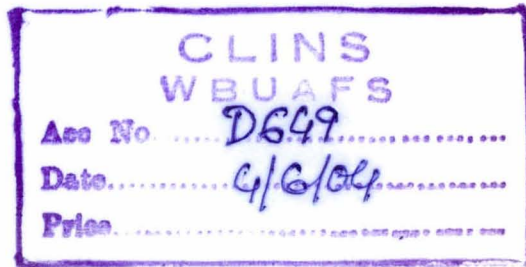


**STUDIES ON EFFECT OF CRYOSURGERY ON
ANAL FISTULA AND VENEREAL
GRANULOMA IN CANINE**

*A thesis
Submitted to the
West Bengal University of Animal and Fishery Sciences
in partial fulfilment of the requirement for the Degree of
Master of Veterinary Science
in*

Veterinary Surgery & Radiology

By
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**DEPARTMENT OF VETERINARY SURGERY AND RADIOLOGY
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DECEMBER, 1999



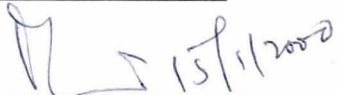


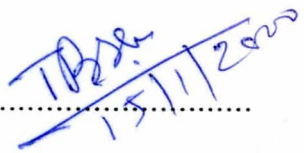
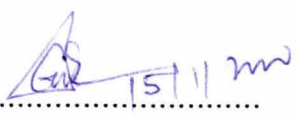
*The Reverent
Dedication to*

My

SISTER

APPROVAL OF EXAMINERS FOR THE AWARD OF THE DEGREE OF MASTER OF VETERINARY SCIENCE

We the undersigned, having been satisfied with the performance of Avijit Dutta in the Viva-Voce Examination, conducted today, the 15th January, 2000, recommended that the thesis be accepted for the award of the Degree.

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CERTIFICATE

This is to certify that the work recorded in the thesis entitled "STUDIES ON EFFECT OF CRYOSURGERY ON ANAL FISTULA AND VENEREAL GRANULOMA IN CANINE." submitted by Avijit Dutta in partial fulfilment of the requirements for the Award of the Degree of Master of Veterinary Science in Veterinary Surgery and Radiology of the West Bengal University of Animal and Fishery Sciences, is the faithful and bonafide research work carried out under my personal supervision and guidance. The results of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

Dated

The 10th Dec, 1999.

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ACKNOWLEDGEMENT

The author expresses his deepest sense of indebtedness and whole hearted thanks to Prof. (Dr.) P. K. Bose, M.v. Sc., Ph. D., Prof., Department of Veterinary Surgery & Radiology, Faculty of Veterinary & Animal Sciences, West Bengal University of Animal & Fishery Sciences for his valuable guidance, constant inspiration, close supervision, Kind advice and encouragement throughout the course of research work.

The author is highly obliged and grateful to Dr. P. K. Samanta, M. Sc. (Vet), Ph. D. (Cal), Reader, and Dr. T. B. Sen, M. V. Sc., Ph. D., Reader & Head, Department of Veterinary Surgery and Radiology; Dr. C. Guha, M. V. Sc., Ph. D., Head, Department of Clinics, West Bengal University of Animal & Fishery Sciences, members of advisory committee for their valuable suggestions, whole hearted encouragement and compassion which enable the author to execute the experiment and preparation of this thesis smoothly.

The author also expresses his deep sense of gratitude and acknowledges his greatfulness to Dr. A. K. Bhattacharya, Vice-chancellor, W.B.U.A. & F. Sc. and Prof. D. P. Banerjee, Dean, U.G. & P.G. studies, Faculty of Veterinary & Animal Sciences, W. B. U. A. & F. Sc.; Dr. D. N. Jana, Director of research Farm & Extension, W. B. U. A. & F. Sc. and other officials of the University and member of the Faculty Council, for the financial assistance and academic facilities extended to the author.

The author records his sincere indebtedness to Dr. D. K. Dey and Dr. N. R. Pradhan, Department of Clinics, W. B. U. A. & F. Sc. for their kind co-operation by referring the cases to the Department of Surgery & Radiology during the course of research work.

The author expresses his sincere gratitude to Dr. D. K. Basak, Head, Department of Veterinary Pathology, for his kind advice during histopathological examination.

The author is very much thankful to Dr. J. Sengupta, M. B. B. S.,

M. D. (Cal) for his learned assistance and encouragement during haematological & biochemical analysis.

It is a gratifying moment for the author to express his heartfelt thanks to Dr. B. Singh, Dr. S. Sanki, Dr. S. Haldar, Dr. P. Bhagat, Dr. A. Raksit and Dr. P. Biswas for their untiring help and encouragement time to time. The author also extends his sincere thanks to Dr. P. Nag, Dr. K. Das, Dr. S. K. Das, Dr. A. Chakraborty, Dr. S. Kumar and Dr. T. Biswas, fellow M. V. Sc. students of this department for their encouragement, active co-operation and help during the course of study and a special thanks goes to Dr. S. Ghatak, Dr. R. Banerjee, Ph. D. students Department of Veterinary Public health and also to Dr. T. Rajak, Ph. D. student, Central Avian Research Institute, IVRI, for their positive support whenever needed.

The author is very much grateful to the staff members of the Central Library of W. B. U. A. & F. Sc. and also to all staff members, Department of Veterinary Surgery & Radiology for their kind co-operation in completing the dissertation work.

The author would like to thanks to M/s. Eastern Industrial Trading Corporation, 92-B Chittaranjan Avenue, Calcutta, for supplying the required instruments during the dissertation work.

Finally, my eternal gratitude and indebtedness to all of my family members for their active co-operation, support, encouragement, patience, alaround sacrifice and blessings which enables the author to complete the task.

Avijit Dutta

(AVIJIT DUTTA)

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ABBREVIATION

b. wt.	-	Body Weight
c. mm.	-	Cubic millimeter
I/M	-	Intramuscular
Kg	-	Kilogram
mg	-	Milligram
No.	-	Number
P.O.D.	-	Postoperative Day
@	-	At the dose rate of
°C	-	Degree Centigrade
°F	-	Degree Fahrenheit
γ	-	Gamma
%	-	Percentage
W.B.U.A. & F.Sc.	-	West Bengal University of Animal and Fishery Sciences.



Chapter I

INTRODUCTION

INTRODUCTION

Dramatic developments in medical technology during the past decade have resulted in improved ability to destroy the diseased tissues, inaccessible to surgical removal and have seen a significant resurgence in interest in the cryonecrosis of the same. Cryo biology, the study of life at subfreezing temperature, is the basic principle behind the cryonecrosis. When it is intended for surgical use, it is defined as Cryosurgery. Thus cryosurgery is the new venture for surgical management of different pathological conditions where the diseased tissues are purpose fully placed in such a biological environment by creating the effect of ultra low temperature, so as to cause their eventual death and destruction.

The utilization of cold temperature for medical therapy is not a new concept rather as early as in 2500 BC the Egyptians used it for treating traumatized patient. The earliest clinical reports of the use of cryosurgery are from the mid 1800s ; ice and brine solutions were applied to human breast and skin tumors (*James Arnott, 1851*) in London. The modern cryosurgery became a reality since the late 1960s by the introduction of cryosurgical apparatus (*Irving Cooper, 1961*) to produce a sustained freezing temperature. Furthermore the availability of different cryogens like liquid nitrogen, nitrous oxide, carbondioxide etc. made it approachable in various diseased conditions. At present cryosurgery is a standard modality in medical science in such disciplines as dermatology to treat skin cancer; in gynaecology for the treatment of chronic cervicitis, in proctology for the treatment of haemorrhoids and also in neurology, ophthalmology and urology.

In the Veterinary science, cryosurgery is well documented and a wide range of its use has developed in such conditions where it is equally effective or superior to the conventional treatments. Practically all neoplastic and nonneoplastic conditions may be treated by freezing in situ. The mostly treated conditions are equine sarcoïd, bovine ocular squamous cell carcinoma, hemangioma, oral papillomas, epulis, granulomas, mast cell sarcomas, interdigital cyst, etc. In fact, inspite of these uses, the technique is still restricted in research level instead of being a extensively practiced surgical technique in field condition. Though the cost to conduct the treat-

ment is minimal, but the high price of the instrument is seemed to be the cause behind its restricted use.

Recently several surgical conditions of animal organs have been tried to cure by cryosurgery not only to evaluate the therapeutic value of the technique but also to find a way for better postoperative management, which is very much important for animal patients. Anal fistula and venereal granuloma are such two conditions, frequently reported in the canine populations.

The therapeutic management of anal fistula or anal sinus or anal furunculosis is a major problem in canines, seen predominantly in adult German shepherd dogs (*Harvey, 1972, Halnan 1976*) and is more common in aged male dogs (*Robins and Lane, 1973*). It has also been reported in Irish setter, Labrador, Cocker spaniel and crossbred animals. Anal fistula is a chronic suppurative inflammatory condition of the perianal, anal and perirectal tissues characterized by discharging sinuses in the perianal region. There are certain etiological factors responsible for this condition namely minute faecoliths lodging in the anal crypts formed by the longitudinal folds of the rectal mucosa, abscessed anal sacs, (*Lacroix and Lacroix, 1945*), trauma associated with foreign bodies and the poor ventilation in the perianal area due to slung, broad based tail in German Shepherd breed (*Lewis, 1968*). Regarding the therapeutic approach, the condition is refractory to medical treatment and the surgical intervention is necessary for a successful outcome. Conventional surgical approaches like - surgical excision (*Harvey, 1972*), chemical cauterization etc. were applied but none of them were satisfactory rather reproduced postoperative complications.

On the other hand ventral granuloma or transmissible venereal tumor is a commonly seen problem in stray dogs of both sexes. The disease is coitally transmitted and mostly noticed in healthy animals within a short period following breeding season, when it protrudes externally with frequent bleeding. The tumor, believed to be derived from cells of the reticuloendothelial system, appears as a multilobulated, cauliflower like, pink, friable mass involving the vestibule and vagina of bitch and the base of the penis with occasional involvement of the prepuce in case of male dog. The tumor is usually benign but occasional metastasis may occur to the

superficial inguinal lymph nodes and to the oral cavity, nasal passage, orbit and skin (*Johnston 1985*). The tumor cells have fewer chromosomes (59) than the normal canine cells (78) and this abnormal number of chromosomes is thought to be responsible for the genesis of tumour. Early treatment by surgical excision or radiation therapy or chemotherapy or immunotherapy is beneficial but not totally satisfactory when the condition aggravates. There are also some harmful side effects following, specially the chemotherapy and radiation therapy, which may be accounted in respect of pet value of the animal patient.

For the aforesaid affections in canine search is still going on to find such a treatment modality which may not be ideal in all aspects but may be nearer to it. Cryosurgery is seemed to be interesting and prosperous in this regard. Before claiming any credit in favour of this, a quick view over its basic principle is necessary for better understanding about it.

The ultimate goal of cryosurgery is to kill all the cells in a diseased area while, causing minimal damage to the surrounding normal tissue. The mechanism of cellular death and destruction is accomplished by a combination of

- direct cellular destruction, and
- anoxia secondary to vascular stasis.

Detailed process of cryonecrosis will be discussed in the subsequent chapter.

Apart from being effective in destroying unwanted tissues, cryosurgery has some remarkable advantages (*Lane, 1974*). As there is no adverse effect on the patient as well as on the operator, it can be repeated as many time as necessary. The overall cryosurgical procedure does not require strict aseptic measures because of the germicidal effect of cryotemperature. During operation hemorrhage is minimal and tissue responses are slight; reactionary hemorrhage and secondary infection in the treated site are not subsequent features. Healing of a cryolesion is not as rapid as first intention healing ; however possibility of dehiscence does not apply and scarring is not marked. Due to the analgesic effect of cold, this procedure is relatively painless. Thus it neither requires general anesthesia nor the postoperative analgesic.

Evidence of immunostimulation following cryosurgery (*Neel, 1980*) is a distinct advantage over the other conventional therapies. Cellular death by freezing is accompanied by antigen formation, probably through the liberation of lipoprotein complex from cell membrane and it rises the circulating antibody against that specific antigen within the body. So the possibility of recurrence as well as metastases may be decreased.

Another important point is that, during treatment of the neoplastic diseases, the cell which could migrate either through blood circulation or lymph flow, when otherwise handled, are frozen in situ by the cryotemperature and thus there is no chance of precipitating metastases.

Like other surgical procedure, cryosurgery also has some limitations like improper cryonecrosis of too large diseased tissues and rare evidences of air embolization (*Harver, 1978*) or subcutaneous emphysema (*Rao et al. 1994*), but only associated with cryospray application.

In view of the above mentioned points, it is expected that the cryosurgical approach may provide lasting relief from certain incurable and/recurable surgical affections which are refractive to the conventional therapies.

Literatures so far available indicate that some works have been taken up on anal fistula and venereal granuloma but unfortunately those studies are incomplete in respect of haematological and biochemical observations.

Therefore the aim of the present study has been primarily focused on clinical, haematological and biochemical observations following cryosurgery on the aforesaid surgical conditions.



Chapter II

**REVIEW OF
LITERATURE**

REVIEW OF LITERATURE

Under this chapter an attempt has been made to review the works on cryosurgery with special reference to its application on anal fistula and venereal granuloma in conjunction with other therapies, on the basis of literatures so far available.

2.1. REVIEW OF LITERATURE ON CRYOSURGERY AND ITS APPLICATION :

The utilization of cold temperature for medical therapy was introduced as long as 2500 BC. when Egyptians used it for treating traumatized patients and both Homer and Hippocrates reported its use in their writings on the control of haemorrhage and edema.

The modern cryosurgery was started with the use of freezing temperature for the therapeutic destruction of tissue in England between 1845 - 1851 when James Arnott described the use of iced salt solutions (about - 18°C to - 22°C) to treat advanced carcinomas of the breast and uterine cervix producing reduction in tumour size and amelioration of pain.

The next step in the development of cryosurgery followed the 30 years period of 1870 to 1900 in which the so-called permanent gases (oxygen, nitrogen, and hydrogen) were liquified. The liquid air was applied in the form of a spray or by a swab dipped into the fluid. White (1899) and Idem (1901) stated that carcinoma of the skin could be cured by these techniques.

Whitehouse (1907) used wash bottle principle to apply a spray application of liquid air for the treatment of skin cancer.

Hass *et al.* (1948) performed experimental studies on human patient, concerned with the production of focal lesions in the brain, liver, and heart by local freezing and demonstrated the feasibility of producing local areas of destruction in those tissues. Same findings were observed by Taylor *et al.* (1951) on heart and Clasen *et al.* (1953) on brain tissue.

Allington (1950) first introduced the freezing method using liquid

nitrogen into clinical practice for the treatment of skin diseases.

Lovelock (1953) first observed the frost-bite effect on cell level following freezing of tissues.

Cooper and Lee (1961) were the pioneer workers who first developed a cryosurgery machine in New York. Liquid nitrogen was circulated through a hollow metal probe which could produce a probe tip temperature -109°C . The probe was applied to the basal ganglion for the treatment of Parkinson's disease.

Zacarian and Adham (1966) stated that the effectiveness of a copper plaque or rod cooled by being immersed in liquid nitrogen is restricted for cryotherapy because although initially there was a temperature of -195.8°C , but it is soon raised to ambient temperature and effectively freezes only a few millimeters of tissue adjacent to the probe.

Cahan (1967) suggested that to produce adequate cryonecrosis, a rapid freezing and slow thawing of the tumour should be applied with twice or thrice or more, according to the size and consistency of the tumour.

Yantorno *et al.* (1967) first reported that the antibody is produced in the body in consequence of immunostimulation following freezing treatment.

Drylie *et al.* (1968) explained the theoretical immunogenic aspects of cryotherapy. They stated that the breakdown of the squamous cell carcinoma of bovine eye by cryotherapy could stimulate the immune system of the body and thus other squamous cell carcinomas that might occur in other areas of cow's body might be suppressed, because of specific sensitization of that tumour. Later this immunogenic aspect of cryotherapy on tumour was supported by Neel (1980) who stated its beneficial effect to cancer therapy.

Stone *et al.* (1969) reported that the malignant cells are more cryosensitive than normal cells of mammalian body.

Edwards (1969) suggested the temperature ranges during cryotherapy

on different conditions. According to him, - 20°C, -30°C, -30°C to -40°C, -60°C to -70°C, below -100°C and -196°C temperatures are required for destruction of thin, superficial structures, removal of crystalline lens, retinal reattachment, destruction of small tumours, destruction of large tumours or small bone tumours and destruction of very large tumours or large bone tumours respectively.

Borthwick (1970) first performed the cryosurgery in the veterinary field in England.

Zacarian (1970) stated that the maximum percentage of cells killed is obtained by rapid freezing and slow thawing of the tissues subjected to cryosurgery.

Gill *et al.* (1971) viewed that all tissue frozen in situ require a final temperature in an around -20°C to achieve the complete destruction. He also stated that the tissue destruction due to cryoapplication is caused by ischaemic infarction of the capillaries in the applied area.

Whitaker (1972) investigated and concluded that the vascular stasis after cryosurgery is the secondary factor for cryonecrosis. He used carbon labelling techniques to study the blood flow in an area after cryosurgery, and mentioned that the stasis was increasingly severe for 2 hours postoperatively but lastly the arteries were remained patent with increased permeability.

Lane (1974) highlighted the advantages of cryosurgery over the sharp surgical excision and other therapies like diathermic cautery including fulguration, radiation therapy, and chemotherapy. Most of the advantages reported by him are :

- (1) It has no adverse effect on both the patient and operator.
- (2) Strict aseptic measures during operation is not obligatory.
- (3) The procedure is relatively painless and thus it rarely requires general anaesthesia.
- (4) Haemorrhage during operation, reactionary haemorrhage, and secondary infection at the treated site are not subsequent features.

- (5) Possibility of dehiscence during healing does not apply and scarring is not marked.
- (6) No chance of precipitating metastasis during application on neoplasia.
- (7) Chance of postoperative immunostimulation following cryosurgery on neoplasia.

Faraci *et al.* (1975) conducted an experiment to assess the differential cellular and humoral immunity after cryosurgery and excision of fibrosarcoma in mice. They observed that lymphocyte mediated cytotoxicity against tumour cells was markedly greater when cells from cryosurgically treated mice were used as compared with cells from the excisional group. Lastly they concluded that both cellular and humoral arms of immune mechanism are augmented by cryosurgery.

