PROCESSED CANINE AND FISH COLLAGEN SHEETS FOR CYSTOPLASTY IN RABBITS AND DOGS

By
C.B. DEVANAND

THESIS

Submitted in partial fulfilment of the requirement for the degree

Doctor of Philosophy

FACULTY OF VETERINARY AND ANIMAL SCIENCES
Kerala Agricultural University

DEPARTMENT OF VETERINARY SURGERY & RADIOLOGY
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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2002

DECLARATION

I hereby declare that the thesis entitled ***"PROCESSED CANINE AND FISH COLLAGEN SHEETS FOR CYSTOPLASTY IN RABBITS AND DOGS"*** *is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.*

Mannuthy,
13-02-2002

C.B.DEVANAND

CERTIFICATE

Certified that the thesis entitled "**PROCESSED CANINE AND FISH COLLAGEN SHEETS FOR CYSTOPLASTY IN RABBITS AND DOGS**" is a record of research work done independently by Sri. C.B. Devanand, under my guidance and supervison and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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We, the undersigned members of the Advisory Committee of Sri. C.B. Devanand, a candidate for the degree of Doctor of Philosophy in Veterinary Surgery, agree that the thesis entitled "PROCESSED CANINE AND FISH COLLAGEN SHEETS FOR CYSTOPLASTY IN RABBITS AND DOGS" may be submitted by Sri. C.B. Devanand, in partial fulfilment of the requirement for the degree.

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C.B. Devanand

This thesis is
Dedicated to my family

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CHAPTER – I

INTRODUCTION

Implants and devices have received an unassailable role in the quest for better and longer life expectancy in animals and man. The expanding use and the high expectation about their performance are matched by increasing complexity of the prosthesis involved. The evaluation of biomaterials, their biocompatibility and functional efficiency has thus assumed greater importance today.

Replacement of lost tissue becomes essential to establish functional status of urinary bladder in conditions like rupture consequent to urinary concretions, congenital defects, chronic interstitial cystitis, weakening of bladder due to urolithiasis and neoplastic inflammations. Hollow visceral organs in the body, though anatomically different, have almost similar functional status. A mild change in anatomical structure of these organs can cause functional disturbances in the system, which necessitates partial or subtotal excision. Reconstruction of defects in hollow organs is an absolute necessity for better re-establishment of functional status and structural integrity. Repair of defects in these organs with homologous or substitute materials have always been attempted.

Reconstructive surgery in human and veterinary field has made considerable advances in the past. Viable functional substitutes like homologous tissue, in emergency, necessitates a second surgical intervention, thereby subjecting the animal/patient to additional trauma and stress on healing process. In such situations use of processed, stored biological substitutes or biomaterials are the next choice. Several biological, functional and synthetic biografts like gut segments, duramater, skin, peritoneum, bladder, pericardium and polymers have been tried for reconstruction of bladder. (Tsuji *et al*, 1967; Kudale and Hattangady, 1971; Prasad *et al*, 1973; Sharma and Khan, 1978; Prasad and Tyagi, 1980; Nair *et al*, 1988 and Shivaprakash *et al*, 1991, and Nandi *et al*, 1995)

Beneficial effects of wound healing by the use of biomaterials such as collagen gel, sponges and membrane have been reported in human beings and animals (Chvapil *et al.*, 1987; Chvapil *et al.*, 1991 and Mian *et al.*, 1992). Highly crosslinked insoluble collagen promotes healing and ensures better appearance in experimental animals (Gao *et al*, 1992) Porcine dermal collagen crosslinked with glutaraldehyde was used in the repair of perineal hernia (Frankland,1986) and chromicised collagen for bladder repair (Sambandam, 1992) in dogs. Management of full thickness cutaneous wounds with porous bovine collagen membrane was evaluated in horses (St. Jean *et. al.*, 1995).

