 0.46 millions/cmm. On the 3rd day after cryosurgery, the RBC count was 4.87 ± 0.45, 5th day 4.85 ± 0.38, 7th day 4.82 ± 0.40, 14th day 4.82 ± 0.43, 21st day 4.78 ± 0.50 and on the 28th day after cryosurgery, the mean value was 4.82 ± 0.49 millions/cmm.

When 5 percent of the body surface area was frozen to a sub-cutaneous temperature of -50°C, the mean red blood cell counts (± standard error) on the 0, 1st, 3rd, 5th, 7th, 14th, 21st and 28th days were found to be 4.60 ± 0.97, 4.71 ± 0.83, 4.60 ± 0.93, 4.62 ± 0.93, 4.65 ± 1.03, 4.67 ± 0.74, 4.77 ± 0.80 and 4.58 ± 0.99 millions/cmm respectively.

Statistical analysis of the above mentioned data revealed no significant difference between the red blood cell counts of the dogs where either 2.5 percent or 5 percent of the body surface area was frozen to a sub-cutaneous temperature of -50°C.

4.4.2.2 Haemoglobin

Before freezing 2.5 percent of the body surface area to a sub-cutaneous temperature of -50°C, the mean ± standard error values of haemoglobin content of the 6 dogs was 9.37 ± 0.88 grams/dl. Following freezing, the corresponding values of the haemoglobin content on the 1st, 3rd, 5th, 7th, 14th, 21st and 28th day were found to be 9.23 ± 0.76, 9.23 ± 0.71, 9.28 ± 0.71, 9.28 ± 0.69, 9.25 ± 0.69, 9.32 ± 0.49 and 9.40 ± 0.86 grams/dl respectively.

When 5 percent of the body surface area was subjected to freezing, the haemoglobin content on the 0, 1st, 3rd, 5th, 7th, 14th, 21st and 28th day after cryosurgery was found to be 9.83 ± 0.93, 9.92 ± 1.03, 9.89 ± 0.83, 9.89 ± 0.81, 10.00 ± 0.88, 9.92 ± 0.91, 9.95 ± 0.88 and 10.01 ± 0.81 grams/dl respectively.

Statistical analysis of the data by completely random design under factorial experimentation revealed no significant difference in the haemoglobin contents of the dogs where 2.5 percent and 5 percent of the body surface area was subjected to cryosurgery.

4.4.2.3 Packed Cell Volume

When 2.5 percent of the body surface area was subjected to cryosurgery, the mean packed cell volume before and on the 1st, 3rd, 5th, 7th, 14th, 21st and 28th day of freezing were found to be 39.33 ± 3.55, 39.17 ± 3.87, 39.00 ± 3.99, 39.33 ± 3.55, 39.0 ± 3.75, 39.17 ± 3.43, 39.67 ± 3.63 and 38.00 ± 1.79 percent respectively.

Before freezing 5 percent of the body surface area to a sub-cutaneous temperature of -50°C, the mean packed cell volume was 38.00 ± 3.28 percent. The packed cell volume on the 1st, 3rd, 5th, 7th, 14th, 21st and 28th day after cryosurgery was found to be 37.67 ± 3.63, 37.83 ± 4.09, 37.50 ± 3.99, 38.17 ± 3.87, 38.00 ± 3.16, 37.00 ± 3.09 and 37.67 ± 4.80 percent respectively.

Statistical analysis of the data revealed no significant difference between the groups or days.

4.4.2.4 Total Leucocyte Count

When 2.5 percent of the body surface area was frozen to a sub-cutaneous temperature of -50°C, the total leucocyte count before and on the 1st, 3rd, 5th, 7th, 14th, 21st and 28th day of freezing was found to be 8.88 ± 1.91, 12.23 ± 1.89, 13.70 ± 2.05, 15.37 ± 2.14, 15.09 ± 3.33, 12.45 ± 3.02, 10.00 ± 1.99 and 9.50 ± 2.29 thousands/cmm respectively.

The leucocyte counts before freezing of 5 percent of the body surface area and on the 1st, 3rd, 5th, 7th, 14th, 21st and 28th day of cryosurgery were found to be 8.31 ± 1.98, 10.87 ± 1.89, 13.19 ± 2.18, 14.38 ± 2.20, 15.48 ± 2.87, 10.24 ± 2.91, 9.47 ± 2.57 and 9.36 ± 2.22 thousands/cmm respectively.

Statistical analysis of the data revealed a highly significant difference ($p < 0.01$) between the group of dogs where 2.5 percent of the body surface area and the group of dogs where 5 percent of the body surface area was frozen. A highly significant difference ($p < 0.01$) in the leucocyte count and significant difference between intervals ($p < 0.05$) were also established by factorial experimentation under completely random design analysis (**Table I**). However, the interaction of the leucocyte count parameter and the various intervals was found to be non significant.

4.4.2.5 Neutrophil Count

The mean absolute total neutrophil counts before and on the 1st, 3rd, 5th, 7th, 14th, 21st and 28th day of freezing 2.5 percent of the body surface area were recorded to be 6.13 ± 0.54, 9.09 ± 0.55, 10.52 ± 0.63, 10.76 ± 0.61, 9.71 ± 0.79, 7.48 ± 0.74, 6.70 ± 0.54 and 6.47 ± 0.64 thousands/cmm respectively.

The average neutrophil counts before and after freezing 5 percent of the body surface area at the said intervals were found to be 5.81 ± 0.56, 8.15 ± 0.58, 10.91 ± 0.69, 10.07 ± 0.63, 10.06 ± 0.76, 5.69 ± 0.63, 6.86 ± 0.80 and 6.36 ± 0.61 thousands/cmm respectively.

Statistical analysis of the data revealed a highly significant difference ($p < 0.01$) between the dogs where 2.5 and 5 percent of the body surface area was frozen to a subcutaneous temperature of -50°C. A significant difference ($p < 0.05$) between the neutrophil parameter of the two groups and a highly significant difference ($p < 0.01$) among the various intervals was also noticed (**Table I**). However, the neutrophil parameter and the interval interaction was not significant.

4.4.2.6 Lymphocyte Count

On freezing 2.5 percent of the body surface area, the average absolute lymphocyte count, which was 2.35 ± 0.24 thousands/cmm changed to 2.93 ± 0.34, 2.70 ± 0.17, 3.07 ± 0.18, 2.26 ± 0.20, 3.00 ± 0.30, 2.49 ± 0.20 and 2.56 ± 0.25 thousands/cmm respectively by the 1st, 3rd, 5th, 7th, 14th, 21st and 28th day.

When 5 percent of the body surface area was frozen, the values of absolute lymphocyte counts at the said intervals were found to be 2.08 ± 0.20, 2.50 ± 0.18, 2.64 ± 0.18, 2.91 ± 0.17, 2.32 ± 0.18, 2.27 ± 0.25, 2.56 ± 0.29 and 2.53 ± 0.24 thousands/cmm respectively.

Statistical analysis of the data revealed that the difference between the two sets of dogs was not significant.

4.4.2.7 Monocyte Count

Following freezing 2.5 percent of the body surface area, the mean monocyte count which was 0.30 ± 0.02, thousands/cmm changed to 0.31 ± 0.06, 0.30 ± 0.02, 1.38 ± 0.08, 3.02 ± 0.72, 1.87 ± 0.02, 0.70 ± 0.05 and 0.38 ± 0.04 thousands/cmm by the 1st, 3rd, 5th, 7th, 14th, 21st and 28th day of freezing respectively.

Similar values of monocyte counts before and at the said intervals following freezing 5 percent of the body surface area were 0.33 ± 0.03, 0.22 ± 0.02, 0.26 ± 0.02, 1.30 ± 0.08, 3.10 ± 0.23, 1.42 ± 0.16, 1.72 ± 0.08 and 0.37 ± 0.04 thousands/cmm respectively.

Statistical analysis of the data revealed a highly significant difference ($p < 0.01$) between the dogs where 2.5 percent and 5 percent of the body surface area was frozen and the various intervals (**Table I**). While a significant difference was found between the monocyte count parameter ($p < 0.05$), no significant difference in the monocyte count and the interval interaction was observed.

4.4.2.8 Eosinophil Count

The eosinophil count which was 0.095 ± 0.02 before freezing 2.5 percent of the body surface area, was found to be 0.09 ± 0.03, 0.12 ± 0.05, 2.20 ± 0.07, 0.11 ± 0.04, 0.13 ± 0.01, 0.10 ± 0.01 and 0.10 ± 0.01 thousands/cmm on the 1st, 3rd, 5th, 7th, 14th, 21st and 28th day respectively.

Similar values of absolute eosinophil count at the various intervals mentioned were found to be 0.08 ± 0.01, 0.11 ± 0.01, 0.08 ± 0.03, 0.14 ± 0.01, 0.10 ± 0.03, 0.10 ± 0.01, 0.10 ± 0.01 and 0.09 ± 0.01 thousands/cmm when 5 percent of the body surface area was frozen.

Statistical analysis revealed that the difference between the two groups was not significant.

4.4.3 Serum Biochemical Examination

4.4.3.1 Aspartate Amino Transferase (AST or SGOT)

The mean aspartate amino transferase levels before and on the 1st, 3rd, 5th, 7th, 14th, 21st and 28th day of freezing 2.5 percent of the body surface area to a sub-cutaneous temperature of -50°C were found to be 46.00 ± 7.27, 54.00 ± 6.65, 68.17 ± 7.25, 70.50 ± 7.58, 56.17 ± 5.97, 53.17 ± 6.01, 49.00 ± 7.34 and 49.67 ± 7.20 units/l respectively.

Similar values before and following freezing of 5 percent of the body surface area at the said intervals were found to be 48.50 ± 1.98, 59.50 ± 2.44, 86.83 ± 6.41, 91.17 ± 5.92, 64.67 ± 5.79, 59.50 ± 8.39, 47.00 ± 3.44 and 46.67 ± 3.08 units/l respectively.

Statistical analysis revealed a highly significant difference ($p < 0.01$) between the two treatments, aspartate amino transferase parameter and the various intervals (**Table I**). However, the aspartate amino transferase and the interval interaction was found to be non significant.

4.4.3.2 Total Serum Protein

Average total serum protein levels before and on the 1st, 3rd, 5th, 7th, 14th, 21st and 28th day of freezing 2.5 percent of the body surface area to a sub-cutaneous temperature of -50°C were found to be 5.82 ± 0.51, 5.85 ± 0.49, 5.92 ± 0.49, 5.82 ± 0.44, 5.85 ± 0.44, 5.82 ± 0.49, 5.83 ± 0.49 and 5.87 ± 0.44 grams/dl respectively.

Similar mean values of total serum protein in the six dogs where 5 percent of the body surface area was frozen to -50°C were 6.52 ± 0.24, 6.50 ± 0.37, 6.20 ± 1.74, 6.32 ± 0.54, 6.50 ± 0.47, 6.50 ± 0.17, 6.47 ± 0.22 and 6.52 ± 0.22 grams/dl respectively.