Bushby (1978) observed the microscopic tissue alterations following cryosurgery of canine skin and noted that the changes occurred in an orderly progression of: edema and erythema, infiltration of inflammatory cells, tissue necrosis, tissue slough, and repair by granulation and reepithelialization. He also observed that inflammatory response was little and thickening of epidermal layer with complete lack of all adnexal structures after healing of the lesion.

Harvey (1978) cited that the fatal air embolization may follow as a sequelae of cryosurgery with liquid nitrogen spray. In an experiment during cryospray on gingival tumour and gingival melanoma in two dogs he observed that the cryosurgical apparatus is capable of delivering liquid nitrogen spray at a pressure upto 30 lb/sq. in. which is potential for driving air or vapour of liquid nitrogen into blood vessel to produce emboli and opined that the possibility of air embolization by any means other than cryospray is remote.

Helpap and Grouls (1979) made a comparative cell analytical investigation on liver tissue reparation after electrosurgery and cryosurgery and stated that there was minimal fibrosis with faster healing after cryosurgery than after electrosurgery.

Neel and Ritts (1979) reported that the differential immunity was

somewhat less when the primary tumours in mice were surgically excised or excised 4 to 24 hours after cryosurgical necrosis than that when the cryosurgically necrosed tumour remained in the host.

Withrow (1980) cited that the monitoring of temperature during cryosurgery can be done by visualization and palpation of the formed tissue ice ball. He also stated that the chance of healthy tissue necrosis during cryosurgery is negligible because the margin of the ice ball if reaches the healthy tissue zone it is not capable to set up cryonecrosis as it posses only 0°C temperature which is far behind the actual temperature (-20°C) required for cryonecrosis.

Holmberg (1980) reported that the cryosurgery is highly effective for the treatment of different eyelid tumours over routine blade excision where there is maximum chance of cicatrical contraction following operation.

Merideth and Gelatt (1980) excellently documented the application of cryosurgery on different surgical conditions of eye like : vitreocorneal adhesion, extraction of crystalline lens, glaucoma, and retinal detachment.

Hoyt and Seim (1981) explained that the cellular death from freezing is a direct result of intracellular & extracellular ice crystal formation, intracellular fluid & electrolyte disturbances, denaturation of cell membrane, and thermal shock and these factors act dynamically to cause overwhelming cellular pathology.

McKibbin and Paraschak (1985) successfully treated bone spavin, splint, and tracted splint bone injuries in standardbred horses by liquid nitrogen cryosurgery for a double freeze - thaw cycles. They concluded that the cryosurgery provides a successful treatment alternative which is safe and humane to the horse, requiring a very short layoff or recovery time.

Munroe (1986) stated that the cryosurgery is not panacea for all ills of equine but is a very useful alternatives to more conventional therapies for sarcoids, squamous cell carcinomas, papillomas, dermoids, excessive granulation tissue, cutaneous habronemiasis or eosinophilic granuloma etc.

Rao *et al.* (1994) reported the occurrence of subcutaneous emphysema in a dog following cryosurgery of mammary tumour using liquid nitrogen spray.

Cozzi *et al.* (1997) conducted a feasibility study of renal cryosurgery in sheep model and concluded that the procedure is safe, efficacious and can cause significant necrosis with minimal disruption of surrounding renal parenchyma.

2.2. REVIEW OF LITERATURE ON ANAL FISTULA AND ITS SURGICAL MANAGEMENT :

Lacroix and Lacroix (1945) stated that the pararectal fistulations are the commonest sequelae of anal sac infection and suppuration.

Lewis (1968) reported that the poor ventilation in the anal area due to stung and broad based tail predisposes the occurrence of anal fistula in German Shepherd breed of dog.

Borthwick (1971), the pioneer worker on cryosurgery in veterinary field successfully treated two cases of multiple perianal sinuses in dog by using an apparatus employing liquid nitrogen as a refrigerant and producing a tip temperature of between -100°C to -180°C.

Palminteri (1971) opined that the high incidence of the anal fistula in German Shepherd dogs is an inherited trait.

Harvey (1972) evaluated a drastic surgical procedure for anal fistula which necessitates the partial or total excision of the anal ring. In his experiment he observed a high recurrence rate (45% out of 54 cases operated on) following that method.

Robins and Lane (1973) employed anal saccullectomy, debridement, and cauterization by 75% silver nitrate or 80% liquified phenol for the treatment of 24 clinical cases of anal fistula and got complete resolution in 19 cases. The postoperative complications observed by them were minimal.

Lloyd - Williams *et al.* (1973) treated haemorrhoids by cryosurgery

and were impressed by the painlessness of the procedure and also by the absence of stricture formation to anus.

Lane and Bruch (1975) successfully treated 40 clinical cases of anal furunculosis in dog by nitrous oxide cryosurgery and got a result that 90% cases were cured and little fibrous tissue was laid down during healing.

Halnan (1976) reported that the German Shepherd breed of dogs are most prone to perianal fistula among the canine population.

Houlton (1980) made a comparative study in between the cutting diathermy and cryosurgery for the treatment of anal furunculosis and reported the success rate of 54% of 24 clinical cases and 50% of 46 clinical cases respectively. He also stated some reasons like improper removal of anal sacs during diathermy and improper freezing of sinus tracts by cryosurgery, for the failure of the respective techniques. Later he employed a combined technique of cutting diathermy and cryosurgery on 35 clinical cases of anal furunculosis and got 97% success.

Liska (1980) cited that the cryosurgery comprising two freeze - thaw cycles below -20°C is significant for maximum cases of anal fistula. He also stated that the healing is commonly resulted within 14 to 30 days after cryosurgery and the anal stenosis may be resulted only in those cases where the 360° perianal tissues are severely affected.

Budsberg *et al.* (1981) reported that the postoperative complications following cryosurgery are minimum and in his experiment he observed occasional flatulence (10%), occasional faecal incontinence (10%), frequent tenesmus (5%), and constipation (5%) in 12 cured animals out of 25 total clinical cases of anal fistula.

Vasseur (1984) viewed that the high recurrence (56.1%) of perianal fistula in 41 animals operated by bilateral sacculotomy and surgical excision of all diseased tissues. He also observed some prominent postoperative complications like faecal incontinence (26.8%), tenesmus (19.5%), flatulence (17.1%), and anal stricture (14.6%) formation.

Holt (1985) stated that the clinical examination is sufficient to confirm the perianal fistula, although biopsy must be taken if malignancy is

suspected. He also stated that the cryosurgery with or without surgical excision gives good result but the chemical cauterization should not be recommended.

vanEe and Palminteri (1987) indicated that the tail amputation is a means of removing the chance of occurrence of anal fistula in German Shepherd breed of dog, as it prevents the continuous soiling of the perianal region and increases the ventilation of the area.

Killingsworth *et al.* (1988) conducted the study of thyroid and immunologic status of dogs with perianal fistula. On the basis of lymphocyte proliferation assays, they concluded that the suppression of cell mediated immunity is probably a result of the affection, rather than a cause of the disease.

Gregory (1990) mentioned that the deroofting anal fulguration in combination with excision of deeper fistulas may give good result for the management of perianal fistula, but the commonest postoperative complications are faecal incontinence and stricture formation.

Day (1993), performed the immunopathological study of anal fistula in dogs. In his study he made immunostaining of the perianal tissues from normal as well as affected animals in order to determine the distribution of T - lymphocyte, B - lymphocyte or plasma cells. He observed that the perianal tissues affected with fistulae have high eosinophilic infiltration. He concluded that the immunological defect is contributing to predispose the dogs of German Shepherd breed to anal fistula.

Kengannavar *et al.* (1996) reported a successful treatment of six clinical cases of anal fistula in dog by carbon dioxide cryosurgery in conjunction with bilateral anal sacculotomy and followed double freeze - thaw cycle of one minute duration of freezing. Later (1997) they made a comparative study between the medical, surgical and cryosurgical therapy on 18 dogs affected with perianal fistula and reported the superiority of the cryosurgical approach regarding its high rate of success and least postoperative complications over the same achieved in medical and conventional surgical therapy.

2.3. REVIEW OF LITERATURE ON THERAPEUTIC MANAGEMENT OF VENEREAL GRANULOMA :

Gleeson (1947) recommended the surgical excision for the treatment of venereal granuloma as the tumour rarely involves more than mucous membrane.

Masaaki *et al.* (1958) performed radiation therapy for venereal granuloma and applied 7 exposures over a period of 22 days for a total unit of 2244 R to kill the total tumour mass. He also observed the side effects of radiation therapy.

Joyce (1976) successfully treated the squamous cell carcinoma of vulva and penis of horse by liquid nitrogen cryosurgery.

Krahwinkel, Jr. (1980) reported that the cryosurgery is effective for the treatment of papilloma on the vulva of dog and results minimal scarring after healing.

Dodi *et al.* (1982) applied cryosurgery without any general or local anaesthesia on condylomata acuminata or venereal warts in 21 human patients of which 19 were cured after first treatment and rest two required further treatment and cured.

Calvert *et al.* (1982) stated the efficacy of Vincristine sulphate, a mitotic inhibitor chemotherapeutic agent, for the treatment of transmissible venereal tumour in dog but at the same time he also reported some salient side effects like anorexia, vomiting, alopecia, dehydration, and constipation. These were later also observed by Hoque *et al.* (1995) when they were working with vincristine and other chemotherapeutic agents like cyclophosphamide and methotrexate for the treatment of venereal granuloma in canine.

Johnston (1985) cited that spontaneous regression of venereal granuloma occurs in some cases and also stated that immunosuppression in the host may explain cases in which local invasion and metastasis occur.

Omara - Opyene *et al.* (1985) conducted cryosurgery on squamous cell carcinoma in the vulva of 62 cows. They carried two freeze - thaw cycles of about -25°C and reported that 88.7% cases were cured success-

fully. They concluded that the cryosurgery is superior to surgical excision for the treatment of bovine vulvar carcinoma.

Idowu (1985) reported successful cryosurgical applications on transmissible venereal tumours in 15 dogs of which all were regressed and did not recur for 9 to 22 months observation. He concluded that the cryosurgery is an effective therapeutic modality for the treatment of canine transmissible venereal tumour.

Hobson (1990) viewed that the transmissible venereal granuloma in male dog is responsive to radiotherapy in most cases and rarely requires partial amputation of penis.

Dinesh *et al.* (1993) reported that the chemotherapy with vincristine for the treatment of venereal granuloma in dog significantly depresses the reticuloendothelial system. This finding was supported by Padile *et al.* (1998).



Chapter III

**MATERIALS AND
METHODS**

MATERIALS AND METHODS

3.1 EXPERIMENTAL MODEL :

In the present plan of study a total number of twenty (20) clinical cases of adult dogs of both sexes were utilized out of which 10 animals were affected with anal fistula and the other 10 animals with venereal granuloma. All the 20 animals were placed in four different groups being serially numbered from 1 to 20. Selections of animals for grouping was random. Out of ten animals affected with anal fistula, six bearing number 1 to 6 were placed in group A and the rest bearing number 7 to 10 were placed in Group A₁. Likewise, other ten animals affected with venereal granuloma were placed in Group B and Group B₁ bearing number 11 to 16 and 17 to 20 respectively. In fact a total of 30 clinical cases of both the surgical affections were presented for treatment, but only the 20 animals were included in the study by placing them in the respective groups as and when they were presented for the treatment in the Department of Veterinary Surgery & Radiology under west Bengal University of Animal and Fishery Sciences, during the period from May to October, 1999. Other 10 animals of which 7 animals were affected with venereal granuloma and 3 animals were affected with anal fistula, were not at all fit for any type of surgical therapy due to their poor physical condition and kept in owner's residence with instruction of daily saline irrigation of the lesion and liver extract with Vit-B complex injection, until their body condition improved to permit any surgical therapy.

In the present study cryosurgery was adopted in animals under Group A and Group B and the animals under Group A₁ and Group B₁ were subjected to conventional surgical excision. All the animals were maintained under identical environment and laboratory condition with standard diet during the entire period of experimentation.

The general performance of the operative procedures with regard to the anaesthesia, control, restrain, operative site, pre and post operative management and all other selected parameters were thoroughly observed during the period upto 28 days postoperatively and the animals were kept under observation upto 90 days postoperatively. The grouping of the ani-

TABLE NO. 1

Showing groupings of the experimental animals

VENEREAL GRANULOMA																			
ANAL FISTULA						GROUP A					GROUP B					GROUP B1			
An. No.	Age (year)	Sex	Breed	Body weight (kg.)	An. No.	Age (year)	Sex	Breed	Body weight (kg.)	An. No.	Age (year)	Sex	Breed	Body weight (kg.)	An. No.	Age (year)	Sex	Breed	Body weight (kg.)
1.	5.2	Male	G.S.D.	18	7.	6.2	Male	G.S.D.	16.5	11.	4.8	Female	Mongrel	12.5	17.	2.8	Female	Mongrel	12
2.	7	Male	G.S.D.	15.5	8.	5.8	Male	G.S.D.	18	12.	3.4	Female	Spitz	10.5	18.	4.2	Female	Spitz	10
3.	6.4	Male	G.S.D.	17	9.	8	Male	G.S.D.	16	13.	10	Male	Mongrel	14	19.	3	Female	Mongrel	11.5
4.	12	Male	Lhasa Apso	15	10.	6	Male	G.S.D.	15.5	14.	5.2	Female	Mongrel	11	20.	6	Male	Boxer	14
5.	4.8	Male	G.S.D.	17.5						15.	4	Female	Mongrel	13					
6.	4	Male	G.S.D.	16						16.	3.2	Male	Mongrel	13.5					

mals is presented in the Table No. 1.

3.2. MATERIALS (Photo No. 1 to 4)

3.2.1. Anaesthetic and preanaesthetic agents :

- (a) Atropine sulphate injection (P & B Labs.)
- (b) Xylazine hydrochloride injection (Xylaxin*, Indian immunologicals, Bangalore).
- (c) Lignocaine hydrochloride - 2% (ASTRA - IDL, Bangalore).
- (d) Dexamethasone injection (Decdan*, Merind, Bombay).

3.2.2. Components of Cryosurgical set :

- (a) Cryogum (Cryo Super AA-4, Appasamy Associates)

(b) Interchangeable multitip cryoprobes (Cryo Super AA-4, Appasamy Associates) : These are phase change cryoprobes, harness the loss of latent heat when a change of phase, usually liquid to gas of cryogen takes place. These are fitted with the cryogum at the time of operation according to the need of size and type of cryoprobe. The tip is made up of stainless steel and the rest part of the probe is teflon coated, as a safety measure and to prevent the loss of cryotemperature during operation. There are -

- (i) Anorectal flat probe (P-0832)
- (ii) General purpose probe (P-1900)
- (iii) Maxiderm probe (P-2500)
- (iv) Curved nasal probe (P-0420)
- (v) Endo/Exo Cone probe (P-1923)

(c) pressure monitoring dial (Appasamy Associates) : This is fitted with the hose of the cryocylinder, situated on its top. It shows the optimum working pressure of the cryogen within the cylinder.

(d) Cryocylinder with Cryogen (British Oxygen Company, Calcutta) : Cryocylinder is a close container for the storage of the cryogen. A 5 liter

water capacity Nitrous Oxide Cylinder was utilized in the present study.

Cryogen is the liquid refrigerant, stored within the cryocylinder. When it is released within the cryoprobe (in probe freezing) through a fine nozzle, a sudden change of the liquid phase to the gaseous phase produces in Joule Thompson effect by which a cooling temperature is created in the tip of the probe. This cool temperature is soon released as cryotemperature from the metal tip to the adjacent target tissue.

The commonly used cryogens are liquid nitrogen, nitrous oxide and carbon dioxide.

The mostly used cryogen in veterinary cryosurgery is liquid nitrogen as it produces a tip temperature - 196°C due to its extreme low boiling point (-196°C). But it can not be stored in closed nonvented container. It requires dewar flask for storage which is costly and the most available ones are only adequate for short term (2 - 3 days) storage with approximately 8% evaporation per day. In addition, because of its greater potency, freezing must be closely monitored to prevent the excessive or accidental destruction of healthy tissue and vital organs.

On the other hand carbon dioxide is easily storable in closed container and does not require close monitoring during freezing process. But its less potency (-78°C) for tissue penetration is the only limitation for its wide use.

The nitrous oxide gas in this aspect is similar with that of carbon dioxide except that it has a better tissue penetration (-89°C) than that of carbon dioxide.

In respect of storability, tissue penetration as well as less chance of healthy tissue destruction, the nitrous oxide was used in the present study and was made available from time to time within the period of study from British Oxygen Company, Calcutta.

(f) Spanner : It is used to torque the nob or regulator of the cryocylinder to turned on the supply as well as to regulate the out flow of cryogen from the cylinder to the cryogun.

3.2.3. Surgical instruments :

Routine surgical instruments were used for operation.

3.2.4. Medications :

- (a) Injection 5% Dextrose saline solution (Alembic, Baroda).
- (b) Injection Styptochrome (Dolphin, Calcutta).
- (c) Injection Dexamethasone (Decdan[®], Merind, Bombay).
- (d) Injection Ampicillin and Cloxacillin (Ampilox[®], Biochem, Bombay).
- (e) Injection Adrenaline (P & B Labs).
- (f) Betadine Liquid 5% (Win - Medicare, New Delhi).
- (g) White petroleum Jelly (Vaseline[®], Hindustan Lever Ltd., Bombay).

3.2.5. Suture materials :

- (a) Chromic catgut No. 2 - 0 (Ethicon, Bombay)
- (b) Medium size silk.

3.2.6. Accessories :

- (a) Sterile drape.
- (b) Roll bandage.
- (c) Absorbent Cotton.
- (d) Sterile gauze.
- (e) Sterile gloves.

3.3. PREOPERATIVE CONSIDERATION :

In all cases, the initial history was studied thoroughly. General physical examination of all the animals were done in a routine manner. Temperature, pulse, respiration were recorded. In each case a piece of tissue from the lesion was taken for histopathological examination. Blood was drawn preoperatively from each animal for haematological and biochemical examination.