In our country a large quantity of materials, which can be converted into collagen, are wasted. These materials can provide a cheap, easily available biomaterial with fairly good shelf life. The processing of these materials is not complicated and the cost involved is marginal. More over it ensures better utilization of presently wasted animal and aquatic tissue. There are reports of processed collagen sheets of animal tissue for repair of defects in animals, but detailed investigation on the aspects of systemic, structural and functional status after reconstruction of defects has not been reported extensively. Hence the present study was undertaken,

(1) To evaluate the host acceptability and healing of processed collagen sheets of animal and aquatic tissue for reconstruction of bladder in rabbits.

(2) To evaluate these collagen sheets clinically for reconstruction of bladder in dogs.

Review of Literature

CHAPTER – II

REVIEW OF LITERATURE

Tizzoni and Foggi (1888) performed two stage ileocystoplasty for the first time on dog with an aim to restore normal bladder capacity and function.

Barness *et al.* (1953) have tried isolated loop of colon for anasatomosing it to the dome of bladder in human beings with the aim of increasing its capacity.

Hammer *et al.* (1953 and 1955) have used viable segments of small intestine for anastomosing it to the trigone of the bladder in human beings.

Arconti (1957) used isolated loop of ileum for the contracted bladder and succeeded in enlarging the capacity of the bladder in human beings.

Marcowitz *et al.* (1959) have described the use of viable bowel segments for increasing the capacity of bladder and also for anastomosing it to the trigone of bladder for reinforcement.

Kudale and Hattangady (1971) transplanted intestinal pedicles to experimentally created rent in the wall on the dorsal aspect of bladder in dogs. Necropsy of animals at various intervals showed perfect union at junctions of transplant with intact blood supply. Histological studies revealed the union of transplant through collagenous fibre bundles traversing from the wall of the urinary bladder to the muscularis interna of the transplant.

Chvapil *et al.* (1973) reported use of reconstituted collagen sheet in medical field to reconstruct bladder wall defects. The collagen was absorbed and regeneration of bladder wall was noted.

Prasad *et al.* (1973) performed partial cystectomy and reconstructed the bladder with autogenous caecal pedicle in twelve male buffalo calves. Radiographical evaluation by pneumocystography revealed functional bladder and viable graft. No significant alteration in serum electrolytes could be noticed in the study. Gross examination of the bladder showed ecchymotic haemorrhage on the mucosa. Microscopically congestion and haemorrhage associated with inflammation were observed in the muscularis on 30th day. Regeneration of uroeptithelium was seen to be more progressive in cases where the inflammatory reaction was active.

Gupta *et al.* (1978) treated experimentally created wound over the back of guinea pigs with chromicised collagen prepared from intestine of cattle. There was complete epithelialization of the wound with good, supple, scar. Collagen implant was well replaced with fibroblast, new blood vessels and epithelial cells.

Sharma and Khan (1978) performed colocystoplasty in eight male buffalo calves and evaluated the success of cystoplasty by clinical manifestations, determination of blood urea nitrogen as well as gross and histopatholoic examination for a maximum period of 75 days. Microscopical studies revealed complete regeneration of uroepithelium over the seromuscular graft on 75th day.

Gera *et al.* (1980) performed transplantaion of formalin preserved bladder grafts in ten buffalo calves. The animals passed blood tinged urine for a period of about 24 hours and it became clear afterwards. Body temperature, respiration rate and pulse rate fluctuated within normal limits. There was transient rise in blood urea nitrogen level and no marked change in serum creatinine level. The serum potassium and sodium concentrations fluctuated within normal range.

Prasad and Tyagi (1980) used formalin preserved buffalo duramatter to replace the anterior third of the bladder wall in 12 dogs. Radiographically areas of increased soft tissue density in the region of graft were demonstrated which gradually diminished by the 90th day. Resorption of the graft occurred by both

exudation and phagocytosis. Proliferation of transitional epithelium was not complete by the end of the period of observation (90 days)

Sharma and Khan (1980) conducted caecocystoplasty on eight male buffalo calves by isolating caecal segment which was then opened longitudinally to form an open sheet from which mucosa was stripped off. The resulting seromuscular graft attached with one side of the caecal wall was sutured to partially cystectomised bladder. The success of cystoplasty was evaluated based on clinical manifestations, estimation of blood urea nitrogen as well as gross and histopathological examination for a maximum period of 75 days postoperatively. All the animals survived well and blood urea nitrogen estimated at 48 hours interval up to 10^{th} postoperative day exhibited slight increase in its level but within normal range. A perfect union of seromuscular graft and the host tissue was evident on 15^{th} day. The development of uroepithelium over the graft was fully marked by 75^{th} day.