Statistical analysis of the data pertaining to total serum protein levels indicated that the difference between the groups of dogs where 2.5 percent and 5 percent of the body surface area was frozen was non significant.

TABLE I

EFFECTS OF CRYOSURGERY ON THE MEAN AST, TOTAL LEUCOCYTE, NEUTROPHIL AND MONOCYTE COUNTS

CLASSIFICATION	PARAMETERS			
	AST iu/l	Total Leucocyte count 10^3/cmm	Neutrophil count 10^3/cmm	Monocyte count 10^3/cmm
Between Freezing				
2.5% Body Surface Area	55.83	12.17	8.36	1.26
5% Body Surface Area	62.98	11.41	7.92	0.94
Mean of Means	59.41	11.79	8.14	1.10
S.E.	2.23	0.34	0.64	0.18
'F' Ratio	**	**	*	*
Between Intervals				
Pre-operative	47.25	8.60	5.97	0.32
POD 1	56.75	11.55	8.63	0.27
POD 3	77.5	13.50	10.41	0.31
POD 5	80.83	14.88	10.42	1.34
POD 7	60.42	15.28	9.89	3.06
POD 14	56.33	10.95	6.59	1.65
POD 21	48.00	10.12	6.78	1.21
POD 28	48.17	9.43	6.42	0.38
Mean of Means	59.34	11.79	8.13	1.06
S.E.	4.36	0.68	0.35	0.35
'F' Ratio	**	*	**	**

* - Significant ($P < 0.05$)   ** - Highly significant ($P < 0.01$)
POD - Post-operative day   S.E. - Standard Error

4.4.3.3 Serum Potassium

The serum potassium level which was an average of 3.65 ± 0.12, mEq/l before cryosurgery, changed to 3.63 ± 0.15, 3.55 ± 0.19, 3.42 ± 0.08, 3.36 ± 0.13, 3.27 ± 0.15, 3.67 ± 0.10 and 3.84 ± 0.19 mEq/l on the 1st, 3rd, 5th, 7th, 14th, 21st and 28th day respectively following freezing of 2.5 percent of the body surface area to a sub-cutaneous temperature of -50°C.

When 5 percent of the body surface area was frozen, the corresponding levels of serum potassium before and at the said intervals was estimated to be 3.65 ± 0.12, 3.63 ± 0.15, 3.55 ± 0.19, 3.42 ± 0.08, 3.36 ± 0.13, 3.27 ± 0.15, 3.67 ± 0.10 and 3.84 ± 0.19 mEq/l respectively.

No significant difference between the serum potassium levels of the two groups of dogs was observed on statistical analysis.

4.5 Group-IV: Effects of Freezing Cornea and Iris During Extracapsular Cryoextraction of Lens

4.5.1 Control

4.5.1.1 Clinical Observations

In the six dogs where extracapsular cryoextraction of lens was done, corneal opacity developed on the fist post-operative day. The opacity, however, was limited to the area around the suture line. The dogs exhibited tendency to scratch the eyes for 3-4 days. The degree of corneal opacity was reduced by the 7th day and by the

14th day, it was still less. Apart from this, no other clinical changes were noticed. The corneoscleral suture line had healed by the 7th post-operative day.

4.5.1.2 *Fluorescein dye test*

Fluorescein dye test conducted on the 7th post-operative day did not reveal ulceration of the cornea in any of the six dogs.

4.5.1.3 Tonometry

The average intraocular pressure before surgery in the control dogs was 22.83 ± 1.47 mm Hg. On the 7th post-operative day, it was 14.83 ± 1.45 mm Hg and by the 14 post-operative day, it was 20.67 ± 1.15 mm Hg.

4.5.1.4 Gross Pathological Observations

Following sacrifice of three dogs on the 7th post-operative day, gross pathological examination revealed neither corneal opacity nor any changes in the iris, except for mild corneal opacity that was limited to the area around the suture line. No adhesions between cornea and iris were found.

4.5.1.5 Histopathological Observations

The changes on histopathological examination were limited to the area around suture line, which comprised of corneal oedema and cellular infiltration.

4.5.2 Freezing of Cornea

4.5.2.1 Clinical Observations

In all the six dogs where cornea was frozen following extracapsular cryoextraction of lens, opacity of the entire cornea was observed during the first 3-4 days of operation (**Plate 38**), which still persisted, albeit to a lesser degree till the 7th post-operative day. In the three dogs that were sacrificed on the 14th post-operative day, the corneal opacity was found to have completely resorbed. The suture line was found to have healed in all the dogs by the 7th post-operative day. Despite the corneal opacity that persisted upto 7 days in all the dogs, no dog was noticed to be blind, as indicated by the menace test.

4.5.2.2 Fluorescein Dye Test

The fluorescein dye test did not indicate corneal ulceration in any of the six dogs where cornea was frozen following extracapsular cryoextraction of lens.

4.5.2.3 Tonometry

The mean intraocular pressure, which was 24.83 ± 1.17 mm Hg pre-operatively, changed to 17.33 ± 0.82 mm Hg by the 7th and to 22.67 ± 0.58 mm Hg by the 14th post-operative day.

4.5.2.4 Gross Pathological Observations

The only gross pathological change noticed in the dogs sacrificed on the 7th post-operative day was mild opacity of the entire cornea.

By the 14th post-operative day, the three dogs sacrificed revealed clear corneas with no signs of corneal opacity. No adhesions between the cornea and iris were noticed in any of the dogs.

4.5.2.5 Histopathological Observations

In the three dogs sacrificed on the 7th post-operative day, slight thickening of the stratified squamous epithelium of the cornea was noticed in some places. A few inflammatory cells were noticed in the epithelial layer and deep stroma (**Plate 39**). The substantia propria was observed to be oedematous and was sparsely devoid of keratocytes. The corneal endothelium was found to be normal and single layered.

The three dogs sacrificed on the 14th post-operative day, depicted a multilayered normal appearing epithelium. In some areas, however, the epithelial cells appeared vacuolated (**Plate 40**). The stroma was sparsely devoid of keratocytes. There was no evidence of oedema. The endothelium was normal and was monolayered.

4.5.3 Freezing of Iris

4.5.3.1 Clinical Observations

In the six dogs where iris was frozen, diffuse opacity of the upper half of the cornea was noticed. There was evidence of haemorrhage in the anterior chamber and this could still be observed in the dogs sacrificed on the 14th post-operative day.

PLATE-38. CORNEAL FREEZING-DIFFUSE OPACITY OF ENTIRE CORNEA. 4th POST-OPERATIVE DAY

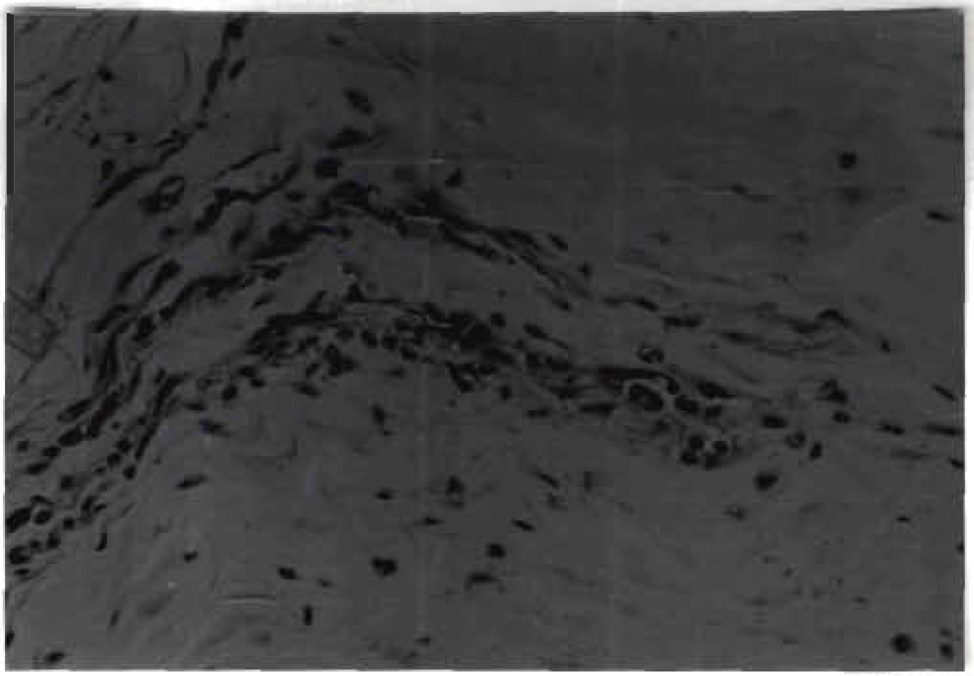


PLATE-39. MICROPHOTOGRAPH SHOWING NEUTROPHIL INFILTRATION IN CORNEAL STROMA AFTER CORNEAL FREEZING. 7th POST-OPERATIVE DAY. H&E X400

Anterior synechia was noticed in all the six dogs where iris was frozen following extracapsular cryoextraction of lens (**Plate 41**).

4.5.3.2 Fluorescein Dye Test

No evidence of corneal ulceration could be noticed in any of the six dogs where iris was frozen following extracapsular cryoextraction of lens.

4.5.3.3 Tonometry

The average intraocular pressure observed pre-operatively and on the 7th and 14th post-operative days was noticed to be 20.33 ± 1.03, 16.33 ± 0.82 and 19.33 ± 0.58 mm Hg respectively.

4.5.3.4 Gross Pathological Observations.

In all the six dogs sacrificed on the 7th and the 14th post-operative day, blood tinged aqueous humor was expelled following incision of the eye ball. In all the six dogs, adhesion of the frozen portion of the iris with the cornea was a consistent finding. Opacity of the cornea was limited to the areas of corneo-iridial adhesions.