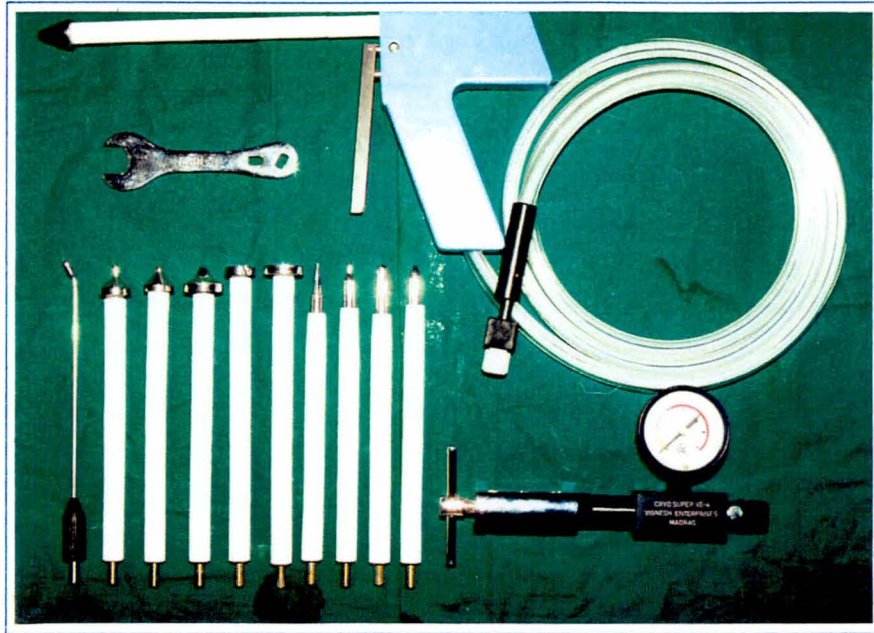


Photo No. 1. Showing components of a cryosurgical set



Photo No. 2. Showing assembled cryosurgical set with Nitrous Oxide cylinder



Photo No. 3. Showing routine surgical instruments used for conventional surgical excision



Photo No.4. Showing medicaments used during cryosurgery & surgical excision.

Surgical risk of each individual animal, those were subjected for general anaesthesia, was evaluated. Accordingly the physical status was determined and graded as (A) Good, (B) Fair, (c) Poor, (d) Emergency good, (e) Emergency poor, and (f) Moribound.

General temperament and appearance of each individual animal was noted primarily including the evidence of shock, state of nutrition, and dehydration. Necessary correction of body fluid deficit was made with intravenous administration of 5% Dextrose saline solutions to make the operation safer for the patient. Routine dewarming of individual animal was done 15 days before operation.

A detail examination of the lesion of individual animal regarding the extent of affection, any discharge, and haemorrhage was done.

As both the surgical conditions are not immediate life threatening, all were undertaken as elective surgical cases. The animals were kept off fed and withdrawal of water for 12 hours and 4 hours respectively before surgery.

3.4. ANATOMIC BASIS FOR SURGICAL APPROACH.

3.4.1. Surgical anatomy of anal & perianal region :

Rectum, the terminal portion of large intestine is soon followed by anal canal caudally. The anal canal is 1 cm. long. The inner side of it is lined by mucous membrane which blends with the skin surrounding the anus. Anal glands are situated at the junction of rectum and anus. A small opening on either side of the anus leads into lateral anal sacs. The skin overlying these contains coil glands. Further the entire skin of the perianal region contains large sebaceous glands and peculiar circumanal glands.

The muscular supports of the anal canal are -

(a) The striated external anal sphincter muscle, extends from the perianal dermis to the origins of the rectococcygeus muscle and overlies the anal sacs. Dorsally a few fibres attach to deep tail fascia and ventrally the muscle joins the bulbocavernosus or constrictor vulvae muscle.

(b) The striated levator ani muscles arise floor of the pelvis, lies around

the sides of the rectum and then narrow again at their insertion on the seventh coccygeal vertebra.

(c) The coccygeus muscles, situated lateral to the levator ani muscle, arise from the pelvic floor and insert on the second of fifteen coccygeal vertebrae.

The blood vessels supplied to the anal and perianal regions are cranial and caudal rectal (haemorrhoidal) artery. These areas are innervated with rectal (haemorrhoidal) nerve, which is a branch of the pudendal nerve.

3.4.2. Surgical anatomy of valva and vestibule :

The valva having two thick labia forms the entrance of the genital passage of bitch and leads into the vestibule, the average length of which is 3 cm. and is directed craniodorsally to connect the vulva with the vagina. Clitoris, the homologous portion of penis lies in a well developed fossa situated in the floor of the vestibule. The external urethral meatus is cranial to this fossa. The vulvar mucous membrane presents small prominences caused by lymph follicles, and is continuous with the same of the vagina.

The uterine artery supplies blood to the vulva and vestibule.

3.4.3. Surgical anatomy of glans penis & prepuce :

The external penile portion presents glans penis, the terminal portion of penis and situated immediately cranial to the scrotum. The glans penis has two parts. The anterior part, pars longa glandis is cylindrical with a pointed free end, behind which the rounded enlargement or bulbus glandis is situated. Both are composed of erectile tissue. The os penis begins caudal to the bulbus glandis and extends forward almost to the tip of the glans. The whole portion of the glans penis is covered with mucous membrane which is closely attached to the pars longa glandis and more loosely to the bulbus glandis. It continues with the mucous membrane of the prepuce forming the internal lamina which is again continuous at the preputial orifice with the external lamina of the prepuce, similar to the skin of the body. Prepuce is a pouch like dermal covering over the entire portion of glans penis. The opening of this pouch is the preputial

orifice.

The blood supply of these area is derived from pudendal artery and the entire area is innervated with the dorsal branches of the pudendal nerve.

3.5. PRINCIPLE OF CRYOSURGERY :

As it has been stated earlier that the cellular death occurs by the freezing temperature during cryosurgery, the best cellular destruction occurs by suitable combination of freezing as well as thawing of the subjected tissue. Best result is achieved by rapid freezing and slow thawing of the target tissue without any external aid. This constitutes the single 'freeze - thaw' cycle. The cycle may be multiple according to the type as well as depth of the target tissue. Tissue frozen to -20°C or below for one minute undergoes cryonecrosis but the duration of freezing depends on the extent of lesion and type of target tissue. Freezing should be monitored carefully (specially when liquid nitrogen is used as cryogen) to prevent accidental contact and damage of healthy tissue and vital organs. Freezing can be monitored by two ways -

(a) Placing two thermocouple needles, one at the periphery of the target tissue and another at the depth of the tissue in between the target tissue and surrounding healthy tissue. The temperature produced during freezing is recorded in the pyrometer, connected with the thermocouple needles.

(b) Visualization and palpation of the ice ball formed by the freezing temperature on the target tissue. The frozen tissue has a white discoloration which is readily visualized. Because of the ice ball temperature is only 0°C at its periphery, cellular death at this point is far from certain. Therefore a freeze margin of 5 mm. normal tissue around the target tissue is recommended.

Major Phases during Cryosurgery :

The major phases during cryosurgery are - Immediate phase, Delayed phase, and Late phase.

● ***Immediate Phase :*** It occurs immediately during the freeze - thaw cycle.

There are four ways in which the cell may be destroyed, all of which may occur simultaneously.

Ice crystal formation : Rapid freezing of the target tissue makes the cells unable to lose water fast enough to establish a hydration equilibrium between the intracellular and extracellular spaces and increases likelihood of small ice crystal formation. These are more lethal than the large extracellular ice crystals which are formed during slow freezing process. Furthermore, the slow thawing of the frozen tissue produces more damaging large ice crystals within the cells by process of recrystallization. Thus the maximum amount of cellular destruction results from a rapid - freeze - slow - thaw cycle.

Dehydration and solute concentration : Ice crystal formation in the extracellular space results a hyperosmolar state with subsequent extraction of water from the intracellular space resulting a toxic concentration of electrolytes within the cells.

Protein denaturation : Freezing causes profound changes in the physical environment of the cell and is sufficient to denature the sensitive lipoprotein complex, thus damages the cellular membrane. The loss of phospholipid renders the cell membrane permeable to ions and extracellular fluid, so that the cells swell slowly and burst. This lysis may occur during the freezing or the thawing stage.

Thermal shock : Thermal shock or cryoshock refers to injury of the cells caused by a rapid change in temperature rather than by the direct effect of freezing. It adversely affects the enzyme system of the cells.

Delayed Phase : It occurs within a few hours after the final freeze - thaw cycle. Cellular destruction during this phase is caused primarily by the effects of vascular stasis. The greatest destruction occurs at the capillary level. Thrombosis and infarction are the results of endothelial damage, erythrocyte clumping, alteration in permeability, platelet aggregation and activation of the clotting mechanism.

The resultant ischemia leads to anoxia and death.

Late Phase : Otherwise known as 'Immunological phase', is of particular interest of cryosurgery on malignant neoplasm. There is evidence that many tumours contain tumour specific antigens that are accessible for immunological recognition and are capable of inducing responses cytotoxic to tumour cells. Thus the destruction of tumour by cryosurgery leads to increased tumour - specific transplantation immunity.

Now on the basis of these principles a successful application was made for cryonecrosis of the target discussed tissue during cryosurgery in the present study.

3.6. CRYOSURGICAL APPROACH FOR THE TREATMENT OF ANAL FISTULA AND VENEREAL GRANULOMA IN DOG.

3.6.1. Preparation of the animal and preanaesthetic medication :

The surgical site did not required elaborate preparation as the cryotemperature is itself germicidal. Excessive and matted hair around the lesion was clipped and shaved for proper visualization of the site. The skin was gently cleansed and an antimicrobial agent was applied prior to freezing.

Injection atropine sulphate @ 0.65 mg/animal was injected subcutaneously 7 - 10 minutes before anaesthetic medication.

3.6.2. Anaesthesia :

Operations were done under epidural anaesthesia by injecting xylazine hydrochloride @ 0.75 mg/kg b.wt. along with normal saline upto 2 ml of total volume within the lumbo - sacral joint.

3.6.3. Positioning of the animal :

In both surgical conditions, except the male dog affected with venereal granuloma, the animal was placed on the table in the perineal position, 10 -12 minutes after anaesthetic medication. Tail was secured dor-

sally over the back. Cloth padding was placed under the pelvis and the table was tilted 10° with the head of the animal down.

Whereas male dog affected with venereal granuloma was placed on dorsal recumbancy.

3.6.4. Preparation of cryosurgical set :

The total set was prepared and the working pressure of the nitrous oxide gas was adjusted to 700 p.s.i.

3.6.5. Procedure : (Photo No. 5 to 17)

Freezing of the target tissue can be achieved by two methods.

- (1) By probe freezing, and
- (2) By spray freezing.

For smaller to medium sized lesions probe freezing is safe and effective, whereas the spray freezing is effective for large to very large sized lesions. Further the probe freezing by the nitrous oxide cryo unit can be applied by three ways.

- (i) Overlapping - probe method - Performed by producing multiple overlapping ice balls to form one large ice ball which increases the total area of necrosis.
- (ii) Base method - Removal of bulk of a large lesion protruding from the surface of the skin by surgical excision and freezing the remaining base of the lesion. This procedure insures maximum cellular necrosis.
- (iii) Punch method : Performed by placing the cryoprobe into the hole left by punch biopsy. This allows ice ball to penetrate deeper into the lesion.

In the present study overlapping probe and base methods were followed during operative procedure. The animal was properly draped to protect the surrounding healthy zone from accidental contact with the super cooled cryoprobe. The normal tissue just adjacent to the lesion was smeared with white petroleum jelly for the same purpose.

3.6.5.A. Procedure for Anal Fistula :

A gauze tampon was inserted into the rectum to minimize the faecal contamination. Each fistula was explored to its full extent. Loosely attached perianal tissues were debrided. Previously selected cryoprobe was either inserted or placed on fistulae and frozen below the - 20°C temperature for 30-40 seconds through two freeze - thaw cycles. The extent of freezing with ice ball formation was visually monitored. The ice ball was then allowed to thaw at room temperature.

After completion of the operation, the animal was removed from the operation table and strict postoperative care, management, and observations were made upto 4 weeks.

3.6.5. B. Procedure for Venereal Granuloma :

The anus was plugged with gauze to minimize the faecal contamination. In case of bitch, where required episiotomy was done aseptically under local infiltration anaesthesia, for clear visualization of the extent of lesion. Similarly in case of male dog, where required, the preputial cavity was opened by giving incision on the prepuce through ventral median raphae. Adrenaline was injected at the base of the tumour to minimize the supply of warm blood and thus to lessen the chance of quick thawing. The 'base' method was applied in some cases where the tumour mass was too large. Cryoprobe was then placed over the tumour mass. Two freeze - thaw cycles were followed at a temperature below - 20°C for 40 to 50 seconds. The ice balls were allowed to thaw at room temperature.

The surgical wound created during operation was closed by applying continuous suture using 2 - 0 chromic catgut and horizontal mattress suture using silk for mucous membrane and skin respectively.

The animal was removed from the operation table after completion of operation and strict post operative care, management, and observations were made upto 4 weeks post operative period.

3.6.6. Post operative management :

Ampicillin and cloxacillin @ 10mg/kg b.wt. thrice daily were given intramuscularly for at least 5 days postoperatively. No painkiller was given.



Photo No 5.

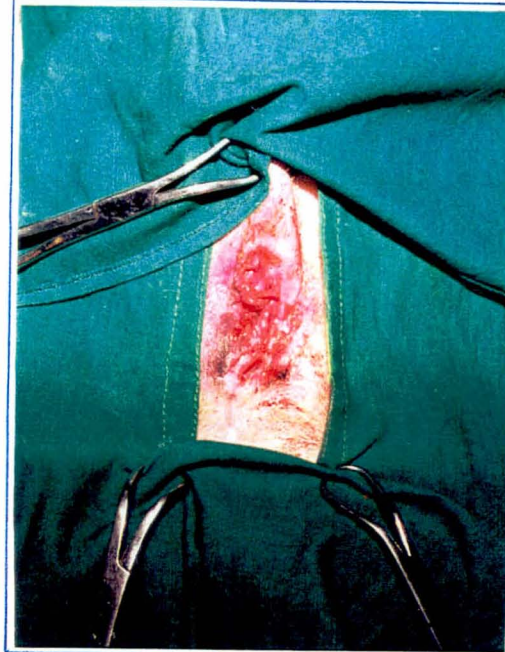


Photo No 6.

Photo No. 5 & 6. Showing perianal fistula in dogs.

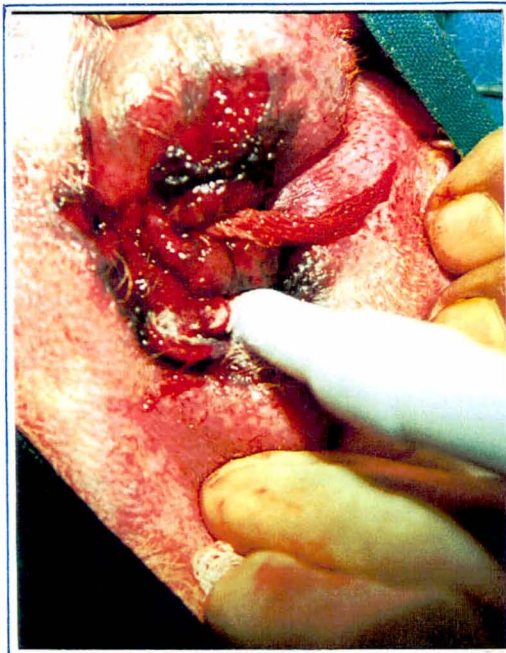


Photo No 7.

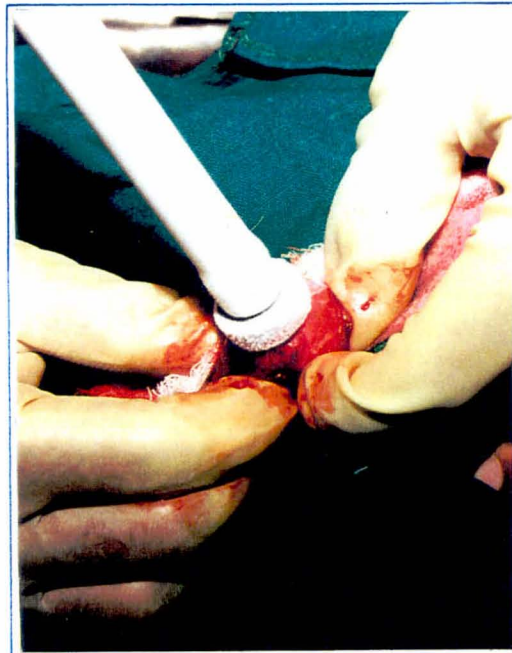


Photo No 8.

Photo No. 7 & 8. Showing probe freezing of fistulous tract during cryosurgery on anal fistula in dog.



Photo No.9. Showing granulomatous mass protruding outside the vulva



Photo No.10. Showing epidural anaesthesia of the animal before cryosurgery .

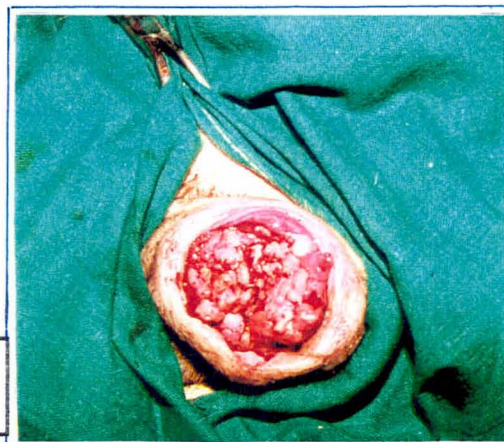


Photo No.11. Showing partial excision of venereal granuloma before cryosurgery.