Gentry *et al.* (1981) studied the haemostatic effect of various collagen preparations and found that collagen had important role in coagulation by promoting the contact activation of coagulation mechanism and platelet activation and aggregation.

Shetty *et al.* (1982) conducted an experimental study to compare the fate of fresh, formalin preserved, Tyrod's solution preserved and enzyme treated arterial xenograft in carotid artery of dogs on the basis of histopathological examination. The enzyme treated arterial graft was found to be the best vascular substitute. Tanning of the enzyme treated graft with glutaraldehyde increased the holding power of the graft and it incorporated well in the host tissue.

Taylor (1982) opined that an ideal graft should have both biocompatible and biodegradable properties. It should not be grossly rejected and at the same time create only minimum adhesion at the site of grafting. It was also stated that all implanted materials initially gave a foreign body inflammatory reaction associated with exudation and prominent lymphocytic infiltration. The vigor of this response

was a measure of biocompatibility. After this initial phase there would be migration of macrophages and fibroblasts into the zone around the implant with vascularisation to form a typical granulation tissue. It was also reported that chromic bovine collagen was slowly absorbed due to slow rate of digestion of collagen by macrophages and giant cells. Glutaraldehyde processing of any allograft or xenograft reduced antigenicity and increased flex resistance.

Sarmah and Holl-Allen (1984) used porcine dermal collagen implants to repair large incisional herniae in 11 human patients. Both single or double layered dermal collagen were used to bridge the defect. The patients had uncomplicated recovery, though antibiotics were not used. It was suggested that dermal collagen implants form good alternative to other methods of repair.

Frankland (1986) repaired 27 perineal herniae in 21 dogs using porcine dermal collagen. The material was generally well tolerated and the overall success rate was 59.3 per cent. The dermal collagen was split to 0.6 mm thickness and chemically depilated to remove all non-collagenous elements. Treatment with glutaraldehyde increased the cross linking between the collagen molecules and ensured an early phase encapsualtion, followed by slow absorption and replacement by host tissue. The vascularity of the site of impantation was an important factor in the rate of absorption and replacement.

Sawyer *et al.* (1987) studied the suitability of glutaraldehyde fixed bovine artery for peripheral vascular surgery in dogs.

Blumenthal (1988) evaluated the effectiveness of collagen membrane placement in substituting gingival epithelium and connective tissue cells and guiding new attachment in surgical treatment of created defects in dogs. Histologic and histometric evaluation at two to twelve weeks postoperatively revealed that collagen membrane replacement encouraged formation of new connective tissue attachment.

Nair *et al.* (1988a) performed cystoplasty in 12 clinically healthy, mongrel dogs using autogenous peritoneum on the ventral wall of bladder. Clinically the dogs remained apparently healthy and voided urine regularly. No significant change in pH, specific gravity and other constituents in urine were recorded. Blood urea nitrogen had significantly increased but was within normal limits and returned to initial values at third week. Gross examination of bladder at the end of second week revealed fibrinous adhesions of omental fat and peritoneum with the external surface of graft and adjoining bladder wall. The wall of the bladder at the grafted site was much thickened and soft in consistency. Histological examination revealed considerable amount of young granulation tissue on the outer surface of the graft and adjoining bladder. Regeneration of uroepithelium and formation of an incomplete lining on the internal surface of the grafted tissue were evident. A few smooth muscle fibres had come up in the interior of the grafted tissue. These findings suggested that the peritoneal graft gradually degenerated and was replaced by the regenerated uroepithelial tissue indicating a provision of temporary scaffolding by the grafted tissue.

Nair *et al.* (1988b) conducted cystoplasty in 12 clinically healthy dogs using autogenous skin on the ventral wall of bladder wall. Histologically necrobiotic changes of grafted tissue, regeneration of the uroepithelium and smooth muscle, and replacement of grafted tissue were observed at the end of second week after cystoplasty. At the end of eighth week the grafted skin was almost completely replaced by uroepithelium and smooth muscle.