4.5.3.5 Histopathological Observations

In the three dogs sacrificed on the 7th post-operative day, adhesions between iris and cornea (anterior synechia) was noticed (**Plate 42**). The iris was intensely hyperaemic. The iris was found to be oedematous, with polymorphonuclear leucocytic infiltration. Haemorrhage was also noticed between the iridial muscles, which

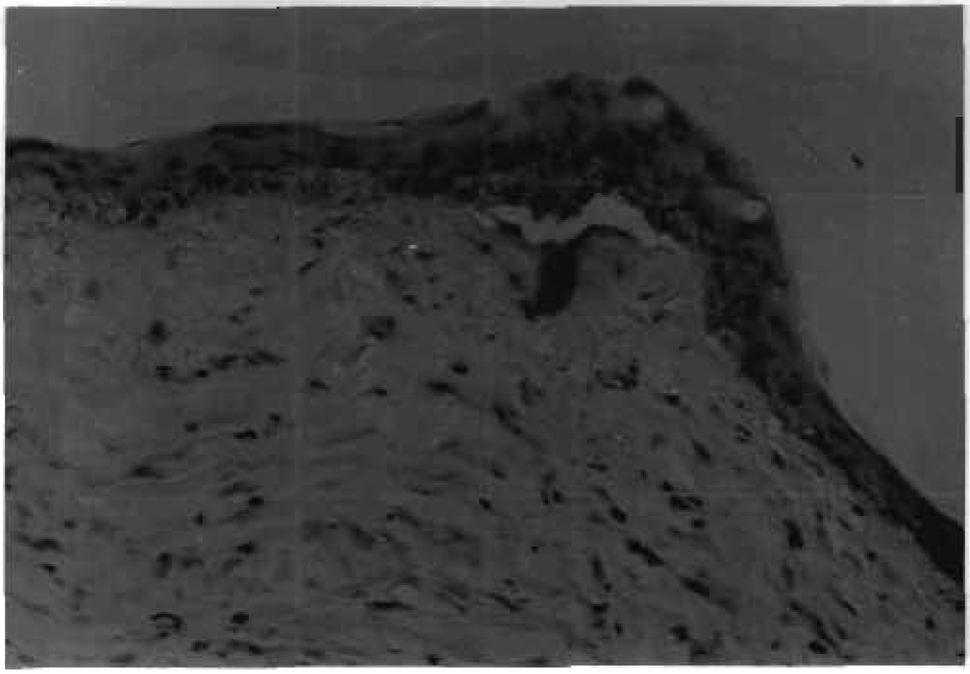


PLATE-40. MICROPHOTOGRAPH SHOWING VACUOLAR CHANGES IN CORNEAL EPITHELIUM AFTER CORNEAL FREEZING. 14th POST-OPERATIVE DAY H&E X320

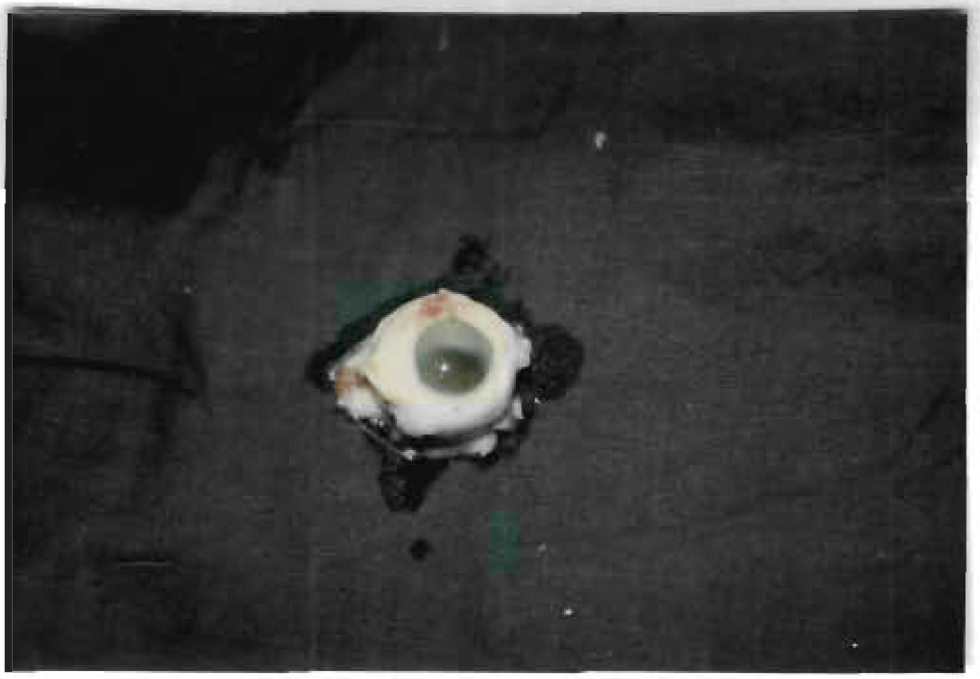


PLATE-41. IRIS FREEZING. ANTERIOR SYNECHIA. 7th POST-OPERATIVE DAY

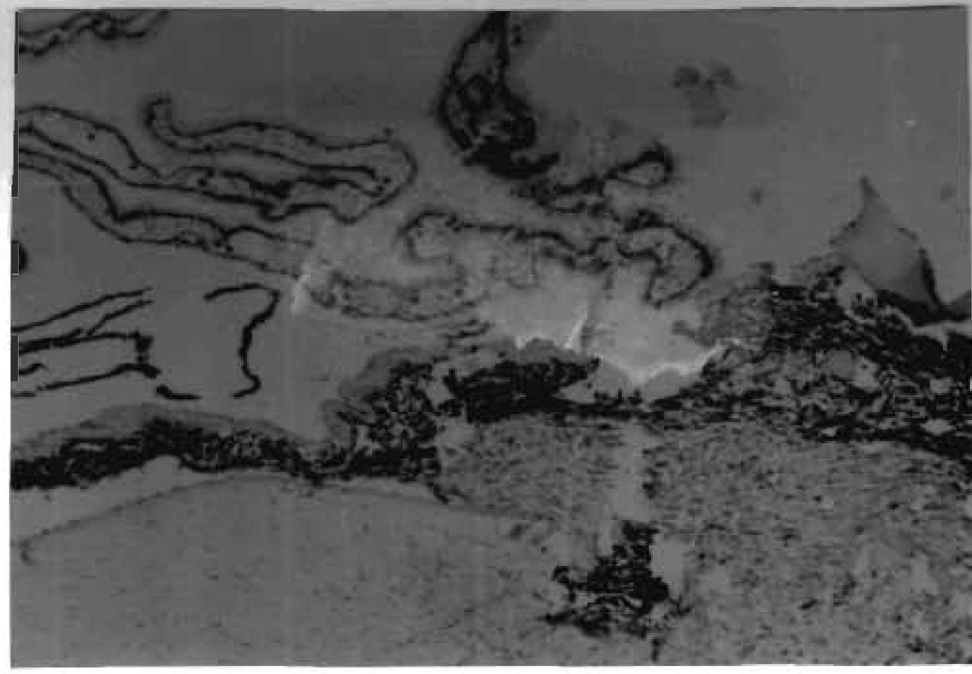


PLATE-42. MICROPHOTOGRAPH SHOWING ANTERIOR SYNECHIA. 7th POST-OPERATIVE DAY. H&E X80

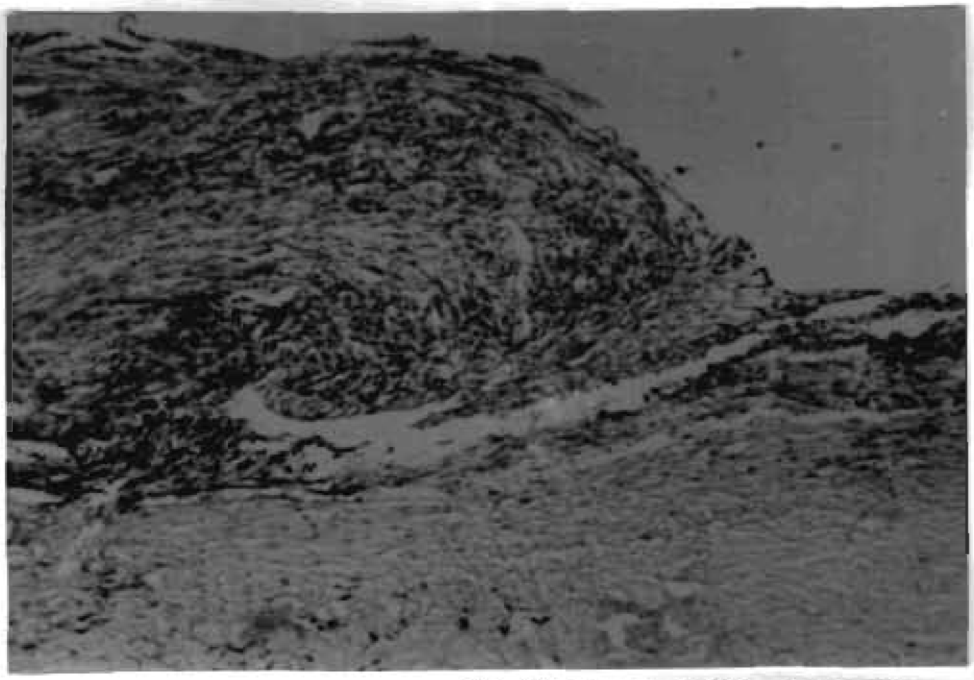


PLATE-43. MICROPHOTOGRAPH SHOWING FREE MELANIN PIGMENT IN IRIDIAL TISSUE. 7th POST-OPERATIVE DAY. H&E X100 appeared necrotic in focal areas. In all the cases, free melanin pigment could be noticed outside the cells and in between the iridial muscles (**Plate 43**). Thickening of the squamous layer of the iris along with infiltration of inflammatory cells was a consistent finding.

The dogs sacrificed on the 14th day also depicted similar changes like adhesions between iris and cornea, hyperaemia and haemorrhage within iridial stroma, presence of free melanin within the iridial muscular tissue and areas of necrosis of the iridial muscle. The oedema, however, was of a lesser degree and the thickening of the squamous layer of the iris had resolved by this time. However, the infiltration with polymorphonuclear leucocytes still persisted.

4.6 Part-C: Clinical Study

The details of the 50 clinical cases cryosurgically treated during the course of the study are presented in Table-**II.**

In the clinical part of the present study, 38 (80.85 percent) of the 47 dogs with various neoplastic and non-neoplastic lesions had complete recovery. All the 3 cases of cataract, that underwent extracapsular cryoextraction of lens in one of the eyes regained vision.