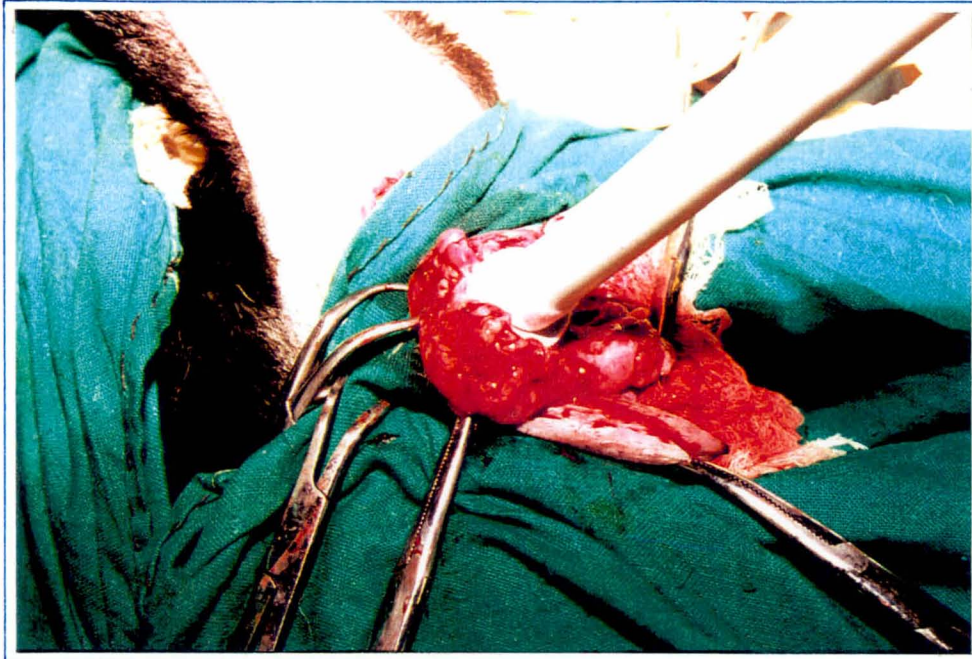


Photo No.12. Showing overlapping probe freezing of venereal granuloma in a bitch

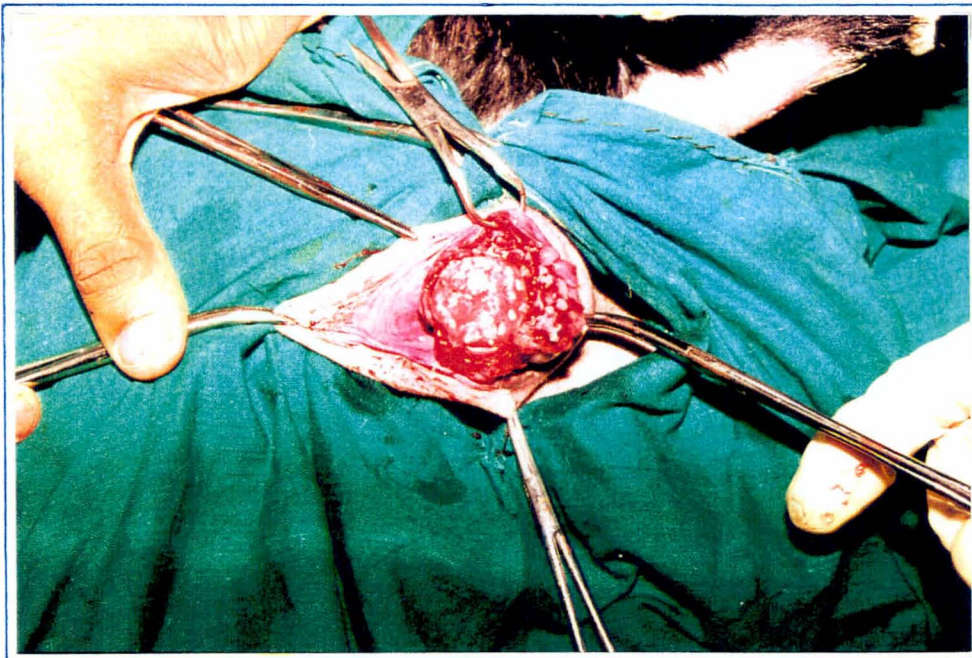


Photo No.13. Showing tissue ice-ball formation immediately after freezing of granulomatous mass in bitch.

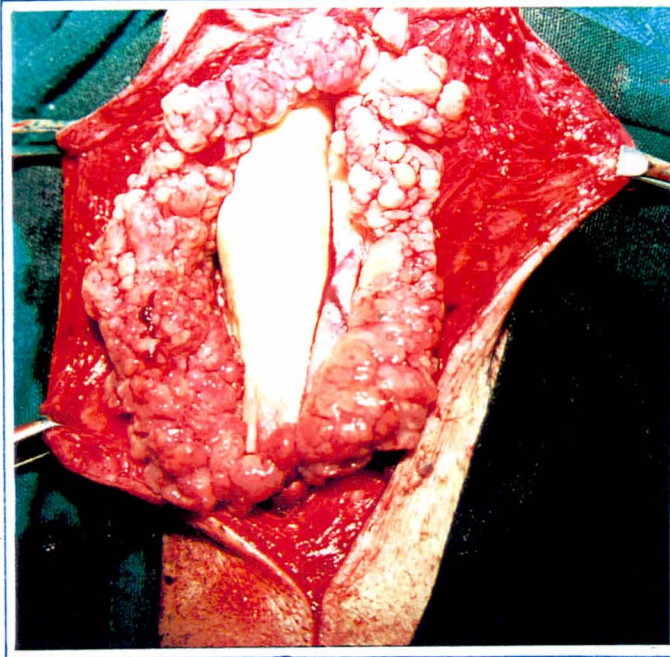


Photo No 14.



Photo No 15.

Photo No.14 & 15. Showing venereal granuloma affecting the prepuce and base of the penis in male dog, respectively.

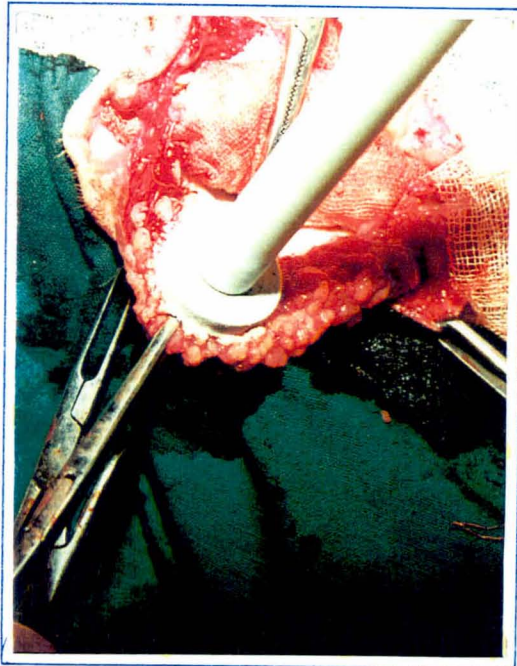


Photo No 16.

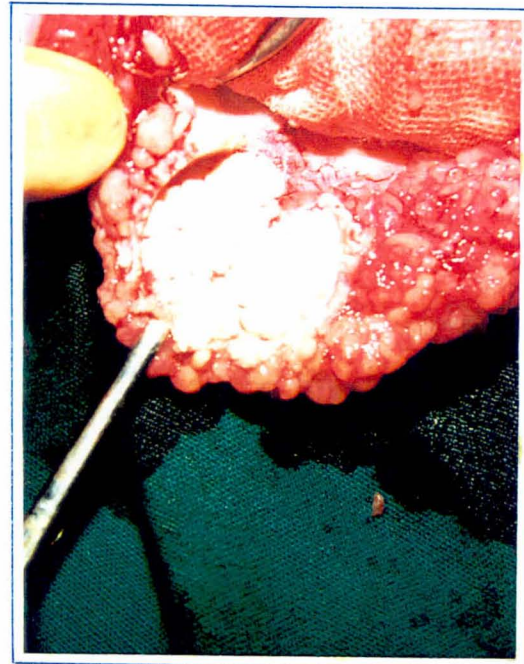


Photo No 17.

Photo No.16& 17. Showing probe freezing of the granuloma & tissue ice-ball formation during cryosurgery in male dog, respectively.

Daily irrigation and cleaning of the operated site was done with normal saline and an antibiotic ointment was applied on the site for at least 10 days postoperatively. All the animals were fed with easily digestible diet along with laxative 4 weeks postoperatively to minimize flatulence as well as straining on the operated site. The skin sutures where applied, were removed after 8 to 10 days. All the animals were maintained and observed upto 4th postoperative week, and during this period clinical, haematological, and biochemical observations were made.

3.7. CONVENTIONAL SURGICAL EXCISION FOR THE TREATMENT OF ANAL FISTULA AND VENEREAL GRANULOMA IN DOG. (PHOTO No.18 To 21)

3.7.1. Preparation of the animal, preanaesthetic and anaesthetic medication :

The operation site is properly shaved and cleaned with antimicrobial agent. Me atropine sulphate @ 0.65 mg/animal was injected subcutaneously 10 minutes before anaesthetic medication with xylazine hydrochloride @ 1.5 mg/kg b.wt. intramuscularly.

3.7.2. Positioning of the animal :

10 minutes after anaesthetic medication, the animal except the male dog affected with venereal granuloma was placed on the table in the perineal position with cloth padding under the pelvis. The tail was secured dorsally over the back. and the table was tilted 10° to down the head of the animal.

3.7.3. Procedure :

The total surgical procedure was performed under strict aseptic condition.

3.7.3.A. Surgical excision for anal fistula :

The rectum was plugged with gauze to prevent the accidental faecal contamination. The fistulous tracts were probed and explored to their full extent. The loose tissues were debrided. The fistulous tracts were excised

and removed and in some cases anal sacs were also removed. Bleeding during operation was controlled by applying styptochrome locally as well as intramuscularly. The fresh wound was then sutured with nonabsorbable suture material.

After the operation was complete, the animal was removed from the operation table and strict postoperative care, management, and observations were made upto 4 weeks postoperative period.

3.7.3.B. Surgical excision for venereal granuloma :

The total tumour masses were excised and removed along its base with a border of healthy mucosa. The wound is then sutured with 2 - 0 chromic catgut. The bleeding during operation was controlled by local application of styptochrome or adrenaline and intramuscular injection of styptochrome.

The animal was removed from the operation table after the completion of operation and strict postoperative care, management, and observations were made upto 4 weeks postoperative period.

3.7.4. Postoperative management :

The postoperative management was followed same as described after cryosurgery.

3.8. FOR ANALYSIS OF THE SELECTED PARAMETERS THE FOLLOWING WERE EVALUATED WHICH ARE OUTLINED AS BELOW :

1. Intraoperative observations :

During the time of operation, the observation like duration of surgery, extent of effective eryonecrosis, bleeding and degree of technical easiness of the surgical procedure and complications if any were recorded in various groups of animals.

2. Clinical observations :

Clinical observations like inflammation, pain, irritation, licking, ooz-

ing, sloughing of necrosed tissue were observed post operatively.

Both the inflammation and painsensation were graded as nil (0), mild (1), moderate (2), and severe (3) and observed upto 9th P.O.D.

Type of healing and complications associated with the healing were also noted.

The preoperative and the postoperative rectal temperature ($^{\circ}$ F), heart rate (beats/min), and respiration rate (per minute) were observed on 1st, 3rd, 5th, 10th, 15th, and 28th P.O.D.

3. Haematological and Biochemical observations :

A total of 5 ml of blood was collected preoperatively and on 5th, 15th, and 28th P.O.D. from each animal operated upon. Out of this 3ml was used for serum collection and remaining 2 ml was collected in a vial containing calcium EDTA. Freshly collected blood was utilized for the determination of TEC (Total Erythrocyte Count), Haemoglobin % (Hb%), & Differential count (Neutrophil, Lymphocyte and Eosinophil).

The serum samples were used for the estimation of total protein and gamma (γ) - globulin to assess the surgical stress and immune response following cryosurgery.

4. Histopathological observation :

A portion of affected tissue was taken from each individual prior to surgery for proper diagnosis of the condition. This was made again on 28th P.O.D. by taking a portion of tissue from the operated site.

3.9. STATISTICAL ANALYSIS.

All the selected parameters were statistically analysed using paired 'F' test and one way analysis of variance (ANOVA) to check the significant changes at 1% & 5% level, if any (*Snedecor and Cochran, 1967*).



Chapter IV

**OBSERVATIONS
AND RESULTS**

OBSERVATIONS AND RESULTS

The selected parameters of the study of both cryosurgical and surgical approaches on anal fistula and venereal granuloma in experimental groups (Group A & B) and control groups (Group A₁ & B₁) with regard to clinical, haematological, biochemical, and histopathological findings were observed on individual merits. The findings were recorded and presented in respective Tables No. from to

The distribution of cases as per breed, sex, and age have been presented in Table No. 3, 4, and 5 respectively.

The observations were graded as intraoperative, postoperative, clinical, haematological, biochemical, and histopathological and presented as detailed under their respective heads.

4.1. INTRAOPERATIVE OBSERVATION :

All the animals were effectively controlled and restrained either under general or epidural anaesthesia on the basis of choice, with xylazine hydrochloride following premedication with atropine sulphate. None of them required additional doses of anaesthetic. Recovery was smooth in all cases without any side effects. Haemorrhage during operation was very less in animals of group A & B, where cryosurgery was employed in comparison to the animals of group A₁ & B₁ where surgical excision was applied. Other observations were presented in Table No. 2.

4.2. POSTOPERATIVE OBSERVATIONS (Photo No.22 to 30).

4.2.1. Group A :

Animal No. 1 suffering from last 4 months was presented having moderate affection in the left perianal region with fistulae discharging blood and occasional mucopurulent discharges. The animal was restless and alert to the affected site. It experienced pain on digital pressure on the perianal region. Both anal sacs were examined and revealed unaffected. The other symptoms were inappetence, tenesmus perianal licking, circling to catch the base of the tail, and occasional rubbing of the

TABLE NO. 2

Showing Intraoperative Observations during Cryosurgery & Surgical excision

PARAMETER	ANAL FISTULA		VENEREAL GRANULOMA	
	GROUP A	GROUP A1	GROUP B	GROUP B1
(1) Duration of operative procedure (minutes)	31.33 (Average)	45 (Average)	31.66 (Average)	47.5 (Average)
(2) Technical easiness	+++	++	+++	++
(3) Loss of blood during operation.	±	++	±	++
(4) Extent of healthy tissue damage	±	+	±	+

TABLE NO. 3

Showing distribution of cases according to breed

Anal Fistula			Venereal Granuloma		
Sl. No.	Breed	No. of Animal	Sl. No.	Breed	No. of Animal
1.	German Shepherd	9	1.	Mongrel	7
2.	Lhasa Apso	1	2.	Spitz	2
			3.	Boxer	1
Total		10	Total		10

Maximum number of cases of anal fistula were German Shepherd breed of dog and maximum number of cases of venereal granuloma were Mongrel dog.

TABLE NO. 4

Showing distribution of cases according to sex

Anal Fistula			Venereal Granuloma		
Sl. No.	Sex	No. of Animal	Sl. No.	Sex	No. of Animal
1.	Male	10	1.	Male	3
2.	Female	0	2.	Female	7
Total		10	Total		10

Maximum number of males were affected with anal fistula and maximum number of females were affected with venereal granuloma.

TABLE NO. 5

Showing distribution of cases according to age

Anal Fistula			Venereal Granuloma		
Sl. No.	Age group (yrs.)	No. of Animal	Sl. No.	Age group (yrs.)	No. of Animal
1.	0 — 2		1.	0 — 2	
2.	2.1 — 4	1	2.	2.1 — 4	5
3.	4.1 — 6	4	3.	4.1 — 6	4
4.	6.1 — 8	4	4.	6.1 — 8	
5.	8.1 — 10		5.	8.1 — 10	1
6.	10.1 — 12	1	6.	10.1 — 12	
7.	> 12		7.	> 12	

Maximum number of cases of anal fistula were between 4 to 8 years of age and maximum number of cases of venereal granuloma were between 2 to 6 years of age.

perianal region. Cryosurgery was done and the procedure took 24 minutes. The animal got uneventful recovery from epidural anaesthesia 35 minutes after operation. Sloughing of the dead and necrosed tissue was evident from 3rd P.O.D. A fresh bed of granulation tissue was observed at about 11th P.O.D. and onward. An appreciable healing was achieved on 21st P.O.D.

The affection of Animal No. 2 and 3 were more or less similar and severe than that of Animal No. 1 and suffering from last 13 and 15 months respectively. The left perianal region was affected along with left anal sac abscessation. They presented symptoms like tenesmus, pain, faecal incontinence, and constipation. The fistulous tracts were discharging pus. The animals had lose temperament. Inappetence, restlessness, and lifting of tail were the other symptoms noted. Freezing was applied and the total procedure took 28 minutes. Recovery from epidural anaesthesia was uneventful and within 40 minutes after operation. Sloughing was evident from 2nd P.O.D. and an appreciable healing was observed from 19th P.O.D.

Animal No. 4 and 5 were presented with 360° perianal fistulae and were suffering from last 19 and 20 months respectively. The tracts were discharging pus with occasional bleeding and a massive pyogenic reaction was evident around the affected tissue. Both the animals were undergone local dressing along with local chemical couterization and topical as well as parenteral antibiotic therapy, before referral to the Department of Surgery, W.B.U.A.F.Sc. The most common signs observed were dyschezia, tenesmus, inappetence, constipation, vigorous perianal licking and rubbing. Both of them were moderately dehydrated and loss of body weight were reported by the owners. Both of them were sedated for proper clinical observation, as they had bad temperament. In both cases merely all the affected tissues were effectively freezed by cryosurgery and the procedure took 32 and 35 minutes respectively. Recovery from anaesthesia were uneventful within 40 and 42 minutes respectively. Sloughing was evident from 2nd P.O.D. and ample healing was observed from 22nd P.O.D.

Animal No. 6 was presented for the treatment of perianal fistula and was suffering from last 25 months. History revealed that the animal was

undergone bilateral anal sacculotomy before referral to the Department of Surgery, W.B.U.A.F.Sc. Left, right, and ventral perianal regions were affected with fistulous tracts discharging blood tinged mucopurulent discharges. The common reported signs were diarrhoea, pain, perianal licking, and flatulence. Cryosurgery was done within a span of 30 minutes and the animal recovered from anaesthesia smoothly within 45 minutes after operation. Sloughing was evident from 2nd P.O.D. and the signs of healing was observed upto 7th P.O.D. but the new tracts discharging pus were evident again on 18th P.O.D. in the surrounding area.

4.2.2. Group A₁ :

Animal No. 7, suffering from last 3 months was presented with anal fistula in the ventral perianal region only affecting the skin and subcutaneous tissue. The affected site was moist and presenting occasional bleeding. Restlessness, perianal licking, circling to catch the anal region, and dyschezia were the most prominent signs. Surgical excision of the fistulous tract was done with 35 minutes operative period and the animal got uneventful recovery from anaesthesia within 55 minutes after operation. Signs of healing were evident from 6th P.O.D. and a complete healing was achieved after 18th P.O.D.

Animal No. 8 and 9 were suffering from perianal fistula for last 9 months and 13 months respectively. Pus discharging fistulae in the entire perianal region made the area ugly looking. Dyschezia, tenesmus, constipation, inappetence, and mild dehydration were the signs observed. Surgical excision of all the diseased tissue was done and required 40 and 45 minutes respectively. Recovery from anaesthesia was uneventful within 60 minutes after operation. Signs of healing in some portion was evident from 5th to 7th P.O.D. but the new tracts were observed in the perianal region from 26th P.O.D. onward.