Roe *et al.* (1990) opined that crosslinked collagen bioprosthesis usually were designed to be inert and non resorbable, resulting in fatigue and wear failure in high-stress environments. Kangaroo tail tendons partially crosslinked with glutaraldehyde (at concentration greater than or equal to 0.05 per cent) were evaluated *in vitro* for resistance to bacterial collagenese digestion and *in vivo* for biocompatibility and resorbability. The intramuscular implant assay revealed that they were collagenase resistant with slow rate of resorption.

Schwarz *et al.* (1991) performed modified "cup-patch" ileocystoplasty using an isolated vascularized segment of ileum devoid of mucosa, in a six month old Golden Retriever suffering from full-thickness wall necrosis involving 90-95 per cent of the urinary bladder consequent to ovariohysterectomy. Serial excretory urography over one year indicated gradual enlargement of the bladder with development of a smooth mucosal surface. At six months after reconstructive surgery, the dog was voiding urine two to three times per day and there was no incontinence. Results of renal function test, urinalysis and bacteriological culture of urine were normal. It was concluded that ileocystoplasty was an effective urinary bladder reconstructive procedure in dogs whenever the bladder neck, proximal portion of the urethra, and their neurovascular supply could be spared.

Srivastava *et al.* (1990) performed *in vivo* evaluation and comparison of collagen, acetylated collagen and collagen / glycosaminoglycan composite films and sponges after implantation into rat lumbar muscle for seven and 14 days. After seven days of implantation, all materials elicited an acute inflammatory cell response characterised by numerous polymorphs and histiocytes. The cell population after 14 days was principally mononuclear i.e., leucocytes, neutrophils, macrophages, lymphocytes and fibroblasts. Native collagen elicited a subacute inflammatory response for seven days. However, 14 days after implantation, a marked infiltration by neutrophils was apparent with subsequent degradation of existing collagen material. Acetylated collagen film evoked a much greater inflammatory response than native collagen.

Shivaprakash *et al.* (1991a) evaluated subtotal cystectomy in 24 goats and bladder reconstruction was performed using four different materials (six goats per group) viz., polytetrafluoroethylene (PTFE) vascular graft, caecal pedicle, fresh autogenous and preserved allogenic bladder grafts. Clinically all the animals were dull initially and their pulse rate, respiration rate and temperature increased significantly upto day seven and thereafter decreased gradually, reaching the preoperative level by day 30. The animals showed continent urination, but straining and blood in urine were observed for three to four days. Alterations in the cellular

and non cellular constituents of urine were non-significant. Transient rise in serum urea nitrogen, and absence of marked changes in serum creatinine levels suggested normal renal function and absence of urinary reflux. The serum sodium, chloride and potassium concentrations increased in the early postoperative period but were well within the normal physiological limits. Complications such as leakage, fistula or calculi formation were not observed in pneumocystogram on day 30 and 60 in any of the animals.

Shivaprakash *et al.* (1991b) performed total cystectomy in 24 goats and bladder reconstruction was performed using polytetrafluoroethylene (PTFE) vascular graft, caecal pedicle, frozen allogenic bladder and fresh autogenous bladder grafts. The PTFE implants were partially and completely covered by the regenerated bladder on the outer surface after 30 and 60 days respectively. However on the inner surface the implant showed partial detachment from host tissue on day 60. The caecal pedicle was deccreased in size with simultaneous regeneration of bladder tissue. The frozen allogenic bladder grafts showed erosions and separation in places. The fresh autografts were almost indistinguishable from the parent bladder on day 60. Microscopic examination showed granulation and fully regenerated bladder tissue over the PTFE implant on days 30 and 60. The uroepithelium failed to cover the caecal graft even on day 60. The frozen allogenic bladder grafts exhibited a host reaction as shown by cellular infiltration, degeneration and necrosis of the mucosal and muscular layers of the graft. By day 60 the allograft showed heavy lymphocytic infiltration and loosening of muscle bundles. Histochemical examination showed abundant collagen fibres in all groups on day 30, which diminished on day 60. Collagen and elastic fibres were more abundant in bladder reconstruction with PTFE and autogenous bladder implants.