TABLE-II

DETAILS OF CLINICAL CASES

Sl. No.	Breed	Sex	Age	Lesion	Location	Size of the lesion	Freezing temp.	Freezing time	Thawing time	Number of treatments	Result
**1	•Dob	M	3.5 yrs.	TVT	Bulbus Glandis	2 cm dia.	-40°C	1 min.	3 min.	3	Cured
**2	•GSD	M	2 yrs.	TVT	-do-	Diffuse	-do-	2 min.	4 min.	2	Cured
**3	•ND	M	1.5 yrs.	TVT	-do-	3 cm dia.	-do-	1 min.	2 min.	3	Cured
**4	•LAB	M	1 yr.	TVT	-do-	1 cm dia.	-do-	1 min.	2 min.	2	Recurred but Cured with 2nd treatment
**5	•ND	M	2 yrs.	TVT	-do-	Diffuse	-do-	2 min.	2 min.	1	Cured
**6	•ND	M	4 yrs.	TVT	-do-	-do-	-do-	2 min.	2 min.	2	Cured
**7	•ND	M	3 yrs.	TVT	Glans Penis	-do-	-do-	2 min.	2 min.	2	Cured
**8	•Pom	M	4 yrs.	TVT	-do-	-do-	-do-	2 min.	3 min.	1	Cured
**9	•ND	M	2 yrs.	TVT	-do-	-do-	-do-	2 min.	3 min.	3	Cured
**10	•ND	M	3.5 yrs.	TVT	-do-	-do-	-do-	2 min.	4 min.	2	Cured
**11	•ND	M	2 yrs.	TVT	Bulbus glandis	-do-	-do-	2 min.	4 min.	2	Cured
*12	ND	F	1 yr.	TVT	Vulval lips	2 cm dia.	-do-	1 min.	3 min.	2	Cured
*13	ND	F	9 m.	TVT	-do-	3 cm dia.	-do-	1 min.	4 min.	2	Cured
*14	GSD	F	4 yrs.	TVT	-do-	1 cm dia.	-do-	1 min.	4 min.	2	Cured
*15	ND	F	3 yrs.	TVT	-do-	2 cm dia.	-do-	1 min.	4 min.	2	Cured
*16	ND	F	6 yrs.	TVT	-do-	1 cm dia.	-do-	1 min.	5 min.	1	Cured
*17	ND	F	2 yrs.	TVT	-do-	Diffuse	-do-	1 min.	5 min.	2	Cured
*18	ND	F	1 yr.	TVT	-do-	-do-	-do-	1 min.	3 min.	1	Cured
*19	ND	M	6 yrs.	Fibro sarcoma	Fore head	10 cm dia.	-140°C	2 min.	8 min.	2	Euthanised

Sl. No.	Breed	Sex	Age	Lesion	Location	Size of the lesion	Freezing temp.	Freezing time	Thawing time	Number of treatments	Result
*20	ND	F	8 yrs.	-do-	Upper gums	8x6 cm	-do-	2 min.	10 min.	2	Euthanised
*21	ND	F	9 yrs.	-do-	-do-	Diffuse	-do-	2 min.	6 min.	2	Euthanised
*22	ND	F	11 yrs.	-do-	Lower gums	-do-	-do-	2 min.	4 min.	2	Euthanised
*23	ND	F	7 yrs.	-do-	Vulval lip	3x2 cm	-90°C	1.5 min.	4 min.	1	Cured
**24	•ND	M	4 yrs.	Hyp.Oti.Ext.	Ext.ear	Diffuse	-40°C	4 min.	8 min.	3	Cured
**25	•Dob	F	6 yrs.	-do-	-do-	-do-	-do-	4 min.	8 min.	2	Cured
**26	•ND	M	3 yrs.	-do-	-do-	-do-	-do-	4 min.	8 min.	3	Cured
**27	•ND	M	5 yrs.	-do-	-do-	-do-	-do-	4 min.	8 min.	3	Cured
**28	•ND	M	7 yrs.	-do-	-do-	-do-	-do-	4 min.	8 min.	3	Cured
*29	Pom	M	5 m	Oral Papi.	Mouth	-do-	Could'nt measure	-	-	2	Cured
*30	ND	M	3 yrs.	-do-	-do-	6 pea sized warts	-do-	1 min.	2 min.	1	Cured
**31	Lab	M	3 yrs.	Perianal fistula	Perianal Region	-	-do-	1 min.	3 min.	2	Cured
**32	GSD	M	6 yrs.	-do-	-do-	-	-do-	1 min.	3 min.	1	Cured
**33	Pom	M	4 yrs.	-do-	-do-	-	-do-	1 min.	3 min.	1	Cured
*34	ND	M	3 yrs.	Peianal adenoma	-do-	3 cm dia.	-80°C	2 min.	4 min.	1	Cured
*35	ND	M	2 yrs.	-do-	-do-	2 cm dia.	-do-	1 min.	3 min.	1	Cured
*36	GSD	M	8 yrs.	-do-	-do-	1 cm dia.	-60°C	1 min.	3 min.	1	Cured
**37	•Dob	M	2 yrs.	Lick Granuloma	3rd Digit Fore Limb	1 cm dia.	-40°C	1 min.	3 min.	1	Recovered but recurred
**38	•GSD	F	6 yrs.	-do-	2nd Digit Fore Limb	1 cm dia.	-do-	1 min.	3 min.	2	-do-
**39	•Lab	F	1 yr.	-do-	4th Digit Fore Limb	2 cm dia.	-do-	1 min.	3 min.	1	-do-

Sl. No.	Breed	Sex	Age	Lesion	Location	Size of the lesion	Freezing temp.	Freezing time	Thawing time	Number of treatments	Result
**40	•ND	M	4 yrs.	-do-	2nd Digit Fore Limb	1 cm dia.	-do-	1 min.	3 min.	1	-do-
**41	•ND	M	3 yrs.	-do-	4th Digit Fore Limb	1 cm dia.	-do-	1 min.	3 min.	2	-do-
**42	ND	M	2 yrs.	Sebaceous adenoma	Ear Base	1 cm dia.	-60°	1 min.	3 min.	1	Cured
**43	ND	F	6 yrs.	-do-	Thigh	1 cm dia.	-do-	1 min.	4 min.	1	Cured
**44	Dasch	F	6 yrs.	Adamanti-noma	Lower Jaw	2 cm dia.	-80°C	1 min.	4 min.	1	Cured
*45	Dob	F	11 yrs.	Fibroma	2nd Digit Fore Limb	2 cm dia.	-do-	1 min.	4 min.	1	Cured
**46	Lab	M	2 yrs.	-do-	Ear Canal	1 cm dia.	-40°C	1 min.	4 min.	1	Cured
**47	GSD	F	6 yrs.	-do-	-do-	2 cm dia.	-do-	1 min.	4 min.	1	Cured
***48	ND	F	6 yrs.	Cataract	Left Eye	-	-	-	-	1	Cured
***49	ND	M	7 yrs.	-do-	Right Eye	-	-	-	-	1	Cured
***50	ND	F	9 yrs.	-do-	Left Eye	-	-	-	-	1	Cured

* Treated with Frigitronics CS-76

** Treated with Cryosuper VE-4

*** Treated with Cryosuper VE-1

• No anaesthesia used

4.6.1 Transmissible Venereal Tumour (TVT)

A total of 18 cases of transmissible venereal tumours were treated during the course of the study. Of these, 11 were in males and 7 in females. The nature and distribution of the lesions is depicted in the table-2.

In all but one of the male dogs, TVT completely resolved following 1-3 weekly episodes of cryosurgical treatment (**Plate 44**). The lesions turned blackish by the 5th day and sloughing of the lesions was observed, followed by complete healing. In one dog, however, the TVT recurred at the same spot on the bulbus glandis three months after apparent resorption of the lesion. However, the dog was free from TVT following second treatment.

Among the bitches that were treated for TVT of vagina, five required a second treatment, since a small rudiment of tumour still remained on the 7th day, when a major portion of the lesion had sloughed off. All the 18 dogs treated by cryosurgery responded well to cryosurgery.

4.6.2 Fibrosarcoma

Out of the five cases treated, four had to be euthanised. In three of them, the response to cryosurgery was not considered satisfactory. These three tumours were in the oral cavity and the treatment produced severe oedema, malodour and the animals were unable to take food and water. In the fourth dog, where fibrosarcoma was present on the forehead, the owner was unable to bear the stench of the cryolesion. Hence, despite apparent improvement in the condition (**Plate 45**), the

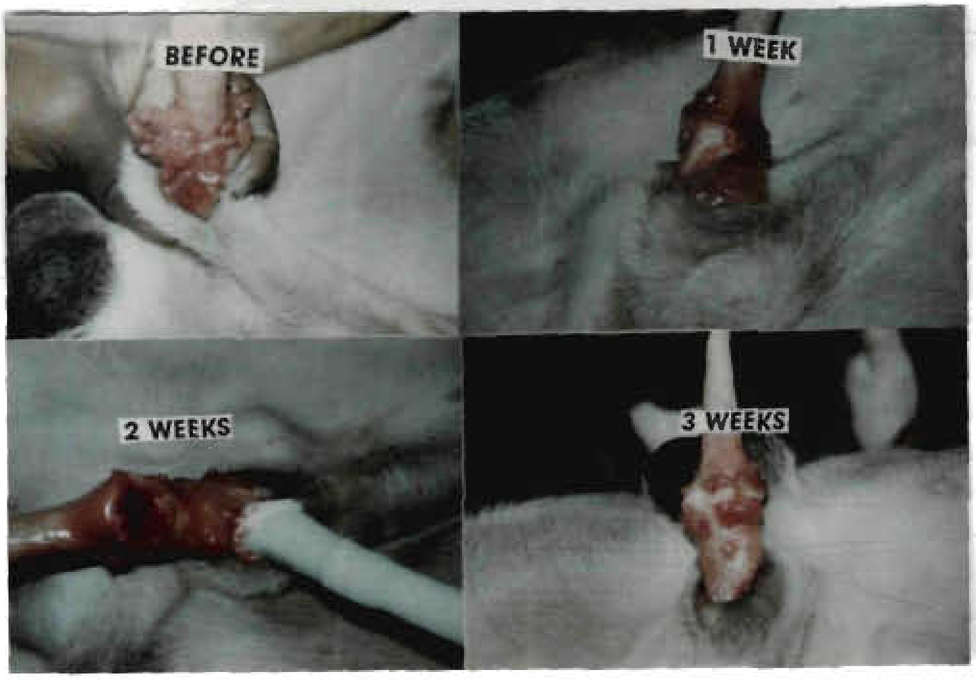


PLATE-44. CRYOSURGERY OF TVT PENIS. REMNANTS DISAPPEARED AFTER 3rd TREATMENT

PLATE-45. CRYOSURGERY OF FIBROSARCOMA ON FOREHEAD. DOG EUTHANISED dog had to be euthanised. The fifth, a case of fibrosarcoma of the vulval lip resolved completely following cryosurgical treatment (**Plate 46**). All the dogs were treated with liquid nitrogen spray using either the 25 mm or 5 mm spray cone.

4.6.3 Hyperplastic Otitis Externa

The external ear canal was completely blocked due to hyperplastic changes and these five dogs were presented with history of purulent discharge from the ears. The medicaments could not reach the horizontal part of the ear canal because of the hyperplastic otitis. In all the five dogs, 3-4 weeks following cryosurgical treatment with Cryosuper VE-4, the ear canal opened up, due to necrosis and sloughing of the hyperplastic skin, enabling medication into the ear. Following antibiotic treatment for otitis, the presenting symptoms disappeared. One day after cryosurgical treatment, the dogs stopped scratching the ear due to palliative effect of cryosurgery.

4.6.4. Oral Papilloma

In both the dogs treated cryosurgically with 2 mm spray cone of Frigitronics CS-76 for oral papilloma, the lesions sloughed off and there was complete recovery (**Plate 47**).

4.6.5 Perianal fistula

In all the three dogs treated with cryosurgery using nitrous oxide with Cryosuper VE-4, the fistulae healed up. There was complete recovery.