Animal No. 10 was suffering from last 16 months with perianal fistulae in the right and left perianal region and anal sac abscessation. Some portions of the affected area were already fibrosed. The signs noted were tenesmus, diarrhoea, flatulence, perianal licking, mucopurulent discharge, inappetence and abrupt loss of body weight along with aggressiveness. Within a 60 minutes operative period, surgical excision of the affected tissue as well as anal sacculotomy were done. Recovery from anaesthe-

sia was smooth within 45 minutes after operation. No signs of healing was noticed rather the condition remained as it was.

4.2.3. Clinical observations :

Moderate to severe degree of perianal inflammation and swelling was observed in all animals preoperatively. In all animals of group A except the animal no. 6 mild degree of inflammation & swelling were noted upto 20 hours postoperative period and then declined by following 6 - 8 hours and completely subsided by 2nd P.O.D. In all animals of group A₁ maximum inflammatory swelling was noted on 3rd P.O.D. and then it gradually declined on 7th P.O.D. only in animal no. 7 and in other animals it persisted throughout the postoperative period. When compared between the group A & A₁ a significant ($P < 0.01$) decrease of inflammatory swelling was observed on 3rd P.O.D. at the operated site in group A than that of group A₁.

Moderate degree of pain at the perianal region of all animal was noted preoperatively due to suppurative inflammatory process. In all animals of group A, quick declination of pain sensation at the operated site was observed from 1st P.O.D. and completely subsided by 2nd P.O.D. but in animal no. 6 it again gradually increased from 14th P.O.D. In animals of group A₁ moderate to severe degree of pain sensation was noted upto 7th P.O.D. and then declined gradually in case of animal no. 7 where as it remained as it was in other animals of group A₁. When compared between group A & A₁ significant reduced pain sensation at the operated site was observed on 1st P.O.D. in group A than that of group A₁.

Moderate to severe degree of perianal discharge was observed preoperatively in all animals which was mucopurulent to purulent in nature with occasional bleeding. In all animals of group A, mild weeping of serous fluid was observed for 48 hours postoperative period where as that of in group A₁ reactionary haemorrhage was observed upto 5th P.O.D. In animals of group A₁ except the animal no. 7 and animal no. 6 of group A, mucopurulent discharge was again noted on the course of recurrence in the postoperative period ranging from 18th to 26th P.O.D. The operated site in animals of group A remained comparatively dry from 3rd P.O.D. than that of group A₁.

Perianal licking was moderate to severe in all animals preoperatively. Tendency of perianal licking was not observed in animals of group A except the animal no. 6, from 3rd P.O.D. Whereas that of group A₁ except the animal no. 7, remained significantly severe throughout the postoperative period.

Bad temperament was observed preoperatively in all animals. Animals of group A exhibited fair temperament from 3rd P.O.D. whereas no significant improvement of temperament was observed in animals of group A₁ except the animal no. 7.

Successful healing with minimal fibrosis was achieved in all animals of group A except the animal no. 6, within 19th to 22nd P.O.D. whereas reasonable healing with stricture formation was noted only in animal no. 7 among the group A₁. No postoperative faecal incontinence or constipation was observed in animals of group A.

Preoperative temperature of all animals of both groups ranged from 99.99 (°F) to 102.0 (°F). No significant changes of temperature were recorded in animals of group A. In the animals of group A₁ the temperature fluctuated within normal range except on 3rd P.O.D. when it was recorded significantly high ($P < 0.05$) and higher than that of group A.

In all animals of both groups, average heart rate (beats/min.) ranged from 100.33 to 101.25 preoperatively. Among the animals of group A, except the animal no. 6 it gradually declined to reach normal value by 28th P.O.D. In contrary it remained high on postoperative periods in the animals of group A₁ except the animals no. 7 where it reached nearly to the normal value by 28th P.O.D. A significant ($P < 0.01$) increase of heart rate was noted on 3rd P.O.D. in group A₁.

The average respiration rate (per minute) in all animals of both groups varied from 37.25 to 38.50 preoperatively. In animals of group A except the animal no. 6 it gradually decreased post operatively and reached to the normal level by 28th P.O.D. whereas in the animals of group A₁ except the animal no. 7, it gradually increased upto 5th P.O.D. and then decreased toward normal level. When compared between the group A & A₁, significant increased respiration rate was observed on 5th P.O.D. in group A₁ than that of group A.

4.3.1. Group B :

Animal no. 11 and 12 suffering from transmissible venereal granuloma since 13 months and 9 months respectively, referred for treatment to the department of surgery, W.B.U.A.F.Sc. In both cases the tumour masses involved vagina and vestibule and appeared as multilobulated, pink, friable, and haemorrhagic mass. The masses were large and well explored from the vulva thus creating problem for urination. The animals were dull and depressed and looked as if anaemic. The other signs were inappetence, dehydration, and loss of body weight. Following episiotomy cryosurgery was done successfully within 35 minutes and 40 minutes respectively. Both of them got uneventful recovery from epidural anaesthesia 50 minutes after operation. Sloughing of the necrosed tissues were evident from 3rd P.O.D. and healing was noticed on 7th P.O.D. and it was completed by 12th P.O.D.

Animal No. 13 was suffering from venereal granuloma at the base of the penis for last 3 months. Dribbling of urine along with occasional bleeding from the tumour mass was observed. The mass was multilobulated around the entire base of the penis, interspaced with normal tissue. Mild dehydration and infrequent licking of the penis were also observed. Cryosurgery was done by 25 minutes. Animal recovered from anaesthesia within 40 minutes after operation. Sloughing of the necrosed tumour mass was observed from 2nd P.O.D. but the condition recurred on 21st P.O.D. and onward.

Animal No. 14 and 15 were suffering from venereal granuloma for last 2 months and 4 months respectively. The tumour masses were small cauliflower like and involved only the vulvar epithelium and were visible only when vulvar lips were separated. Bleeding from the mass was negligible. Vulvar licking was most prominent. Cryosurgery was done within a span of 20 minutes and both of them got uneventful recovery from anaesthesia 35 minutes and 42 minutes after operation respectively. Sloughing was noticed from 2nd P.O.D. and reasonable healing was achieved on 9th P.O.D.

Animal No. 16 was suffering from venereal granuloma for last 7 months. The tumour masses were cauliflower like and extensive throughout the preputial epithelium. Difficulty during micturation along with fre-

quent bleeding was observed. Severe dehydration and panting were observed as most significant signs. Cryosurgery was done following opening of the preputial cavity through midventral raphae and the total procedure took 50 minutes. The animal uneventfully recovered from anaesthesia within 45 minutes after operation. From 2nd P.O.D. sloughing of necrosed tumour tissues were noticed and an appreciable healing was achieved on 10th P.O.D.

4.3.2. Group B₁ :

Animal No. 17,18 and 19 were suffering from venereal granuloma since 14 months, 8 months, and 11 months respectively. In animal no 17 & 18 the tumour masses were extensive throughout the vulvar epithelium, whereas in animal no. 19 the mass involved only the floor of vagina and was protruding through vulva. Difficulty in micturation and occasional bleeding were observed. All of them were undergone for surgical excision of tumour masses and took the time ranging from 45 - 57 minutes. Recovery from anaesthesia were smooth and uneventful within 50 minutes after operation. In animal no. 17 & 18 the signs of healing were evident from 9th P.O.D. and completed on 15 th P.O.D. In animal no. 19 although healing of the operated site was completed on 16th P.O.D. but the tumour reappeared on the vulvar epithelium and noticed on 28th P.O.D.

Animal No. 20 was suffering from last 3 months with venereal granuloma at the base of the penis. Multiple tumour masses were present in the same location repaired by normal tissue. The observed signs were occasional bleeding, inappetence, and mild dehydration. Within 37 minutes operative period, tumour masses were removed by surgical excision. The animal got uneventful recovery from anaesthesia within 45 minutes after operation. Signs of healing was evident from 7th P.O.D. and completed on 14th P.O.D.

4.3.3. Clinical observation :

In all animals of group B, a mild degree of inflammation and postoperative oozing were observed upto 24 hours postoperative period and then declined by following 8-10 hours, and completely subsided by 2nd P.O.D. On the otherhand in all animals of group B₁ a moderate degree of inflammation and oozing were observed upto 3rd P.O.D. and then sub-

Fig.No.1:MEAN of inflammation (score) in animals of group A & B (Cryosurgery) and group A1 & B1 (Surgical excision) during different intervals of the postoperative period.

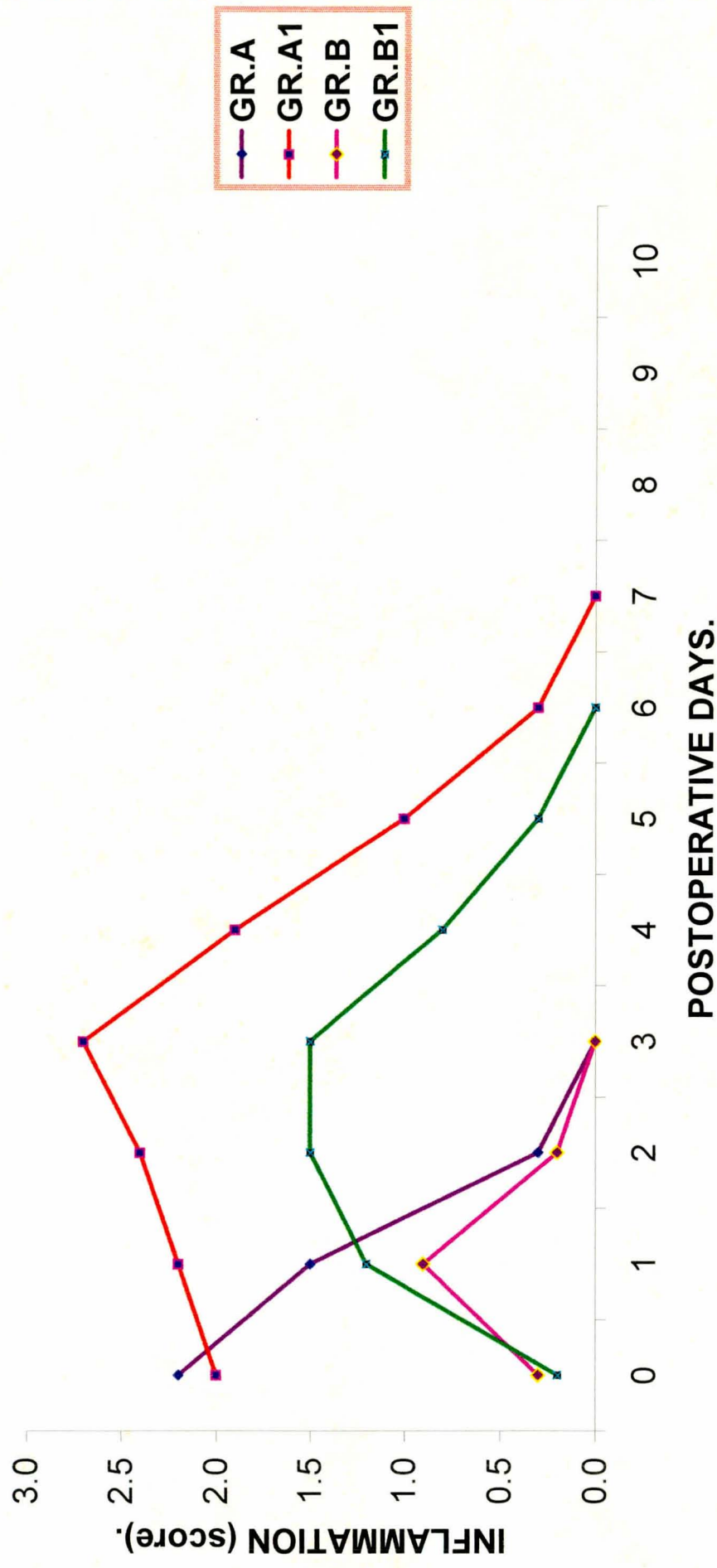
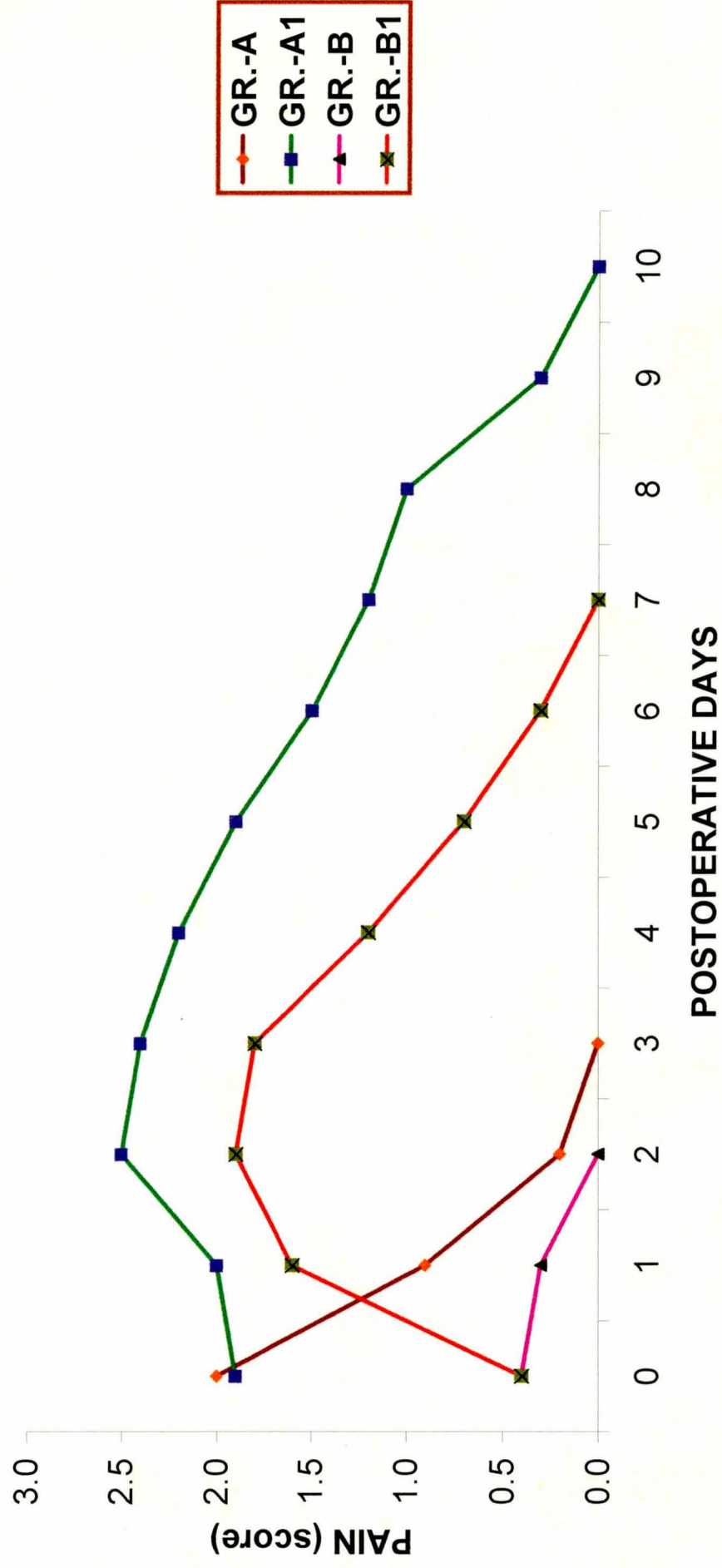


Fig.No.2: MEAN pain (score) in animals of group A & B (Cryosurgery) and group A1 & B1 (Surgical excision) during different intervals of the postoperative period.



sided by 5th P.O.D.

No signs of pain and discomfort were observed postoperatively in all animals of group B. In all animals of group B₁ moderate degree of pain was noted upto 3rd P.O.D. which significantly high than that of group B when compared between the groups.

In all animals of group B no tendency of licking of the operated site was observed on the postoperative days and the same in all animals of group B₁ was observed upto 3rd P.O.D.

Excellent healing of the operated site was observed within 9th to 12th P.O.D. in all animals of group B except the animal no. 13 and that of all animals of group B₁ except animal no. 19 was achieved within 14th to 15th P.O.D. No postoperative urinary incontinence were observed in animals of group B.

The average preoperative temperature (⁰F), heart rate (beats /min), and respiration rate (pre minute) in all animals in both groups were ranged from 101.27 (⁰F) to 101.28(⁰F), 100.25 to 101.16 and 29 to 29.5 respectively. No significant changes of temperature in the animals of both groups were observed on postoperative days rather fluctuated within normal range. Except in the animal no. 13 of group B and animal no. 19 in group B₁, the average heart rate and respiration rate decreased gradually and reached nearly to the normal value by 28th P.O.D. When compared between group B and B₁ no significant differences among these parameters were observed.

4.4. HAEMATOLOGICAL OBSERVATION

Total Erythrocyte count (TEC)

The average erythrocyte count recorded preoperatively in animals of group A & A₁ were 3.71 ± 0.183 and 3.35 ± 0.221 respectively and in group B & B₁ were 3.16 ± 0.346 and 2.65 ± 0.312 respectively. In group A & B the TEC increased significantly ($P < 0.01$) on 5th P.O.D. and reached nearly to the normal value by 28th P.O.D. No significant increase of the postoperative TEC was observed in group A₁ & B₁. When compared between group A & A₁ the value increased significantly ($P < 0.05$) on 5th



Photo No 22.



Photo No 23.

Photo No.22 & 23. Showing sloughing of the necrosed tissue on the 10th P.O.D following cryosurgery on anal fistula in canine and appreciable healing on 22nd P.O.D, respectively



Photo No 24.