Sambandam(1992) opined that chromicised collagen could be an ideal implant for cystoplasty because of reduced antigenicity and better regeneration with minimal cellular infiltration. The healing process was characterised by well vascularised collagenous matrix and minimal cellular reaction.

Rameshkumar (1993) performed experimental replacement cystoplasty in 18 buffalo calves using autogenous peritoneal membrane, chromicised allogenic bovine bladder and glutaraldehyde procesed xenogenic human amniotic membrane. Significant elevation of rectal temperature, pulse rate and respiration rate were noticed in all the animals on the second postoperative day and total leukocyte count, blood urea nitrogen, creatinine, inorganic phosphorus and total protein values on the third postoperative day. But serum chloride level declined significantly in all animals. Contrast cystographic studies using 10 per cent barium sulphate suspension revealed marked adhesions at the cystoplasty site in all the animals. Normal contour of the bladder was noticed in autogenic group, moderate adhesion with thickening of the bladder wall at the repaired site in allogenic group and irregular bladder contour with adhesions in xenogenic group on the 30^{th} day. On gross and histopathological studies incomplete mucosal proliferations were noticed in all the groups on 15^{th} day. The healing was complete with autogenous peritoneal graft on 30^{th} day while it was moderate and incomplete with allogenic bladder and xenogenic human amniotic membrane respectively.

Ruijgrok *et al.* (1994) reported that glutaraldehyde crosslinking of collagenous tissue is a widely used method for the preparation of implantable tissue to be used as biomaterials.

Nimni and Cheung (1994) stated a new method for producing pure collagen from collagenous animal tissue containing non-collagenous material. The collagen network so obtained can be crosslinked using a chromium salt, tannin or aldehyde. The animal tissues used for producing collagen were tendon, heart valve, pericardium, ligament, skin, blood vessel, fascia, cartilage or intestine.

Feinberg and Mc Donnell (1995) evaluated a biocompatible preformed collagen sheet as a disc replacement after temporomandibular joint (TMJ) surgery in New Zealand White rabbits. The study demonstrated that collagen sheet replacement after discectomy acted in a protective capacity and helped to retard

early degenerative changes normally seen in the articular surfaces of discectomised temporomandibular joints.

Mohanty (1995) suggested that the tissue response to implants and their functional acceptance were important modalities of biological evaluation of prosthetic materials. The studies comprised of surgical implantation of materials in soft or hard tissues followed by gross and microscopical evaluation of the response in the surrounding tissue at different time intervals.

Nandi *et al.* (1995) performed bladder reconstruction in apparently healthy, 12-14 month old female goats using preserved homogenous pericardial grafts. Gross examination of bladder on day 21 and day 45 revealed mild adhesions between the site and omental fat, mesentery, uterus and peritoneum. The external suture line on the bladder was much thickened and soft in consistancy. Histologically the bladder wall was lined by multilayered transitional epithelium, good amount of collagen fibres, and granulation tissue by 21st day.

Santillan *et al.* (1995) employed pericardial tissue preserved in 0.5 per cent glutaraldehyde for reconstruction of surgical defects in the thoracoabdominal wall in dogs.

Sharma (1995) used formalin preserved urinary bladder grafts for cystoplasty in nine healthy male buffalo calves. During the postoperative period blood urea nitrogen level was within normal range upto 20 days. On 30th postoperative day, a fibrous tissue covering with mild adhesions of mesentery was observed on the outer surface of the graft. The line of union of graft with bladder was indistinguishable from the mucosal surface. Gradual regeneration of bladder tissue with softening and thickening of the graft margin was marked. Resorption of the prosthetic material was comparatively less.

St.Jean *et al.* (1995) evaluated the effect of a porous bovine derived collagen membrane (PBCM) for the repair of surgically created full thickness cutaneous wounds on the distal extremities of horses and it was found to be not detrimental to wound healing.