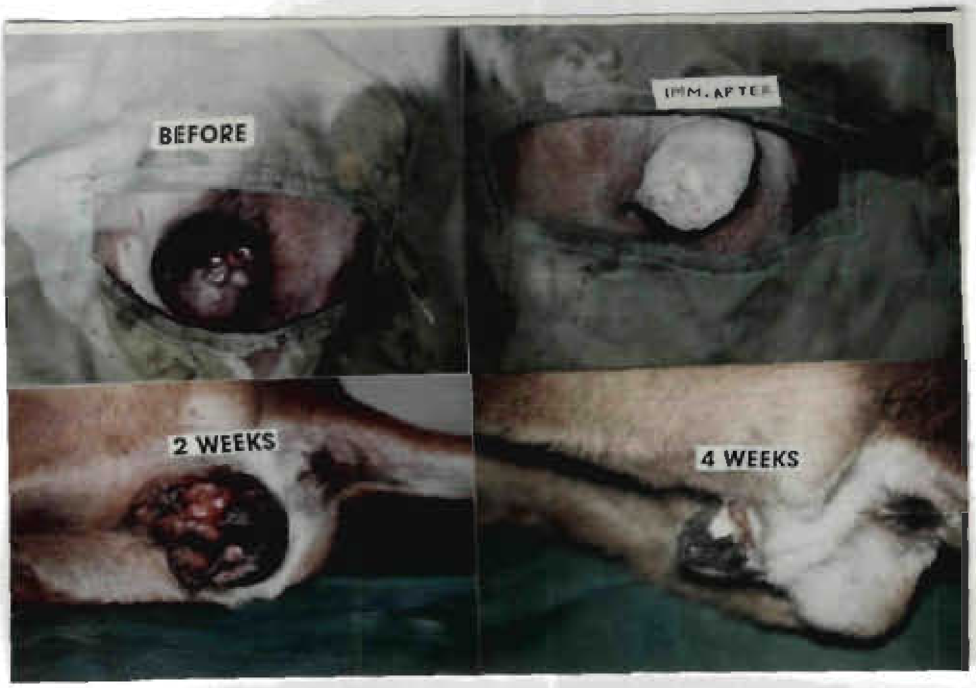


PLATE-46. CRYOSURGERY OF FIBROSARCOMA OF VULVAL LIP

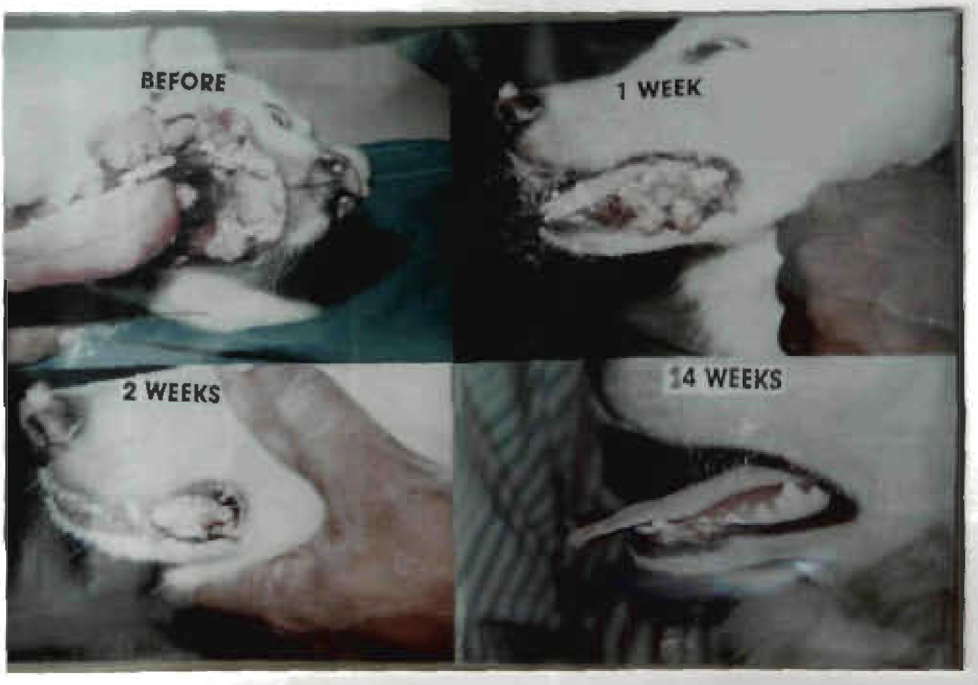


PLATE-47. CRYOSURGERY OF ORAL PAPILLOMA

4.6.6 Perianal Adenoma

In the three dogs with perianal adenoma, the condition was considered inoperable because of their proximity to anal sphincter and hence cryosurgery was attempted. The lesions turned black, sloughed and healed completely. A representative case is presented in plate **48**.

4.6.7 Lick Granuloma

In 5 cases of lick granuloma, measuring from 1-2 cm in diameter, the lesions completely disappeared following cryosurgical treatment with cryosuper VE-4 using carbon dioxide. However, the lesions recurred after 3-5 months in all the cases.

4.6.8 Sebaceous Adenoma

There was complete recovery in both the cases. They were treated with Cryosuper VE-4 using the punch method. One of the two cases is depicted in plate **49**.

4.6.9 Adamantinoma

A lesion of adamantinoma, where the tumour was located between the lower left canine and premolar, sloughed and disappeared completely following cryosurgical treatment with nitrous oxide using Cryosuper VE-4.

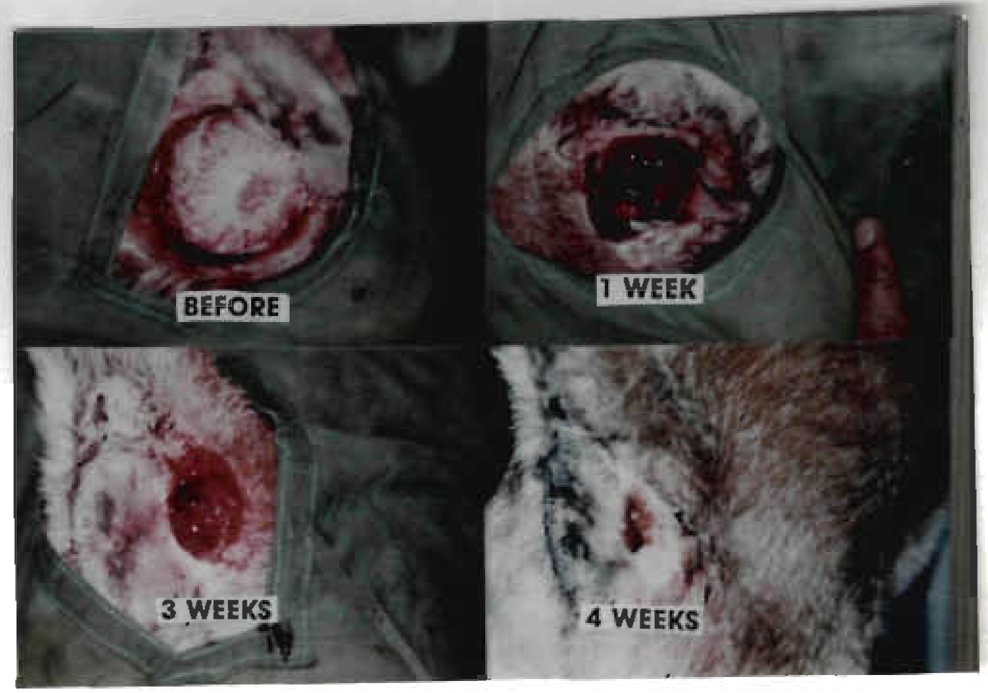


PLATE-48. CRYOSURGERY OF PERIANAL ADENOMA

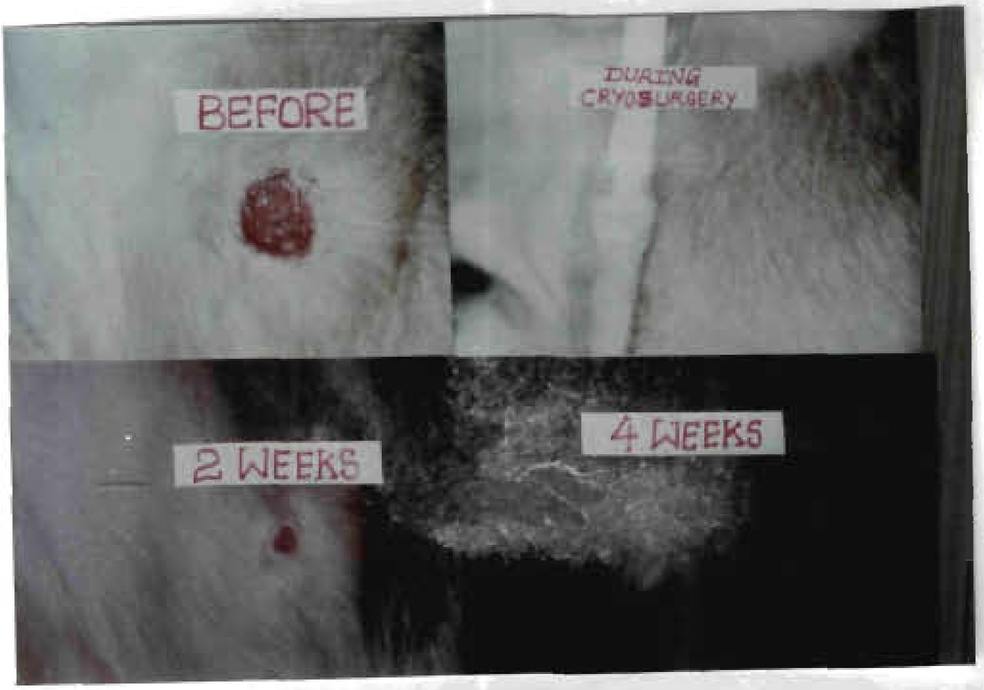


PLATE-49. CRYOSURGERY OF SEBACEOUS ADENOMA OF THIGH

4.6.10 Fibroma

The three fibroma lesions regressed following cryosurgical treatment. One of the three cases, which was located on the digit and treated by liquid nitrogen spray using Frigitronics CS-76 cryosurgical unit is presented in plate **50**. In the other two dogs, the tumours were located in the ear canal and were treated with Cryosuper VE-4. One of these two cases in depicted in plate **51**.

4.6.11 Cataract

In three dogs with mature bilateral cataract, extracapsular cryoextraction of lens was performed using the Cryosuper VE-1 with nitrous oxide as the refrigerant. All the three dogs regained vision in the operated eye. The sutured limbal incisions healed uneventfully following antibiotic therapy. The post-operative appearance of one of these three dogs is depicted in plates **52 & 53**.