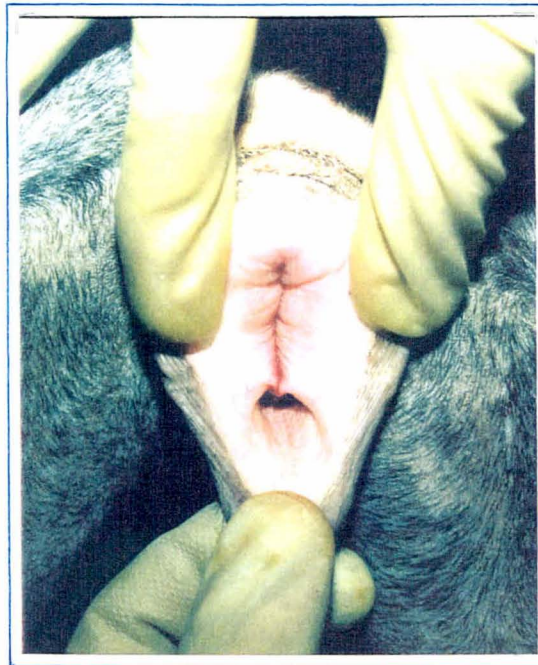


Photo No 25.

Photo No.24 & 25. Showing necrosed as well as reduced tumor mass on 5th P.O.D. following cryosurgery on venereal granuloma in bitch & a complete healing on 12th P.O.D, respectively.

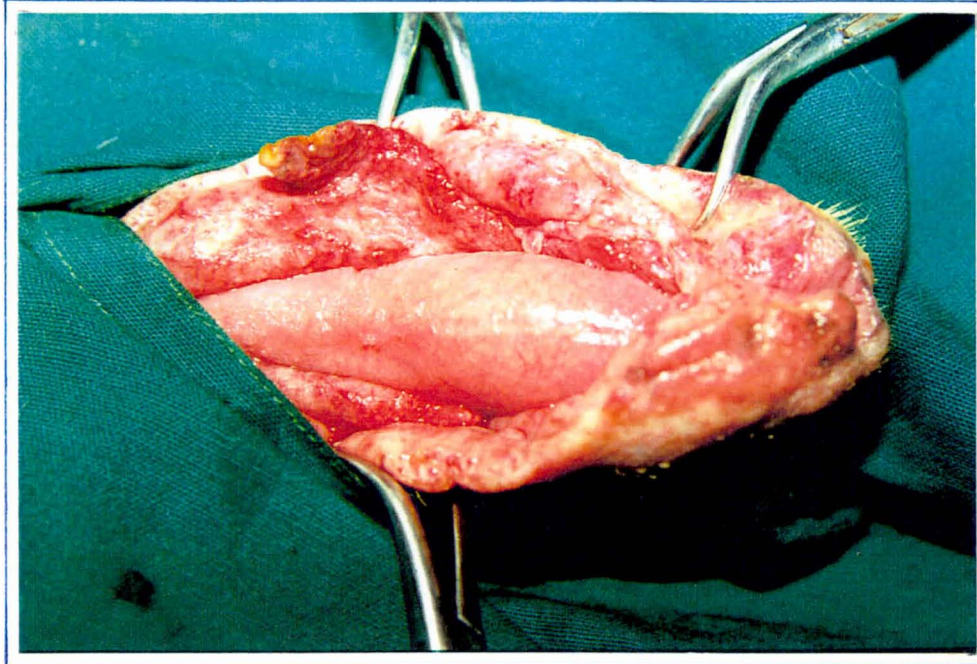


Photo No 26.

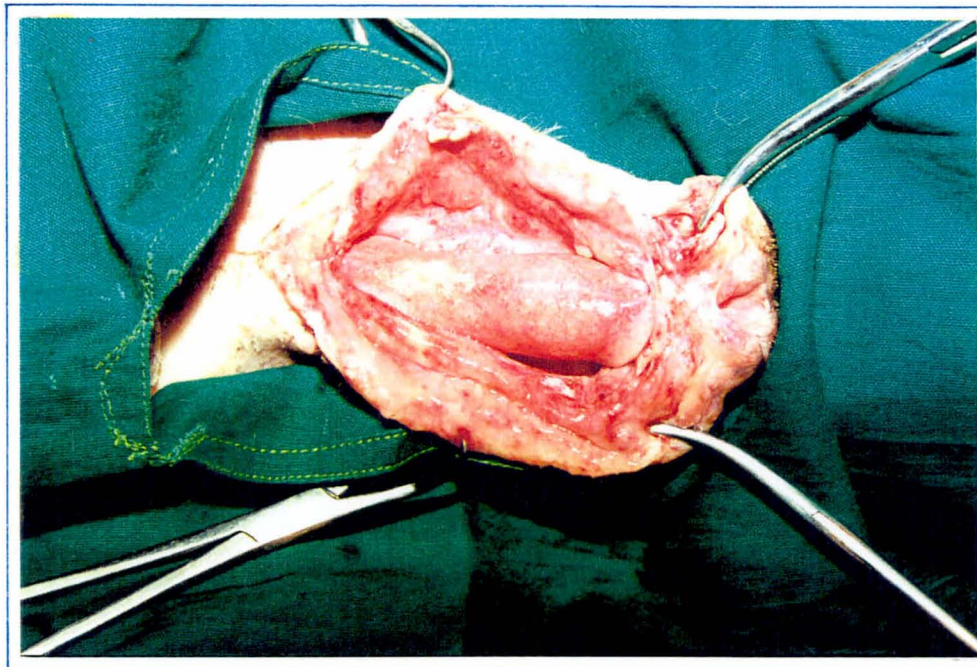


Photo No 27.

Photo No.26 & 27. Showing sloughing of necrosed tumor masses on 5th P.O.D following cryosurgery on venereal grannuloma of prepuce in dog and an appreciable healing on 12th P.O.D, respectively.



Photo No.28. Showing complete healing with stricture formation on 18th P.O.D following surgical excision of anal fistula in dog.



Photo No.29. Showing recurrence of the venereal granuloma in bitch on 28th P.O.D following surgical excision.



Photo No.30. Showing complete healing at the base of the penis on 28th P.O.D. following surgical excision of venereal granuloma in dog.

P.O.D. in group A than that of group A₁. No significant difference was seen at different intervals when compared between group B & B₁.

Haemoglobin Percentage

The preoperative mean Hb% in group A & A₁ were 11.13 ± 0.599 and 9.45 ± 0.290 respectively and in group B & B₁ were 9.11 ± 0.423 and 9.05 ± 0.880 respectively. The postoperative value increased significantly ($P < 0.05$) in group A and reached normal value by 28th P.O.D. The value also increased significantly ($P < 0.01$) in group B and reached nearly value by 28th P.O.D. no significant increase of the postoperative value were observed in group A₁ & B₁. When compared between group A & A₁ significant ($P < 0.05$) increased Hb% was observed on 5th P.O.D. in group A than that of group A₁. No significant differences were seen between group B & B₁ at different intervals.

Neutrophil count (Percentage)

The average preoperative neutrophil count recorded in animals of group A & A₁ were 72.16 ± 2.574 and 74.75 ± 2.561 respectively and in group B & B₁ were 72.16 ± 2.242 and 71.50 ± 1.322 respectively. In all animals of group A & B the postoperative value decreased significantly ($P < 0.01$) and reached nearly to the normal value by 28th P.O.D. In animals of group A₁ no significant decrease of neutrophil count was observed postoperatively, whereas the value in group B₁ declined significantly ($P < 0.05$) and reached normal value by 28th P.O.D. When compared between group A & A₁ a significant ($P < 0.05$) decrease of the value was observed from 15th P.O.D. to 28th P.O.D. in group A than that of group A₁. Similarly the same when compared between group B & B₁, it decreased significantly ($P < 0.05$) on 28th P.O.D. in group B than that of group B₁.

Lymphocyte count (Percentage)

The preoperative mean lymphocyte count recorded in animals of group A & A₁ were 23.50 ± 1.784 and 24.25 ± 1.250 respectively and in animals of group B & B₁ were 22.33 ± 2.538 and 21.25 ± 2.495 respectively. In all animals of group A & B the postoperative value increased significantly ($P < 0.01$) on 5th P.O.D. and reached normal level by 28th

P.O.D. no significant change of the value was observed in group A₁ & B₁ postoperatively. When compared between group A & A₁ significant increase (P<0.05) of postoperative lymphocyte count was observed on 28th P.O.D. in group A than that of group A₁. On the otherhand this value significantly increased from 15th to 28th P.O.D. in group B in comparison to that of group B₁.

Eosinophil Count (Percentage)

Mean eosinophil count recorded preoperatively in animals of group A & A₁ were 3.66 ± 0.333 and 3.00 ± 0.408 respectively and in animals of group B & B₁ were 2.00 ± 0.365 and 2.50 ± 0.288 respectively. In animals of group A the postoperative value decreased significantly (P<0.01) on 5th P.O.D. and declined thereafter and reached nearly normal value by 28th P.O.D. In animals of group A₁, B and B₁ no significant changes of the postoperative count were observed.

4.5. BIOCHEMICAL OBSERVATION

Total Serum Protein

The preoperatively recorded total serum protein in animals of group A & A₁ were 5.88 ± 0.153 and 6.10 ± 0.204 respectively and in group B & B₁ were 6.28 ± 0.177 and 6.45 ± 0.290 respectively. No significant changes in the postoperative value were observed in animals of any group.

Serum gamma (γ) Globulin

The mean serum γ globulin level recorded preoperatively in animals of group A & A₁ were 1.96 ± 0.054 and 2.40 ± 0.242 respectively and in group B & B₁ were 1.94 ± 0.047 and 1.97 ± 0.66 respectively. In animals of both group A & B, the postoperative value increased significantly (P<0.01) on 5th P.O.D. and reached a highlevel on 28th P.O.D. No significant postoperative changes were observed in animals of group A₁ & B₁. In between group A & A₁ difference of postoperative value at different intervals was not significant. But when compared between group B & B₁ significant (P<0.05) increased γ globulin level was observed on 5th P.O.D. in group B than that of group B₁.

TABLE NO. - 6

MEAN \pm SE. of Total Erythrocyte Count (Million/C.mm.) in animals of different groups during different time intervals.

GROUP	PREOPERATIVE	POSTOPERATIVE DAY		
		5	15	28
A	3.71 ^a \pm 0.183	4.55 ^{bA} \pm 0.182	4.85 ^{bcA} \pm 0.133	5.23 ^{cA} \pm 0.147
A1	3.35 \pm 0.221	3.52 ^B \pm 0.286	3.72 ^B \pm 0.319	3.52 ^B \pm 0.377
B	3.16 ^a \pm 0.346	3.91 ^b \pm 0.274	4.16 ^{bc} \pm 0.226	4.43 ^c \pm 0.190
B1	2.65 \pm 0.312	3.17 \pm 0.301	3.42 \pm 0.301	3.62 \pm 0.356

a, b, c mean values bearing at least one common superscript within same row does not differ significantly.

A, B mean values bearing at least one common superscript within same column does not differ significantly.

TABLE NO. - 7

MEAN \pm SE. of Haemoglobin percentage (gm/100ml blood) in animals of different groups during different time intervals.

GROUP	PREOPERATIVE	POST OPERATIVE DAY		
		5	15	28
A	11.13 ^a \pm 0.599	12.46 ^{abA} \pm 0.602	13.06 ^{bA} \pm 0.523	13.85 ^{bA} \pm 0.377
A ₁	9.45 \pm 0.290	9.75 ^B \pm 0.417	9.97 ^B \pm 0.436	9.77 ^B \pm 0.471
B	9.11 ^a \pm 0.423	10.36 ^b \pm 0.392	11.11 ^{bc} \pm 0.320	11.73 ^c \pm 0.308
B ₁	9.05 \pm 0.880	9.55 \pm 0.887	9.92 \pm 0.881	10.25 \pm 0.940

a, b, c mean values bearing at least one common superscript within same row does not differ significantly.

A, B mean values bearing at least one common superscript within same column does not differ significantly.

TABLE NO. - 8

MEAN \pm SE. of Neutrophil count (percentage) in animals of different groups during different time intervals.

GROUP	PREOPERATIVE	POST OPERATIVE DAY		
		5	15	28
A	72.16 ^a \pm 2.574	64.83 ^b \pm 2.257	61.66 ^{bcA} \pm 2.155	58.33 ^{cA} \pm 1.725
A ₁	74.75 \pm 2.561	72.25 \pm 3.037	69.75 ^B \pm 1.436	71.25 ^B \pm 1.314
B	72.16 ^a \pm 2.242	65.50 ^b \pm 1.607	62.83 ^b \pm 1.351	60.66 ^{bX} \pm 1.201
B ₁	71.50 ^a \pm 1.322	68.25 ^{ab} \pm 1.314	67.00 ^b \pm 0.912	65.25 ^{bY} \pm 1.314

a, b, c mean values bearing at least one common superscript within same row does not differ significantly.

A, B, X, Y mean values bearing at least one common superscript within same column does not differ significantly.

TABLE NO. - 9

MEAN \pm SE. of Lymphocyte count (percentage) in animals of different groups during different time intervals.

GROUP	PREOPERATIVE	POST OPERATIVE DAY		
		5	15	28
A	23.50 ^a \pm 1.784	30.00 ^b \pm 1.483	32.00 ^b \pm 1.154	33.50 ^{bA} \pm 0.921
A ₁	24.25 \pm 1.250	26.50 \pm 1.190	28.25 ^B \pm 1.030	28.75 ^B \pm 0.853
B	22.33 ^a \pm 2.538	31.33 ^b \pm 2.403	34.66 ^{bX} \pm 2.076	37.66 ^{bX} \pm 1.563
B ₁	21.25 \pm 2.495	23.75 \pm 2.954	25.50 ^Y \pm 1.258	24.50 ^Y \pm 1.190

a, b, mean values bearing at least one common superscript within same row does not differ significantly.

A, B, X, Y mean values bearing at least one common superscript within same column does not differ significantly.

TABLE NO. - 10

MEAN \pm SE. of Eosinophil count (percentage) in animals of different groups during different time intervals.

GROUP	PREOPERATIVE	POST OPERATIVE DAY		
		5	15	28
A	3.66 ^a \pm 0.333	2.50 ^b \pm 0.223	2.16 ^b \pm 0.166	2.33 ^b \pm 0.210
A ₁	3.00 \pm 0.408	2.50 \pm 0.288	2.75 \pm 0.250	2.50 \pm 0.288
B	2.00 \pm 0.365	2.16 \pm 0.166	2.16 \pm 0.307	2.33 \pm 0.210
B ₁	2.50 \pm 0.288	2.00 \pm 0.408	1.75 \pm 0.250	1.50 \pm 0.288

a, b, mean values bearing at least one common superscript within same row does not differ significantly.

TABLE NO. - 11

MEAN \pm SE. of Total Serum Protein (gm/100ml serum) in animals of different groups during different time intervals.

GROUP	PREOPERATIVE	POST OPERATIVE DAY		
		5	15	28
A	5.88 \pm 0.153	6.36 \pm 0.199	6.55 \pm 0.251	6.65 \pm 0.234
A ₁	6.10 \pm 0.204	5.95 \pm 0.132	5.925 \pm 0.170	6.10 \pm 0.108
B	6.28 \pm 0.177	6.83 \pm 0.190	6.91 \pm 0.181	6.88 \pm 0.144
B ₁	6.45 \pm 0.290	6.47 \pm 0.314	6.55 \pm 0.295	6.57 \pm 0.278

TABLE NO. - 12

MEAN \pm SE. of Serum Gamma Globulin (gm/100ml serum) in animals of different groups during different time intervals.

GROUP	PREOPERATIVE	POST OPERATIVE DAY		
		5	15	28
A	1.96 ^a \pm 0.054	2.55 ^b \pm 0.116	2.59 ^b \pm 0.108	2.42 ^b \pm 0.104
A ₁	2.40 \pm 0.242	2.28 \pm 0.210	2.18 \pm 0.173	2.13 \pm 0.163
B	1.94 ^a \pm 0.047	2.37 ^{bX} \pm 0.099	2.46 ^{bX} \pm 0.124	2.43 ^{bX} \pm 0.133
B ₁	1.97 \pm 0.066	2.00 ^Y \pm 0.054	2.02 ^Y \pm 0.032	1.95 ^Y \pm 0.054

a, b, mean values bearing at least one common superscript within same row does not differ significantly.

X, Y mean values bearing at least one common superscript within same column does not differ significantly.

TABLE NO. - 13

MEAN \pm SE. of Temperature ($^{\circ}$ F) recorded in animals of different groups.

GROUP	PREOPERATIVE	POST OPERATIVE DAY					
		1	3	5	10	15	28
A	101.16 \pm 0.317	101.36 \pm 0.334	101.25 \pm 0.337	101.23 \pm 0.340	101.30 \pm 0.321	100.91 \pm 0.238	100.63 \pm 0.341
A ₁	101.25 ^a \pm 0.193	101.80 ^{ab} \pm 0.108	102.25 ^b \pm 0.202	101.75 ^{ab} \pm 0.104	101.22 ^a \pm 0.325	101.12 ^a \pm 0.363	101.20 ^a \pm 0.348
B	101.28 ^{ab} \pm 0.181	101.46 ^a \pm 0.202	101.31 ^{ab} \pm 0.142	101.26 ^{ab} \pm 0.183	101.10 ^{ab} \pm 0.177	100.95 ^{bc} \pm 0.140	100.58 ^c \pm 0.135
B ₁	101.27 ^{ac} \pm 0.085	101.65 ^{ab} \pm 0.064	101.85 ^b \pm 0.850	101.55 ^{ab} \pm 0.155	101.40 ^{ac} \pm 0.091	101.32 ^{ac} \pm 0.154	101.12 ^c \pm 0.232

a. b. c mean values bearing at least one common superscript within same row does not differ significantly.

TABLE NO. - 14

MEAN \pm SE. of Heart Rate (beats/minute) recorded in animals of different groups.

GROUP	PREOPERATIVE	POST OPERATIVE DAY					
		1	3	5	10	15	28
A	100.33 ^a \pm 2.444	100.00 ^a \pm 2.352	100.50 ^a \pm 1.945	99.83 ^a \pm 2.329	95.16 ^{ab} \pm 2.227	89.50 ^{bc} \pm 2.565	81.50 ^c \pm 3.222
A1	101.25 ^{ab} \pm 2.954	103.75 ^a \pm 2.528	107.75 ^a \pm 2.136	106.75 ^a \pm 1.493	100.75 ^{ab} \pm 2.056	93.75 ^b \pm 2.594	100.75 ^{ab} \pm 2.594
B	101.16 ^a \pm 1.990	101.83 ^a \pm 1.796	102.66 ^a \pm 1.855	101.50 ^a \pm 1.431	98.66 ^a \pm 1.333	89.16 ^b \pm 2.151	82.33 ^b \pm 2.728
B1	100.25 ^a \pm 1.493	102.25 ^a \pm 1.376	104.25 ^a \pm 1.652	102.25 ^a \pm 1.547	99.50 ^a \pm 1.322	89.75 \pm 2.174	83.25 ^b \pm 2.174

a, b, c mean values bearing at least one common superscript within same row does not differ significantly.