PLATE-50. CRYOSURGERY OF FIBROMA OF DIGIT

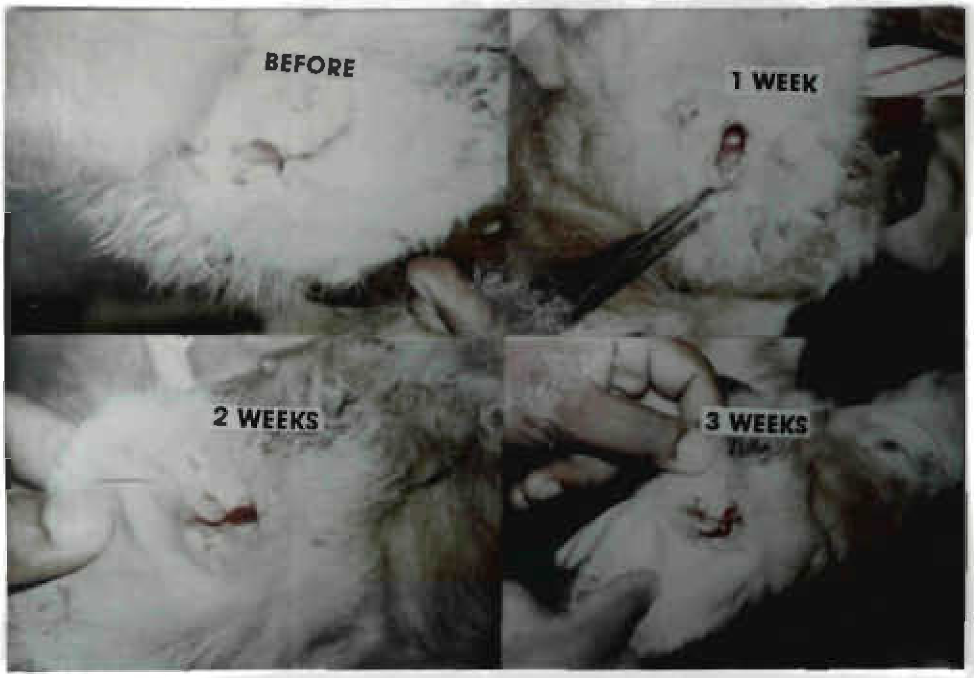


PLATE-51. CRYOSURGERY OF FIBROMA OF EAR CANAL

PLATE-52. MILD CORNEAL OEDEMA NEAR SUTURE LINE. 7th POST-OPERATIVE DAY

PLATE-53. CORNEAL OEDEMA REDUCED. 14th POST-OPERATIVE DAY

Discussion

CHAPTER V

DISCUSSION

Cryosurgery, defined generally as the controlled destruction of tissues by extreme cold, can also be used for producing cryoadhesions between two different tissues or between the cryoprobe and the target tissue. Therefore, it is used in the treatment of various neoplastic and non-neoplastic conditions and for extraction of lens in cases of cataract.

The art and science of cryosurgery, though introduced into veterinary surgery in the early 1970s, has come a long way in the treatment of various neoplastic and non-neoplastic conditions in domestic animals. The monitoring of cryosurgical treatment with newer imaging equipment like CT, ultrasound scanners, MRI (Reiser ***et al.,*** 1983; Isoda, 1989; Onik ***et al.,*** 1991) and its beneficial combination with chemotherapy (Goldstein and Hess, 1976; Homasson ***et al.,*** 1992) have added new impetus to its continued usage. Further, as stated by Gage (1989), the therapeutic modality of cryosurgery, in which interest had apparently been lost over the last few years, needs further experimentation and clinical usage. A collaboration of cryobiologists and cryoengineers has been advocated by the same author, to infuse vitality into this field and hence the current interest in the field of cryosurgery. In addition, several new clinical possibilities have been suggested and put to practice by several workers; viz., its use in cardiac surgery (Holman ***et al.,*** 1983; Misaki ***et al.,*** 1983), orthopaedic surgery (Kramek ***et al.,*** 1986) and in the treatment of abdominal

(Reiser *et al.*, 1983; Isoda, 1989), thoracic (Neel *et al.*, 1973[a]; Schlinkert and Chambers, 1990) and urogenital neoplasms (Richart *et al.*, 1980; Hemmingsson *et al.*, 1981; Idowu, 1985; Matsunaga *et al.*, 1987; Kristiansen *et al.*, 1991; Onik *et al.*, 1991). Certain observations made through the literature published on cryosurgery like ambiguity in the accepted lethal temperature of tissues, on the effect of freezing blood vessels and the lack of published reports on the systemic effects of cryosurgery and the effects of freezing ocular structures, prompted the selection and execution of the present experimental and clinical study on cryosurgery in dogs.

5.1 Part-A: Evaluation of Cryosurgical Units

Goldstein (1977) opined that nitrous oxide cryosurgical units produced ice balls whose nature of spread was more predictable, as compared to liquid nitrogen cryosurgical units, the ice balls of which penetrate rapidly and tends to over-freeze tissues. In the opinion of Crane (1978), cryosurgical units should be evaluated for their individual suitability. Therefore, the three cryosurgical units used in the present study were evaluated.

The results indicated that the Frigitronics CS-76 cryosurgical unit produced the lowest temperatures within the shortest period of time both at the probe tip and the sub-cutaneous levels. The fact that the Frigitronics CS-76 cryosurgical unit continued to function after a prolonged usage of 5 minutes indicated its versatility and its efficient thermal insulation mechanism. The Cryosuper VE-4, on the other hand, evidently due to inadequate thermal insulation, allowed ice formation and jamming of the movable parts of the cryogun or handle and the exhaust outlet thereby limiting the minimal probe tip and the sub-cutaneous temperatures that could be achieved. This also limited the application of Cryosuper VE-4 to treatment of only small and superficial lesions. Routh (1992) also observed that nitrous oxide cryosurgical units freeze small areas of tissues to -40°C. However, the probe tip temperatures attained (-55°C) both with the use of nitrous oxide and carbon dioxide were considered adequate for treatment of diffuse and superficial lesions like TVTs in male dogs, hyperplastic otitis externa and lick granulomas. Nevertheless, the inadequate insulation which caused malfunction of the Cryosuper VE-4, prevented the advantages that could have otherwise been derived from the use of nitrous oxide, which as stated by (Podkonjak, 1982[a]), is capable of producing cooler temperatures than the comparatively warmer carbon dioxide.

The Cryosuper VE-1 resulted in cryogenic temperatures of -80°C and -70°C at the probe tip with the use of nitrous oxide and carbon dioxide respectively, which are comparable with the results published (Podkonjak, 1982[a]). The cooler temperatures produced by this cryosurgical unit probably resulted due to the location of the probe and the control foot switch at a comparatively longer distance from the exhaust out let. This probably resulted in a larger area of heat exchange from the atmosphere and prevented ice formation at the foot switch and the out let.

It was concluded that Frigitronics CS-76 cryosurgical unit was more suitable for large and deeply infiltrated lesions, while the use of Cryosuper VE-4 was limited to the treatment of superficial lesions. This was in conformity with the observations of Goldstein (1977) who stated that liquid nitrogen cryosurgical units tend to over-freeze tissues. Therefore, the Frigitronics CS-76 cryosurgical unit was used for the treatment of large lesions and the Cryosuper VE-4 cryosurgical unit for the smaller ones.

Podkonjak (1982[a]) stated that an ideal cryosurgical unit should be capable of freezing delicate tissues like cornea and large lesions like equine sarcoids. However, none of the three cryosurgical units tested were considered to be suitable for the treatment of all kinds of lesions encountered in the present study. While Frigitronics CS-76 cryosurgical unit was noticed to have produced too rapid a fall in the temperature (Fig. 1), Cryosuper VE-4 was not capable of producing temperatures below -55°C. Additionally, the inherent defects in thermal insulation mechanism of the latter abolished the differences between nitrous oxide and carbon dioxide as cryogens. Hence, during treatment of clinical cases, no distinction was made between nitrous oxide and carbon dioxide since both produced similar probe tip and sub-cutaneous temperatures.

The Cryosuper VE-1 cryosurgical unit was found to be suitable for ophthalmic cryosurgery. Divine and Anderson (1983) also considered that nitrous oxide cryosurgical units are more suitable for ocular cryosurgery than liquid nitrogen cryosurgical units.

5.2 Part-B: Group-I: Local Effects of Freezing

5.2.1 Freezing Skin

Irrespective of the temperature to which skin was frozen, the events that ensued following cryosurgery depicted changes that were common to all the cryolesions. By about 6 hours following double freeze-thaw cycle, oedema and intense erythema of the lesions was a consistent finding. During the next five days, all the lesions gradually turned blackish in colour and appeared to be necrosed on gross pathological examination. Several workers have also reported similar findings (Fraser and Gill, 1967; Joyce, 1976; Bushby ***et al.***, 1978; Burge ***et al.***, 1986; Natiella ***et al.***, 1987; Burge and Dawber, 1990). Sloughing of the necrosed parts of the cryolesions started by the fifth day and was complete in all the cases by the eighth post-operative day. It was also reported by Joyce (1976) that the cryolesions sloughed by the 7th to 10th post-operative days.

The similarities in the cutaneous cryolesions were limited to gross pathological observations and they differed from one another in the histopathological sections, depending on the freezing temperature. The biopsy specimens were obtained on the 5th post-operative day, because at this time, all the cutaneous cryolesions appeared to be necrosed in all the dogs and no apparently necrosed portions remained by the end of the 7th day. The collection of biopsy specimens on the 5th post-operative day were therefore considered ideal for microscopic examination to assess whether necrosis was complete. The sections obtained on the 5th post-operative day, from the sites frozen to -10°C and -20°C depicted that the necrosis was incomplete and islands of live and viable tissues still persisted in some areas of the sections. Several sebaceous glands and hair follicles were found to have remained viable, indicating that freezing of skin upto -20°C was inadequate in producing complete necrosis of all the dermal structures, although they appeared to be necrosed on gross pathological observation. This observation was contrary to the report of Gill ***et al.*** (1971), who stated that all frozen tissues die, and those of Borthwick (1972), Joyce (1976), Seim

(1980), Chambers and Slatter (1984) and Seim and Hoyt (1985), who stated that necrosis resulted due to double freeze-thaw cycle to temperatures of upto -20°C. On the other hand, complete necrosis of all the structures including glands and hair follicles as noticed by the lack of cellular details in all the sections, indicated that skin should be frozen atleast to a temperature of -30°C in a double freeze-thaw cycle to ensure complete necrosis of the skin. These findings corroborate well with those of Goldstein and Hess (1976) and Bojrab (1978). The results indicated that although lesions might appear black and exhibit symptoms of necrosis when frozen to temperatures of -20°C and above, a few cells might remain viable. The clinical implications of discontinued freezing on achieving temperatures upto -20°C, during treatment of cutaneous neoplasms can well be imagined. The outcome of discontinued freezing, due to reassurance from the gross pathological appearance of the cryolesion, is tantamount atleast to leaving some viable neoplastic cells during surgical excision as stated by Joyce (1976), and to inadequate destruction of neoplastic tissues by cryosurgery as stated by Kristiansen ***et al.*** (1991). Further, the observation of Burge ***et al.*** (1986), who found that melanosomes still remained viable after gross pathological assessment of complete necrosis of the cryolesion, emphasised the need for freezing skin to atleast - 30°C in a double freeze-thaw cycle, to ensure complete necrosis. This observation in the experimental part of the present study seemed even more important, especially when seen in the light of the observations of Krahwinkel (1980), who stated that the most common of all the neoplasms seen in dogs are cutaneous neoplasms. In addition, the report of Macdonald and Critchlow (1987), which stated that two or more episodes of cryosurgery in itself is tumour potentiating, emphasised the need for ensuring adequate necrosis at the first attempt.