TABLE NO. - 15

MEAN \pm SE. of Respiration Rate (per minute) recorded in animals of different groups.

GROUP	PREOPERATIVE	POST OPERATIVE DAY					
		1	3	5	10	15	28
A	38.50 ^a \pm 2.348	37.83 ^a \pm 2.663	33.50 ^{ab} \pm 1.727	32.50 ^{abA} \pm 1.360	29.66 ^{bcA} \pm 0.666	28.50 ^{bcA} \pm 0.763	25.33 ^c \pm 1.021
A1	37.25 ^a \pm 0.629	38.25 ^a \pm 0.853	38.75 ^a \pm 0.946	39.75 ^{aB} \pm 0.478	36.25 ^{aB} \pm 0.750	32.25 ^{bB} \pm 0.853	31.25 ^{bB} \pm 1.701
B	29.00 ^{ab} \pm 0.577	29.66 ^a \pm 0.802	28.83 ^{ab} \pm 1.013	27.50 ^{abcX} \pm 1.118	27.00 ^{abcX} \pm 0.577	26.16 ^{bc} \pm 0.600	24.50 ^c \pm 0.670
B1	29.50 ^a \pm 1.190	30.25 ^a \pm 0.629	31.25 ^a \pm 0.853	32.25 ^{aY} \pm 1.314	30.50 ^{aY} \pm 1.040	28.50 ^{ab} \pm 1.190	25.75 ^b \pm 0.670

a. b. c mean values bearing at least one common superscript within same row does not differ significantly.
A. B. X. Y mean values bearing at least one common superscript within same column does not differ significantly.

TABLE NO. - 16

ANOVA of different haematological, biochemical and clinical observations in animals of different groups between Cryosurgery and Surgical excision at pre and postoperative days.

Observations	Source of Variance	GROUP - A		GROUP - A ₁		GROUP - B		GROUP - B ₁	
		Degree of Freedom	Mean Sum Square	Degree of Freedom	Mean Sum Square	Degree of Freedom	Mean Sum Square	Degree of Freedom	Mean Sum Square
Total Erythrocyte Count	Between time	3	2.491**	3	0.093	3	1.783**	3	0.710
	Within time	20	0.159	12	0.376	20	0.156	12	0.406
Haemoglobin Percentage	Between time	3	7.891*	3	17.666	3	7.609**	3	27.833*
	Within time	20	1.709	12	19.583	20	0.798	12	6.041
Neutrophil Count	Between time	3	209.388**	3	17.666	3	149.486**	3	27.833*
	Within time	20	29.016	12	19.583	20	16.324	12	6.041
Lymphocyte Count	Between time	3	116.500**	3	16.562	3	264.222**	3	13.166
	Within time	20	11.350	12	4.770	20	28.466	12	17.958
Eosinophil Count	Between time	3	2.777**	3	0.229	3	0.111	3	0.729
	Within time	20	0.350	12	0.395	20	0.449	12	0.395
Total Serum Protein	Between time	3	0.694	3	0.035	3	0.537	3	0.014
	Within time	20	0.272	12	0.099	20	0.182	12	0.347
Serum γ - globulin	Between time	3	0.497**	3	0.053	3	0.354**	3	0.004
	Within time	20	0.060	12	0.160	20	0.068	12	0.011
Temperature	Between time	6	0.403	6	0.721	6	0.522*	6	0.23*
	Within time	35	0.616	21	0.263	35	0.169	21	0.073
Heart Rate	Between time	6	319.103**	6	86.904**	6	371.936**	6	243.488**
	Within time	35	36.557	21	22.630	35	22.742	21	11.690
Respiration Rate	Between time	6	140.436**	6	43**	6	19.744**	6	17.952**
	Within time	35	16.728	21	3.678	35	3.771	21	5.428

4.6. HISTOPATHOLOGICAL OBSERVATION (Photo No.31 to 34)

This was not included as a routine parameter within the present study and was not studied at different intervals. This was performed by taking a piece of tissue from the affected area of individual animal before employing any surgical therapy to diagnose the disease perfectly. The preoperative histopathological study in animals of group A & A₁ revealed the chronic suppurative inflammation of the perinal tissues, and that of group B & B₁ revealed the granulomatous growth on epithelial lining of vulva, penis or prepuce. The postoperative study on 28th P.O.D. revealed the normal texture of perianal tissue and vulvar or penile or preputial epithelium in successfully cured animals of group A & A₁ and group B & B₁ respectively.

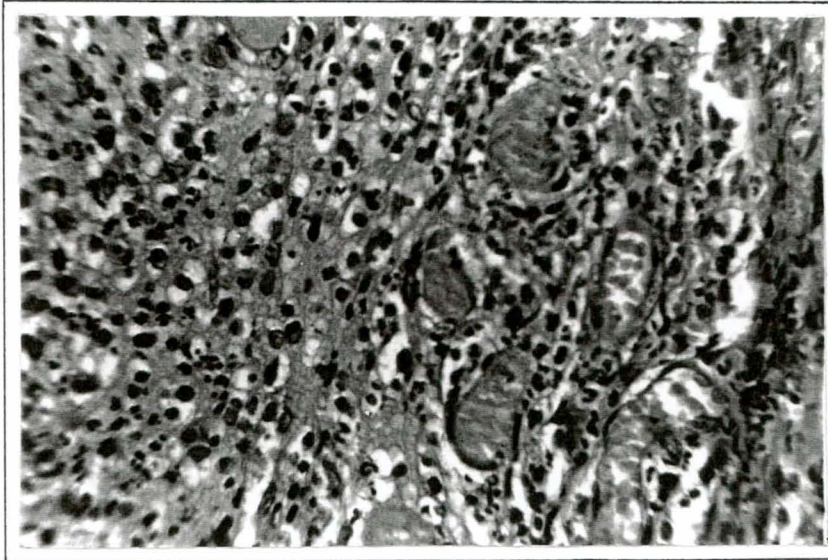


Photo No.31. Showing section of perianal fistula in dog (H&E, $\times 450$).

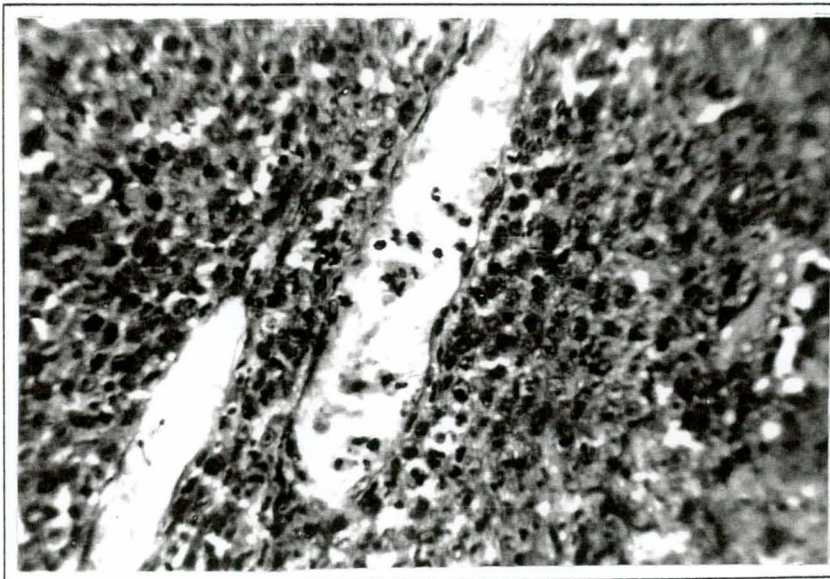


Photo No.32. Showing section of venereal granuloma in dog (H&E, $\times 450$).

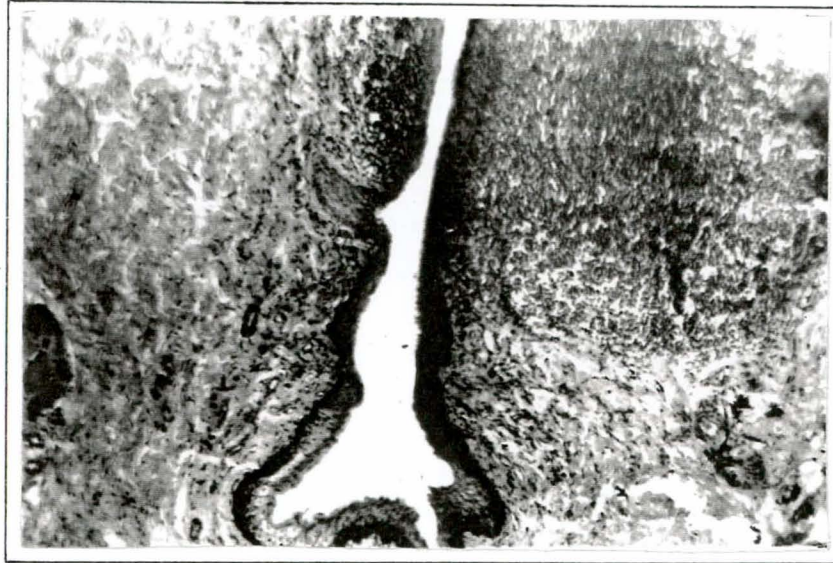


Photo No.33. Section shows normal histological texture of the perianal tissue following cryosurgery.(H&E, $\times 100$).

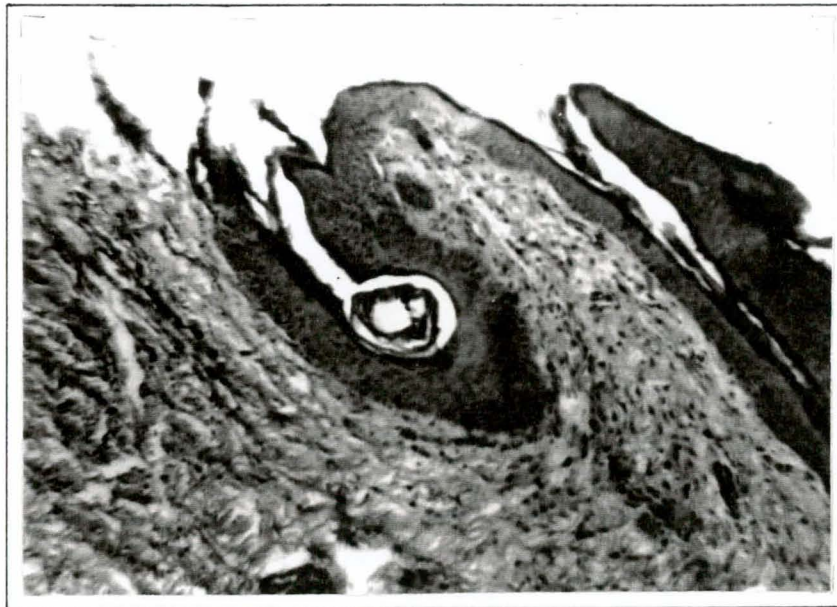


Photo No.34. Section shows normal histological texture of the vulvar tissue following cryosurgery(H&E, $\times 100$)



Chapter V

DISCUSSION

DISCUSSION

Being refractive to the medical therapy the treatment of anal fistula is soully dependent on surgical therapy. The aim of the treatment is emphasised mainly on the removal of the affected tissue as well as to stop the diseased process and then to achieve the normal aratomical texture of the treated site. With the radical surgical approach, some other techniques were also applied for the management of this surgical condition but search is still going on 10 find a better effective treatment for that.

Venereal granuloma in this respect differs slightly. Medical therapy with chemotherapeutic agents or the conventional surgical approach can't be said ineffective but as a rule neither is ideal and nor can claim free from drawbacks. Thus here also interests of the researchers have been paid to search a newer venture for the surgical management of this pathology.

In this race, cryosurgery is not a new conception. Recently interests of the workers and followingly their works on different fields have proved its efficacy on such conditions which are poorly managed by conventional therapies with greater postoperative stress and hazards.

In the present study the efficacy of the cryosurgery in comparison to the conventional surgical excision was evaluated in respect of clinical, haematological and biochemical observations, for the treatment of anal fistula and venereal granuloma.

5.1. BREED, SEX AND AGE DISTRIBUTION :

In the experimental model of the present study 10 clinical cases of anal fistula of 2 to 12 years old having body weight ranging from 15-18 kg and 10 clinical cases of venereal granuloma of 2 to 10 years old having body weight ranging from 10-14 kg, had been used. Occurance of anal fistula was observed 100% among the male of which 90% were German Shepherd breed of dog and 80% having age distribution between 4 to 8 years (*Houlton, 1980*).

Occurance of venereal granuloma was observed 70% among the fe-

male and 30% among the male dog of which 70% were mongrel dog and 90% having age distribution between 2 to 6 years (*Nayak and Samaddar, 1987*).

In the present study, males were absolutely reported having anal fistula of which German shepherd breed of dogs were mostly reported. This observation corroborated the findings of *Lane and Bruch, (1974)*. Male and female ratio in case of venereal granuloma was 7 : 3 and most of this was reported in mongrel dog.

5.2. INTRA OPERATIVE OBSERVATIONS :

Although the ultimate goal of cryosurgery and surgical excision are the removal, either immediate or delayed, of all the affected tissues but the aim of each type of operation is distinct and that of cryosurgery is to put total diseased tissues effectively in the process of cryonecrosis, in contrast to that of surgical excision where the aim is to remove all the diseased tissues and appropriate suturing of the wound for proper healing. The later type is a composite procedure of different steps like incision, excision, suturing and overall maintenance of asepsis. But the cryosurgery includes only the application of freezing temperature with temperature monitoring. Thus this has more technical easiness and takes less time. In the present study the cryosurgical approach required much less time averaging 31.33 minutes for anal fistula and 31.66 minutes for venereal granuloma than that of surgical excision which required average 45 minutes for anal fistula and 47.5 minutes for venereal granuloma.

In both affections the surgical sites are highly vascularised. During cryosurgery in both varieties bleeding was minimal and this was due to the vascular stasis on application of cold temperature (*Whitaker, 1972*) on capillaries and smaller blood vessels. Whereas the intraoperative bleeding was comparatively greater during the surgical excision and removal of the affected tissue in both conditions. This bleeding was obviously due to incision and thus disruption of the capillary bed, deeply embedded within the soft tissue.

Healthy tissue damage was comparatively greater during surgical excision than that of during cryosurgery. During the procedure of surgical

excision on anal fistula, affected tissues along with a thin margin of surrounding healthy tissue had to be sacrificed even at the cost of haemorrhage, otherwise the appropriate suture could not be applied, since the affected tissue had practically poor tensile strength. Similarly the same procedure when employed on venereal granuloma, the underneath epithelium of the tumour masses were also removed to create a fresh wound for proper healing. But when cryosurgery was employed merely the diseased tissues were subjected to freezing along with a thin margin of surrounding healthy tissue where the margin of ice ball was visible. This healthy tissue later returned to the normal condition as the margins of ice ball attained 0°C temperature (*Withrow, 1980*), which could not set cryonecrosis of the healthy tissue.

5.3. POST OPERATIVE OBSERVATIONS :

Unfortunately scarcity of earlier records of comparable work has made it almost impossible to compare the observations and findings of the present study with those of other workers. Author had to formulate and standarize his findings with the support of the related references available in this literature.

5.3.1. Clinical observations :

Group A and A₁ : In all animals of both the groups moderate to severe degree of inflammation and inflammatory swelling was observed preoperatively. In all animals of group A where cryosurgery was employed mild degree of inflammation persisted upto 24 hours postoperative period and then declined gradually and dropped by next 24 hours where the healing process started. But in all animals of group A₁ where surgical excision was employed, the inflammatory stage persisted upto 3rd P.O.D. and then gradually declined and subsided by 7th P.O.D. but again increased on 18th P.O.D. Comparative assessment between group A and A₁ revealed that the inflammatory stage was relatively more marked and persisted for longer period in group A₁, Particularly on 3rd P.O.D., where it was significantly ($P < 0.01$) higher in group A₁ than group A. This finding may be attributed to relatively poor healing process as well as progression of pathogenesis of the disease in the animals of group A₁ following surgical excision in con-

trast to the less tissue reaction and early signs of healing in the animals of group A.

Moderate degree of pain was noted in all animals of both the groups preoperatively. The postoperative pain in the operated sites of the animals of group A declined quickly on 1st P.O.D. and disappeared completely by 2nd P.O.D. But in all animals of group A₁, a moderate to severe degree of pain sensation was noted upto 7th P.O.D. The comparison between group A and group A₁ revealed significant reduction of pain sensation on 1st P.O.D. in group A than that of group A₁ and this indicated that the cryo-surgical approach is relatively painless in the post operative period which corroborated with the findings of *Lloyd - Williams, (1973)*. The marked postoperative reduction of pain sensation in animals of group A may be due to the destruction of nerve endings by freezing effect. Whereas it persisted longer in the postoperative periods in animals of group A₁ and may be due to pressure on nerve endings, exerted by the inflammatory swelling.

Weeping from the operated sites in all animals of group A was observed only for 48 hours postoperative period in contrast to the animals of group A₁ where it was noted upto 5th P.O.D. This phenomenon followed the course of inflammatory response in animals of both groups. When compared between these two groups, the operated site remained comparatively dry in animals of group A than that of group A₁ from 2nd P.O.D. The reason for less weeping in group A may be due to ischaemic infarction in the capillary bed following cryosurgery. (*Gill et al., 1971*). This attributed a quick set up of healing process in the animals of group A than those of group A₁.

Moderate to severe degree of perianal licking and bad temperament were observed on an average in all animals of group A and group A₁, preoperatively. Mild perianal licking was observed upto 3rd P.O.D. in animals of group A in comparison to that of group A₁ where it persisted throughout the postoperative period, except in the animal where healing of the operated site was achieved. The temperament was also observed same like licking. The better picture observed in animals of cryosurgical group, may be due to the early relief from perianal tissue irritation, experienced by these animals following earlier and better prevention of local

infections. Additionally the suture applied in the perianal tissues may be a reason of irritation in the animals of group A₁.

Successful healing was achieved in 5 animals out of 6 of group A, whereas in one animal out of 4 in group A₁. The complete resolution of clinical cases of anal fistula following cryosurgery was achieved among 83.33% animals and recurrence occurred in 16.67% operated animals. These findings justified the findings of *Lane and Bruch, (1975)*. The resolution and recurrence rate of surgical excision on anal fistula was 25% and 75% respectively. This high recurrence rate of anal fistula following surgical excision corroborated with the findings of *Vasseur, (1984)*. The postoperative fibrosis was observed as minimal following cryosurgery whereas anal stricture formation was evident following surgical excision in the present study. These findings corroborated with the findings of *Lane (1974)* and *Robins and Lane (1973)* respectively.