However, the observations of Macdonald and Critchlow (1987) needs further investigation.

Once the lesions had sloughed off, they again appeared similar on gross pathological examination. The histopathological evidence of the presence of a few hair follicles and glands in the lesions frozen to -10°C and -20°C obtained on the 28th post-operative day confirmed the earlier finding of the presence of islands of live tissue. However, lesions frozen to -30°C and below showed complete necrosis of the entire skin. The histopathological observations of the present study are in corroboration with those published by Bushby ***et al.*** (1978), Burge ***et al.*** (1986) and Natiella ***et al.*** (1987), who, however, did not study the effects of freezing skin to different temperatures.

It was concluded from the results of the present study that gross pathological observation of necrosis of the cryolesions was misleading and for attaining assured necrosis of all the structures of the skin, it should be frozen twice to temperatures of -30°C, preferably below. It can also, perhaps, be concluded that freezing should continue till 2-3 mm of the healthy surrounding tissue attained a temperature of atleast -30°C irrespective of the temperature at the center of cutaneous neoplastic lesions during their cryosurgical treatment.

5.2.2 Freezing Gingiva

The results indicated that severe oedema of the gums, lips and cheek occurred following cryosurgery of the gums.

The results showed that even a temperature of -10°C was adequate to produce complete necrosis of the gums. This probably resulted due to thin gingival tissue and the underlying maxillary bone, which resisted withdrawal of heat, as indicated by its normal appearance. Attempts to rewarm the frozen gums were also perhaps limited by the underlying bone (maxillary), which is reported to be resistant to cryosurgical damage (Goldstein and Hess, 1976 and 1977). Another contributory factor for necrosis of gums at comparatively higher temperatures resulted due to higher water content of gums (as compared to skin). This explanation was based on the observations of Szyszkowska ***et al.*** (1983), who stated that cryonecrosis also depended on the water content of tissues. Taking into consideration the severity of oedema of the lips and cheeks, as observed in the present experimental study and also the reports of complications due to oral cryosurgery by Harvey (1980) and Withrow (1982), it is advisable not to over freeze oral structures. Therefore, it can be concluded that during treatment of oral neoplasms, especially of the gums, freezing can be safely discontinued once the 2-3 mm of the healthy gingival area surrounding a lesion, attained a temperature of -10°C. This, while ensuring necrosis of gums, also minimised oedema and the accompanying discomfort to the animal. It was also observed that the gingival cryolesions healed with minimal scar formation.

5.3 Group-II: Freezing Femoral Artery and Cephalic Vein

5.3.1 Freezing Femoral Artery

Following freezing of the femoral artery, it was observed that the artery thawed within 10 seconds and blood supply through the frozen segment resumed thereafter. Holman ***et al.*** (1983) observed that the thawing time of coronary artery in dogs was

29 seconds. Marsland **et al.** (1983) and Rekhter and Mironov (1990) observed changes like necrosis of arterial wall, myointimal thickening etc., which could have resulted due to small size of the artery and prolonged thawing time.

The results revealed the presence of small amounts of haemorrhage around the femoral artery on the 7th post-operative day which reduced further by the 14th post-operative day. In one dog sacrificed on the 7th post-operative day, however, extensive extravasation around the femoral artery covering the entire medial aspect of the thigh was noticed. These changes were thought to have resulted due to increased permeability of the arterial wall, since no sign of arterial rupture was evident in any of the dogs. Whitaker (1972) following Cobalt 57 labelling studies, stated that arterial wall permeability increased due to cryosurgery. While various workers (Borthwick, 1972; Seim, 1980; Holman **et al.,** 1983) have stated that arteries are unaffected by cryosurgery, the reports of Whitaker (1972), Anderson **et al.** (1983), Marsland **et al.** (1983) and Rekhter and Mironov (1990) documented some changes in the arterial wall.

The radiographic and histopathological evidence of thrombosis in two of 12 dogs and the mild changes noticed in the arterial walls on histopathological examination suggested that arteries are not totally resistant to cryosurgical damage, although it does not lead to arterial rupture or necrosis and sloughing and consequent haemorrhage. This finding is contrary to those reported by Fretz and Holmberg (1980) who stated that severe haemorrhage might result due to freezing of arteries that lie near or run through lesions under cryosurgical treatment. Borthwick (1972) stated that the white and elastic connective tissue content of arteries rendered them resistant to cryodamage. However, present findings are in agreement with those of Whitaker (1972), Anderson *et al.* (1983) and Marsland *et al.* (1983), who reported similar changes like thrombosis and degenerative changes in the arterial walls of dogs, foetal lambs and rats respectively. Following experimental cryosurgical damage of myocardium in close proximity to the coronary arteries, Misaki *et al.* (1983) observed no changes in the coronary artery. In the present study, however, cryodamage was directly inflicted on the artery and hence the changes in the femoral artery.

In the present study, the quick thawing (10 seconds) due to warming by arterial blood provided some protection to the artery against the cryoinjury. It is well known that quick thawing resulted in lesser extent of cellular damage. Roberts *et al.* (1984[a]) reported that when thawing was quick, the small intra and extracellular ice crystals melted before they could recrystallise into larger crystals, thereby giving a greater chance for the cells to survive. This perhaps happened in the present instance which accounted for the minimal changes seen in the femoral arterial wall of all the dogs.

5.3.2 Freezing Cephalic Vein

The thawing time which was recorded as 1-2 minutes in the present study, was considered sufficient to result in necrosis of the cephalic vein. The cryosurgical insult inflicted on the veins resulted in complete occlusive thrombosis and necrosis of the cephalic vein. However, absence of severe haemorrhage, development of a network of capillaries and venules anastomosing the proximal and distal parts of the cryonecrosed portion of the cephalic vein and minimal clinical symptoms accompanying these changes indicated that lesions near or around large veins can be safely frozen without fear of any resultant severe haemorrhage. The clinical observations of oedema seen during the early post-operative period were considered as the natural sequelae of venous occlusion. In addition, the disappearance of the swelling and oedema by the 14th post-operative day rendered these symptoms insignificant. The limited observation period of 14 days precluded the assessment of the ultimate fate of the necrosed portion of the vein. However, the intensity of the inflammatory cellular infiltration around the necrosed portion of the veins suggested the animal's response of phagocytosis aimed at subsequent resorption of the necrosed vein.

5.4 Group-III: Systemic Effects of Freezing

The fact that no complications like sepsis or infection occurred at the frozen site despite provision of no post-operative care of any kind indicated the safety of cryosurgery. Following the sloughing of parts of the lesions, serosanguinous discharges noticed were transient and of no consequence. The scar formation following healing was minimal. In the dogs where 2.5 percent of the body surface area was frozen, the RBC count, haemoglobin content and PCV remained within normal limits throughout the observation period. The total leucocyte count began to increase from the first post-operative day as a response to the cryosurgical insult. The fact that neutrophil count began increasing upto the 7th post-operative day and thereafter the increase in monocyte counts indicated the attempts by the animal to phagocytose and remove the necrotic areas. However, once the lesions sloughed off, the need for the increase in these phagocytic cells also rescinded and hence these values came back to near normal levels by the 21st post-operative day. The increase in AST was also due to its release from the necrosed tissue and AST levels remained high as long as the necrosed tissues remained attached to the body. Once the lesions sloughed, the AST levels also returned to pre-operative levels. The serum potassium and total serum protein levels remained within normal limits throughout the observation period.

When 5 percent of the body surface area was frozen, the changes were similar to the above mentioned findings, but to a greater degree because of the more pronounced cryosurgical insult. But for the difference in the degree of increase in the AST, total leucocyte, neutrophil and monocyte counts, the animal's response was found to be similar. In these six dogs also the serum potassium and total serum protein were within the normal limits. Total serum protein was estimated to examine whether the serosanguinous discharges affected its level. It has been reported by Coles (1982) that serum potassium levels increase whenever there is necrosis of the tissues.

The results of the haematological examination revealing no significant difference between the pre-operative and post-operative levels of RBC, haemoglobin and PCV and that of serum proteins on biochemical examination indicated that haemorrhage was not an accompanying feature of cryosurgery and that the serosanguinous discharge was also of no consequence because of normal serum protein levels, in both the groups of dogs where 2.5 and 5 percent of the body surface area was subjected to cryosurgery. The significant increase in the AST levels indicated the normal response to necrosis, especially of muscles. Since some sub-cutaneous muscles were also necrosed, it accounted for the increased AST levels. The observation of the normal levels of serum potassium throughout the observation period indicated that the potassium released from necrosed tissues was promptly excreted.

All the above mentioned findings indicated that cryosurgery was indeed the most physiological method of producing necrosis as also stated by Balthasar (1957).

The increase of total leucocyte counts upto the 14th post-operative day and the increase of neutrophil count upto the 7th day indicated the normal body response to injury. The increase in the levels of monocytes by the 7th day, i.e. till the necrosed portions were still attached to the animals body were considered to be the normal response of phagocytosis directed against necrotic portions. Therefore, the cryosurgical method of causing necrosis was considered to be associated with very little systemic response. At the same time, the observation of increased levels of AST, total leucocyte, neutrophil and monocyte counts in the present study and the reports of Neel ***et al.*** (1973[a]) and Weyer ***et al.*** (1987) who suggested an increase in the immune response in the animals clearly showed that cryosurgical lesions were by no means 'biologically inert' as stated by Fraser and Gill (1967). The present study confirms the findings of Balthasar (1957) who stated that cryosurgery was the most physiologically accepted method of creating necrosis.

5.5 Group - IV : Effects of Freezing Cornea and Iris during Extracapsular Cryoextraction of Lens

5.5.1 Control

The results indicated that following extracapsular cryoextraction of lens in control dogs, the clinical, gross and histopathological changes were limited to the areas around the suture line. This suggested that the changes were due to incision and suturing the cornea and were considered its normal consequence. The decrease in the intraocular pressure also was surmised to be the result of incising the cornea and entry into the eye ball.

5.5.2 Effects of Freezing Cornea

Following freezing of cornea the fluorescein dye test showed no evidence of ulceration or erosion of corneal epithelium, suggesting that cornea is resistant to cryoinjury. However, the observation of corneal opacity for 7 days following cryosurgery indicated the normal corneal response to injury. Histopathological examination of the cornea revealed changes like slight thickening of the corneal epithelium, mild inflammatory cell infiltration and oedema which are in conformity with the finding of Farris ***et al.*** (1977), Holmberg ***et al.*** (1986) and Chan ***et al.*** (1990). Farris ***et al.*** (1977) further noticed corneal oedema following its freezing which spontaneously resorbed by the 7th post-operative day.

In this group also the decreased intraocular pressure was thought to be due to surgical entry into the eye ball. The results indicated that all these changes, because of their transient nature, could be ignored. Vision was not affected in any animal.

5.5.3 Freezing of Iris

The changes noticed in the iris like necrosis of muscles, free melanin pigment in the iridial musculature and anterior synechia were in agreement with the findings of Sudarsky ***et al.*** (1965) and Barrie ***et al.*** (1980). However, unlike the report of the former, no full thickness holes were noticed in the iris during the present study.

It was concluded from the results of the present experimental study, that during extracapsular cryoextraction of lens in dogs, all possible care should be taken to prevent freezing of iris since the changes in iris were found to be more serious than the changes in cornea. Changes in iris following cyclocryosurgery were also observed by Vestre and Brightman (1983[b]).

It was concluded that during extracapsular cryoextraction of lens, although it is important not to freeze any other ocular structures, such a possibility exists. This finding, although seems trivial, gains importance due to the fact that a very small space of a few millimeters is available between cornea and iris for the manipulation of the cryoprobe. Even within this limited space available the present experimental study indicated that it is better to keep the cryoprobe as far away from the iris as possible, since the iridial response to cryosurgery was considered to be serious.

5.6 Pre-operative Preparation, Post-operative Care and Anaesthesia for Cryosurgery

With the exception of dogs where extracapsular cryoextraction of lens was done, the pre-operative preparation was very minimal and consisted of shaving and cleaning the areas and lesions (in clinical cases) to improve thermal contact. It was found that this minimal pre-operative preparation was sufficient. Post-operative care consisted of administration of local and parenteral antibiotics in the dogs where extracapsular cryoextraction of lens was done. In all the other cases, both experimental and clinical, no post-operative care of any kind like wound dressing and administration of antibiotics was considered necessary. It was also reported by Farris ***et al.*** (1975), Podkonjak, (1982[b]) and Henk (1985) that cryosurgery needed little pre-operative preparation and no post-operative care. It has been suggested by Joyce (1976) and Podkonjak (1982[a]) that the necrosed portion itself acted as a biological dressing thereby obviating the need for wound dressing. The administration of antibiotics during ocular cryosurgery in the present study was considered necessary because the procedure involved surgical incision of the cornea, which is likely to get infected if no antibiotic cover is provided. In all the other cases (both experimental and clinical), no instance of infection was recorded inspite of not providing antibiotic cover. This confirmed the findings of Farris ***et al.*** (1975), Fretz and Holmberg (1980) and Withrow (1982) that cryolesions seldom get infected. However, Fretz and Barber (1980) reported infection following cryosurgery in one of the horses being treated for equine sarcoids, which can be ignored as an exception rather than the rule.

In all the experimental dogs, general anaesthesia was administered because the procedures involved creation of multiple defects and cryosurgery of delicate structure like blood vessels. In the clinical cases, wherever possible, anaesthesia was avoided and it was found that the procedures were well tolerated. In instances where anaesthesia was administered, it was because of the location of the lesion or because the dogs were uncooperative. Several workers have also reported that for cryosurgery, anaesthesia was not necessary (Crane, 1978; Bryson ***et al.***, 1985; Matsunaga ***et al.***, 1987). Further, Rao (1990) also reported that unless the location of lesions so demanded, anaesthesia was often unnecessary for cryosurgery in dogs.

5.7 Part-C : Clinical Study

The results of 80.85 percent cure rate, i.e. 38 of the 47 dogs with various neoplastic and non-neoplastic lesions compared well with the published results. (Lane and Burch, 1975; Lane, 1977; Farris, 1980; Fretz and Barber, 1980; Budsberg ***et al.***, 1981; Hemmingsson ***et al.***, 1981; Idowu, 1985; Omara-Opyene ***et al.***, 1985; Lanigan and Robinson, 1987). One case of TVT which recurred 3 months after its initial resorption was probably due to overlooked infiltrated parts of the lesion, as suggested by Kristiansen ***et al.*** (1991).

The failure of cryosurgery in resolving 4 out of the 5 cases of fibrosarcoma indicated its unsuitability in the treatment of such lesions. The resultant oedema of the oral cavity hindering food consumption was found to be the complication that should be kept in mind while treating such lesions by cryosurgery. Similar complication were also encountered by Harvey (1980). Withrow (1982) reported formation of holes in the palate following cryosurgery of the oral tumours in dogs. Cryosurgery was also found unsuitable for the treatment of lick granulomas, since all of them recurred following initial resorption. As also stated by Krahwinkel ***et al.*** (1976), this probably resulted due to regeneration of sensory nerve endings following their destruction by cryosurgery. The other cases treated had no complications and the lesions were found to have healed with minimal scar formation. The cases of perianal adenomas, lying in close proximity to the anal sphincter, had sloughed off and healing was considered satisfactory without any damage to the anal sphincter. Orth ***et al.*** (1992) also reported that cryosurgery of rectal cancer in man obviated the need for an artificial anus in several cases. The outcome of extracapsular cryoextraction of lens was considered satisfactory, since vision was restored in all the three dogs.

5.8 Conclusions

The following conclusions were drawn from the present study:

1. Frigitronics CS-76 Cryosurgical unit was suitable for large and infiltrative lesions while Cryosuper VE-4 was found suitable for small superficial lesions. Cryosuper VE-1 was considered suitable for ocular cryosurgery.

2. Skin should be frozen to temperatures of -30°C or below to ensure complete necrosis.

3. Necrosis of the gingiva was achieved by freezing to temperatures of -10°C or below.

4. Lesions around large arteries and veins can be treated by cryosurgery. Though degenerative changes and thrombosis of the artery and necrosis and thrombosis of cephalic vein occurred, it did not lead to extensive haemorrhage in the present study.

5. Cryosurgery produced necrosis of tissues accompanied by minimal and transient systemic changes.

6. Cornea was found to be comparatively more resistant than iris to cryosurgery.

7. Cryosurgery required very little pre-operative preparation and no post-operative care.

8. Oedema of the oral mucous membrane, hampering food intake following cryosurgery of oral tumours and malodour from treatment of large neoplasms were common complications of cryosurgery.

Summary

CHAPTER VI

SUMMARY

The present experimental and clinical study on cryosurgery in dogs was conducted on 60 experimental dogs and 50 clinical cases presented for treatment of various neoplastic and non-neoplastic conditions at the Madras Veterinary College Clinics.

The layout and design of the present experimental and clinical study was as follows

PART		NUMBER OF DOGS
PART A		
Evaluation of the cryosurgical units		6
PART B		
Group I	Local effects of freezing	12
Group II	Effects of freezing femoral artery and cephalic vein	12
Group III	Systemic effects of freezing	12
Group IV	Effects of freezing cornea and iris during extracapsular cryoextraction of lens	18
PART C		
Clinical study		50 Clinical cases

Part-A: Evaluation of Cryosurgical Units

It was observed that Frigitronics CS-76 Cryosurgical unit produced spray cone tip and probe tip temperature of -195°C and -160°C within 25 and 40 seconds respectively and sub-cutaneous temperatures of -170°C and -130°C within 40 and 65 seconds respectively with the use of spray cone and closed end probe. Liquid nitrogen was used as the refrigerant.

The cryosuper VE-4 cryosurgical unit produced minimal probe tip temperature of -55°C within 30 and 35 seconds and sub-cutaneous temperature of -40°C within 50 and 60 seconds when nitrous oxide and carbon dioxide respectively were used as cryogens.

The Cryosuper VE-1 cryosurgical unit produced minimal probe tip temperatures of -80°C within 40 seconds and -70°C within 50 seconds with the use of nitrous oxide and carbon dioxide respectively.

It was concluded that while Frigitronics CS-76 was suitable for the treatment of large and infiltrative lesions, Cryosuper VE-4 was suitable for small superficial lesions. Cryosuper VE-1 was suitable for ocular cryosurgery.

Part-B: Group-I

In this group the skin and gingiva were frozen to six different temperatures of -10°C, -20°C, -30°C, -40°C, -50°C and -60°C in double freeze thaw cycle at six different places in 12 dogs.

The results indicated that skin should be frozen to atleast -30°C in double freeze-thaw cycle to ensure complete necrosis of all its structures. On the other hand, freezing of gingiva to a temperature of -10°C in a double freeze-thaw cycle resulted in complete necrosis. Both the skin and gingival cryolesions healed with minimal scar formation.

Group-II

In the 12 dogs of this group, the femoral artery and cephalic vein were frozen in double freeze thaw cycle. The effects were studied by observing the clinical, gross pathological, contrast radiographic and histopathological changes.

It was observed that while thrombosis was evident in 2 dogs, all the 12 dogs depicted mild degenerative changes in the femoral artery. Cephalic vein showed necrosis and complete occlusive thrombosis, accompanied by well developed collateral circulation. It was concluded that lesions around large blood vessels can be treated by cryosurgery without fear of serious haemorrhage.

Group-III

In this group of 12 dogs, 2.5 and 5 percent of the body surface area was frozen skin deep to a sub-cutaneous temperature of -50°C in 6 dogs each.

The results indicated no changes in the total RBC, haemoglobin, packed cell volume, total serum protein and serum potassium levels. However, increase in the levels of total leucocyte, neutrophil and monocyte counts and AST levels were noticed in both the sets of dogs.

It was concluded that though cryosurgery produced minimal systemic changes, the cryolesions were not biologically inert.

Group-IV

This group comprised of 18 dogs. In six dogs which served as controls, extracapsular cryoextraction of lens was done. In six dogs each, this procedure was followed with freezing of cornea and iris.

It was concluded that while cornea was comparatively resistant to cryoinsult with changes like corneal oedema and mild cellular infiltration, iris was more susceptible to cryodamage. The changes in iris included intense cellular infiltration, necrosis and presence of free malanin pigment in the iridial musculature. Anterior synechia was also noticed in all the six dogs.

Part-C: Clinical Study

This comprised of 50 dogs. A cure rate of 80.85 percent (38 dogs) among the 47 dogs treated for various neoplastic and non-neoplastic conditions was achieved. Severe oedema of the oral mucosa during treatment of oral neoplasms and malodour from large cryolesions were the adverse effects noticed. It was observed that treatment of lick granulomas by cryosurgery resulted in recurrence. Extracapsular cryoextraction of lens in three cases of cataract restored vision.

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