Recurrence of anal fistula in one animal of cryosurgical group probably due to improper freezing of the affected tissues by the cryoprobe. The behaviour of the animal also boisterous due to age factor. Recurrence of the surgical condition in excision group was obviously due to tendency of constant licking, and biting and rubbing of the wound due to greater irritation persisted in the site of operation and chance of reinfection.

No significant change of rectal temperature was observed after cryosurgery in animals of group A in the postoperative days. But this was significantly high on 3rd P.O.D. in animal of group A₁ following surgical excision. The heart rate following cryosurgery decreased gradually and reached normal level whereas no significant declination of heart rate was observed in animals after surgical excision and this may be due to persistent pain sensation in the operation site. The respiration rate after cryosurgery and surgical excision in group A and A₁ respectively, decreased and reached to the normal level by 28th P.O.D. But a significant increase of respiration rate was observed on 5th P.O.D. after surgical excision than that of after cryosurgery, and this may be due to normal physiological response of the body to the more surgical stress.

Group B and B₁ : In all animals of group B, mild inflammatory swelling was observed on 1st P.O.D. and completely subsided by 2nd P.O.D.

But this was found more severe and persisted upto 5th P.O.D. in all animals of group B₁. This may be attributed to the early starting of healing process in the animals of group B after application of cryosurgery. The reason behind the greater inflammatory response in the animals of group B₁ following surgical excision may be due to greater soft tissue manipulation during operation than that of during cryosurgery.

The postoperative oozing in the animals of cryosurgical group was notably lesser than that of in the animals where surgical excision was applied and in both groups, the post operative oozing followed the same course of inflammation. A mild reactionary haemorrhage was found in all animals of group B₁ for first 48 hours postoperative period. The comparative dryness of cryosurgical lesion may be due to an extreme clumping, packing and adherence of red blood cells of each other and to the endothelium and thus plugging the lumen of the capillaries (*Seim, 1980*). The another cause behind the less postoperative oozing may be that the necrosed tissue resided at the operated site and acted as dressing on the cryolesion and thus did not leave any potential space for oozing (*Yamamoto and Sano, 1982*).

No remarkable pain, licking of the operated site and discomfort were observed in the animals of group B on postoperative days whereas these were notably higher upto 3rd P.O.D. in the animals of group B₁. Sutures applied on the epithelial lining may be a cause of irritation, pain, licking and thus discomfort, experienced by the animals of group B₁. In contrast this was absent in group B. Moreover the cryodestruction of the nerve endings in the operated site may be a cause of absence of pain sensation in the animals of group B.

Early healing (within 9th to 12th P.O.D) without fibrosis was observed in animals of group B. In group B₁ comparatively longer period (14th to 15th P.O.D) of healing was noticed. Resolution of cases following cryosurgery achieved in 83.33% cases in contrast to 75% cases following surgical excision on venereal granuloma.

No significant postoperative increase of temperature, heart rate and respiration rate were observed in the animals where complete resolution occurred in both groups. These declined gradually and reached normal

level by 28th P.O.D.

5.3.2. Haematological and biochemical observations :

Due to nonavailability of any earlier records regarding changes in the blood picture and estimation of total serum protein and γ - globulin in a systemic manner and at regular intervals following cryosurgery on anal fistula and venereal granuloma, no comparison could be made, rather author had tried to formulate and standardized his own findings in the present study.

5.3.2.A. Total Erythrocyte count (TEC) and Haemoglobin % :

In all animals either affected with anal fistula or venerea granuloma, the preoperative total erythrocyte count and haemoglobin (Hb) percentage were below the normal level. In animals of group A where cryosurgery was applied, both increased significantly and reached normal level by 28th P.O.D. But no significant increase of postoperative values were observed. Increase of TEC and Hb % were significant on 5th P.O.D. in group A than that of group A₁. In all animals of group B these values increased significantly and reached normal level by 28th P.O.D. and these were not significant at different intervals than that of group B₁, where the values increased gradually and reached nearly to the normal level by 28th P.O.D. However the quick inclination of these values and early return to the normal level following cryosurgery may be due to the overall improvement of physical condition.

5.3.2.B. Neutrophil count :

In all animals of all groups the preoperative neutrophil count was high. In both cryosurgical group (group A and B) this value decreased significantly and reached normal level by 28th P.O.D. In group A₁ no significant decrease of postoperative value was observed and it may be due to persistent infection and greater inflammatory response following surgical excision. But in group B₁ the postoperative value decreased significantly and reached nearly to the normal level by 28th P.O.D. When compared between group A & A₁ and between group B & B₁, the decrease of this value was significant on 28th P.O.D. in group A and B than that of group A₁ and B₁ respectively. This difference may be due to surgical stress, greater pain

sensation in the postoperative period on animals of group A₁ and B₁ than that of group A and B respectively as these conditions stimulate the release of corticosteroid which in turn causes neutrophilia (Coles, 1986).

5.3.2.C. Lymphocyte Count :

The preoperative lymphocyte count was below the normal level in all animals of group A, A₁, B and B₁. In all animals of group A and B lymphocyte count increased significantly in the postcryosurgical period and reached normal level by 28th P.O.D. Following surgical excision no significant increase of this value was observed in postoperative period in the animals of group A₁ and B₁. When compared between group A and A₁ and between group B and B₁ a significant elevation of this count were observed on 28th P.O.D. in group A and B then that of group A₁ and B₁ respectively. This relative lymphocytosis following cryosurgery may be due to cryoimmunostimulation in the animals of group A and B as lymphocytosis due to increase T - lymphocyte release following antigenic stimulation (Coles, 1986).

5.3.2.D. Eosinophil count :

The preoperative eosinophil count in all animals of group A and A₁ was observed higher than the normal level and that in group B and B₁ was observed more or less normal. The postoperative change of eosinophil count significantly decreased in animals of group A but remain high in animals of group A₁. The postoperative value didnot changed in all animals of group B and B₁, rather fluctuated within the normal level. As there is eosinophil chemotaxis in anal fistula (Day and Weaver, 1992), the preoperative high eosinophil count in the animals affected with anal fistula soon returned to normal level following cryosurgery due to effective necrosis of the affected tissue.

5.3.2.E. Total serum protein :

The normal level of total serum protein in dog is 5-7.5 gm/100ml serum. The preoperative and postoperative values in all animals of all groups fluctuated within the normal level. This may be indicative that neither cryosurgery nor surgical excision created remarkable surgical stress on the animals, because if there was any surgical stress, there should be sig-

nificant decrease in total serum protein concentration (*Schalm et al., 1975*).

5.3.2.F. Gamma (γ) Globulin :

The normal level of γ globulin in dog is 0.8 gm/ 100 ml serum. The preoperative values in all groups were noted higher than that of normal level and it may be due to chronic bacterial infection (in animals of group A & A₁) and neoplasia (in animals of group B & B₁) as reported by *Coles (1986)*. The postoperative value in animals of group A & B were increased significantly from 5th P.O.D. to 28th P.O.D. and significantly higher than that of group A₁ & B₁ respectively at different intervals. In group A₁ & B₁ the post operative value remained unchanged. *Coles (1986)* reported that the level of immunoglobulins (γ -globulin) may be elevated following immunostimulation. Thus the significant increase of its level in group A & B following cryosurgery may be due to cryoimmunostimulation (*Neel and Ritts, 1979*).



Chapter VI

SUMMARY

SUMMARY

With the advancement of science the definitions of the management of different surgical conditions are still changing. These are developed and instituted in an around a basic principle with an ultimate aim to be free from the disease with minimum side effects. This phenomenon is prominent regarding those surgical conditions which are refractive to the medical therapy and poorly managed by conventional surgical approaches. Amongst the many such therapies in different fields, cryosurgery is such modality which has proved its superiority over the conventional therapies in many fields like dermatology, gynaecology, urology, proctology, ophthalmology and neurology.

Cryotherapy, the therapeutic use of cold in medical science when intended for surgical purpose is known as cryosurgery.

Anal fistula and venereal granuloma are two surgical conditions frequently reported in the canine population and still a problem to cure uneventfully. In the present study it was tried to find a better way for the management of these surgical conditions.

The experiment was conducted on 10 animals affected with anal fistula and 10 animals with venereal granuloma. The choice of the clinical cases were random and numbered from 1 to 20 being placed in four different groups like 1 to 6 in group A, 7 to 10 is group A₁, 11 to 16 in group B, and 17 to 20 in group B₁. The clinical, haematological and biochemical and the overall performance of cryosurgery in comparison to the conventional surgical excision was evaluated by applying these on the animals of group A & B and group A₁ & B₁ respectively.

The age of the 10 animals affected with anal fistula was ranging from 4 to 12 years with body weight between 15 to 18 kg. All the reported animals were male of which 9 were German Shepherd breed of dog. The age distribution of the 10 animals affected with venereal granuloma was ranging from 2.8 to 10 years with body weight between 10 to 14 kg. Among these 7 were bitch and 3 were male dog and the maximum reported cases (7 animals) were mongrel dog.

In all cases the initial history was studied thoroughly. General physical examination of all the animals were done in routine manner. Detailed examination of the lesions of individual animal was done. Temperature, heart rate, and respiration rate were recorded. Evidence of shock, state of nutrition, and dehydration were also observed and the necessary correction of body fluid deficit was made with 5% dextrose. Blood drawn pre-operatively and tissue pice from the lesion of individual animal was taken for haematological, biochemical, and histopathological examination respectively.

Nitrous oxide cryounit was used for cryosurgical approach in all animals of group A & B under epidural anaesthesia with xylazine hydrochloride @ 0.75 mg/kg b.wt., premedicated with atropine sulphate @ 0.66 mg/animal. Strict aseptic measures were not imperative. In all animals of group A the perianal affected tissues were debrided and freezed by probe freezing method using two freeze - thaw cycles where 30 to 40 seconds freezing was applied below - 20°C in each cycle. In all animals of group B, the granulomas in the external genitalia were effectively freezed below -20°C for 40-50 seconds in each of two freeze - thaw cycles. Routine postoperative care, management, and observations were made upto 4 weeks postoperative period.

In group A₁ & B₁ conventional surgical excision was done under general anaesthesia with xylazine hydrochloride @ 1.5 mg/kg b.wt. premedicated with atropine sulphate @ 0.65 mg/ animal. In all animals of group A₁, all the affected perianal tissues were excised and the surrounding healthy tissues were sutured. In all animals of group B₁ the total mass of tumour was removed from the base after ligation of the bleeding vessels and the epithelial wound was sutured. Strict postoperative care, management, and observations were made upto 4 weeks postoperative period.

In all groups routine antibiotic coverage was done for 5 postoperative days using ampicillin & cloxacillin. Daily irrigation of the operated site was done by normal saline and an antibiotic ointment was applied on the site for at least 10 days postoperative period. All the animals were fed with easily digestible diet and laxative upto 4 weeks postoperatively to minimize the straining on the operated site, and during this period clinical, haematological, and biochemical observations were recorded.

The intraoperative observations revealed that there was minimum bleeding and healthy tissue damage during cryosurgery on animals of group A & B than that of during surgical excision of animals of group A₁ & B₁. Furthermore cryosurgery was technically more easy to employ and took less time (average 31.33 mins. for group A and average 31.66 min for group B) than that of surgical excision (average 45 min. for group A₁ and average 47.5 min. for group B₁). All animals recovered from anaesthesia smoothly and uneventfully.

The clinical observations studied were inflammatory response, pain, irritation, oozing, licking of the operated site, temperament of the animal, temperature, heart rate, respiration rate, and healing of the operated site.

Inflammatory response and the postoperative oozing was mild and noted only upto 2nd P.O.D. in the animals of cryosurgical groups (group A & B) in comparison to the excision group (group A₁ & B₁) where these were significantly high and persisted upto 5th to 7th P.O.D. The early termination of the inflammatory stage with less postoperative oozing for quick set up of healing process was attributed by cryosurgery.

Moderate to severe degree of pain, tissue irritation, licking and discomfort were observed preoperatively, specially in animals affected with anal fistula. The temperament was also noted bad in these animals. Post-operatively these observations were negligible in animals of cryosurgical groups (group A & B) whereas these persisted comparatively longer in excision groups (group A₁ & B₁).

In all animals of group A & A₁ and group B & B₁ the average preoperative temperature ranged from 99.9 (°F) to 102.0 (°F) and 101.27 (°F) to 101.28 (°F) respectively. The postoperative temperature in all animals of group B & B₁ were within normal range but among group A & A₁ it was significantly high (P<0.05) on 3rd P.O.D. in group A₁ than that of group A.

The mean preoperative heart rate (beats/min). in all animals of group A & A₁ and groups B & B₁ ranged from 100.33 to 101.25 and 100.25 to 101.16 respectively. The postoperative heart rate declined gradually and reached normal level by 28th P.O.D. in animals of group A, whereas in

animals of group A₁ a significant ($P<0.01$) increase of heart rate was observed on 3rd P.O.D. and remained high throughout the postoperative period. The increase of heart rate in animals of group B & B₁ were not observed rather decreased gradually and reached nearly to the normal level by 28th P.O.D.

The mean preoperative respiration rate (per min.) in all animals of group A & A₁ and group B & B₁ ranged from 37.25 to 38.50 and 29.0 to 29.5 respectively. The postoperative value decreased gradually and reached normal level by 28th P.O.D in animals of group A, B, and B₁. But this increased upto 5th P.O.D. in animals of group A₁ and then decreased gradually to reach the normal level by 28 the P.O.D. When compared between group A & A₁ a significant increased of this value was observed on 5th P.O.D. in group A₁ than that of group A.

The average erythrocyte count recorded preoperatively in animals of group A & A₁ were 3.71 ± 0.183 and 3.35 ± 0.221 respectively and in group B & B₁ those were 3.16 ± 0.346 and 2.65 ± 0.312 respectively. The postoperative value increased significantly ($P<0.01$) on 5th P.O.D. in animals of group A & B but that of group A₁ & B₁ remained unchanged. A significant ($P<0.05$) increase of the value on 5th P.O.D. was observed in group A than that of group A₁.

The average preoperative Hb% in animals of group A & A₁ were 11.13 ± 0.599 and 9.45 ± 0.290 respectively and in group B & B₁ were 9.11 ± 0.423 and 9.05 ± 0.880 respectively. The postoperative changes were same as total erythrocyte count.

The mean preoperative neutrophil count was recorded high in all groups and that of group A, A₁, B, and B₁ were 72.16 ± 2.574 , 74.75 ± 2.561 , 72.16 ± 2.242 , and 71.50 ± 1.322 respectively. The significant decrease of the postoperative value was observed in group A & B after cryosurgery.

The mean preoperative Lymphocyte count recorded in all groups were below the normal level and that of group A, A₁, B, and B₁ were 23.50 ± 1.784 , 24.25 ± 1.250 , 22.33 ± 2.538 , and 21.25 ± 2.495 respectively. The postoperative lymphocyte count increased significantly in group

A & B following cryosurgery.

The mean preoperative eosinophil count was high in animals of group A & A₁ (3.66 ± 0.333 and 3.00 ± 0.408 respectively) and that of group B & B₁ were within normal range (2.00 ± 0.365 and 2.50 ± 0.288 respectively). In all groups except group A, the postoperative value remained unchanged and that of group A declined significantly and reached normal value by 28th P.O.D.

The total serum protein recorded preoperatively in animals of all groups were normal and the postoperative value factuated within normal range.

The mean γ - globulin level recorded preoperatively in all animals of all groups revealed high and that of group A, A₁, B and B₁ were 1.96 ± 0.054 , 2.40 ± 0.242 , 1.94 ± 0.047 , and 1.97 ± 0.66 respectively. The postoperative value increased significantly in animals of group A & B than that of group A₁ & B₁ respectively.

Healing after cryosurgery was uneventful with minimal scarring and took an average period 22 days for anal fistula and 9 to 12 days for venereal granuloma. The period of healing after surgical excission was 18 days for anal fistula and 14 to 15 days for venereal granuloma. Stricture formation was observed in animals operated by surgical excision. The complete repair of clinical cases was high after cryosurgery (83.33 % cases of anal fistula and 83.33% cases of venereal granuloma) than that of surgical excision (25% cases of anal fistula and 75% cases of venereal granuloma). The rate of recurrence was less after cryosurgery (16.67% in both anal fistula and venereal granuloma) than of surgical excision (75% in anal fistula and 25% in venereal granuloma).



Chapter VII

CONCLUSION

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- (1) Strict aseptic measures during cryosurgery were not obligatory.
- (2) It did not required general anaesthesia.
- (3) The method was easy to apply and caused minimum bleeding and soft tissue manipulation.
- (4) Significantly less inflammatory response, pain, and irritation in the operated site were observed following cryosurgery. The animal did not experienced any remarkable discomfort and paid little attention to the operated site. The postoperative management was simple with feeble chance of secondary infection. Early improvement of general body condition was observed.
- (5) The surgical stress during cryosurgery was insignificant.
- (6) Healing after cryosurgery was uneventful with minimum scarring. Thus it did not resulted postoperative faecal or urinary incontinence following its application on anal fistula and venereal granuloma respectively.
- (7) Chance of recurrence after cryosurgery was minimum.
- (8) Chance of immunostimulation was indicated following cryosurgery.
- (9) The treatment was economical and thus may gain popularity.



Chapter VIII

**FUTURE SCOPE
OF RESEARCH**

FUTURE SCOPE OF RESEARCH

- (1) Estimation of cell mediated and humoral immunity following cryosurgery on anal fistula and venereal granuloma.
- (2) Precise effect of cryosurgery on reticuloendothelial system.
- (3) Change in the thyroxin level in blood to determine the change of metabolic activity following cryosurgery.
- (4) Influence of male hormone upon anal fistula in canine and its alteration following cryosurgery.
- (5) Effect of cryosurgery on transmissible venereal granuloma in comparison to chemotherapy and in conjunction with chemotherapeutic agents.
- (6) Cryosurgery along with immunomodulator for the treatment of anal fistula and venereal granuloma.
- (7) Therapeutic effect of cryosurgery on some surgical conditions in the Veterinary field like - Prostatic carcinoma in canine, and horn cancer in bovine.



Chapter IX

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