Al-Khateeb *et al.* (1996) evaluated the biocompatibility and effectiveness of a modified bovine type I collagen membrane as a graft material in experimental wounds on labial mucosa of dogs. Clinical and histopathological evaluation at one, two and three weeks post operatively demonstrated its acceptability to the oral mucosa. Improved rate of wound healing and topical haemostatic effect at the time of application, made it an excellent wound graft material.

Jayakrishnan and Jameela (1996) reported glutaraldehyde as a versatile cross linking agent of great value in the preparation of bioprostheses using heart valves, vascular tissue, elastic cartilages, tendon xenografts, artificial skin, pericardial patches and burn dressings. Glutaraldehyde cross linking of collagenous tissue significantly reduced biodegradation, making them biocompatible, non-thrombogenic and non antigenic while preserving anatomic integrity, leaflet strength and flexibility.

Sharma (1997a) performed partial cystectomy on nine healthy male buffalo calves by resecting the anterior half of the bladder. The margin of cystectomy wound was sutured to the already prepared invaginated portion of caecum. All the animals survived for the observation period and no change in blood urea nitrogen was observed postoperatively upto 30^{th} day, estimated at five day interval. Complete regeneration of bladder tissue was observed by 90^{th} day on histological examination.

Sharma (1997b) conducted experimental bladder prosthesis with terylene lined thin hollow plastic balls after partial cystectomy in nine buffalo calves. The prosthetic material was covered completely by fibrous tissue on day 30 and minimal adhesions were observed on day 60 and 90. Regeneration of serosa and muscularis was noted on 30^{th} and 60^{th} day and poorly developed epithelium with distribution of

papillae like projections on 90th day. Marked regeneration of transitional epithelium was noted but it was thin and incomplete.

Balagopalan (1998) evaluated the efficacy of chrome/glutaraldehyde crosslinked canine aortic tissue for surgical implantation in experimentally created cervical oesophageal defect in dogs.

Sreenu *et al.* (1998) studied the gross and histological changes in dogs, after cystoplasty, using allogenic bladder grafts treated with chromic sulphate and glutaraldehyde . Adhesions between omentum, parietal peritoneum and suture line were observed in all animals following surgery. Detachment and shrinkage of grafts and complete epithelialization of bladder were observed on 30th day.

Portis *et al.* (1998) studied the effectiveness of different biodegradable organic materials such as porcine bowel acellular tissue matrix(ATM), bovine pericardium(BPC), human placental membranes (HPM) and porcine small intestine submucosa (SIS) for laparoscopic augmentation cystoplasty in minipigs. Bladders were evaluated by cystoscopy at 6 and 12 weeks and harvested at 12 weeks. In all the groups, except for BPC group, grafts remained in place and were seen incorporated with bladder. There was patchy epithelialization of grafts with a mixture of squamoid and transitional epithelium without evidence of significant inflammatory response.

Materials and Methods

CHAPTER – III

MATERIALS AND METHODS

Plan of Study

A. Experimental Study

The experimental study was conducted in twelve apparently healthy adult New Zealand White rabbits of either sex weighing 2.1- 3.0 kg. The animals were housed in separate cages, under identical conditions of feeding and management, and were kept under observation for a period of two weeks prior to the experiment.

The animals were randomly divided into two groups of six each viz.group I and group II. The programme of study was as given hereunder:

Group I

Animals in this group were subjected to partial cystectomy followed by cystoplasty using glutaraldehyde processed canine collagen sheet.

Group II

Animals in this group were subjected to partial cystectomy followed by cystoplasty using diacetyl processed fish collagen sheet.

B. Clinical Study

Group III

Clinical studies were conducted in six male dogs and were included as group III. In this group cystoplasty was carried out using collagen sheets of canine origin in

three dogs and of fish origin in three dogs for reconstruction of ruptured urinary bladder.

Preparation of graft material

Canine Collagen sheet

Collection of material

Urinary bladder collected from recently died dogs was washed with sterile normal saline and stored in sterile bottles containing isotonic saline. The collected samples were processed at the Bioproducts Laboratory, Central Leather Research Institute, Adayar, Chennai.

Processing

The tissue samples were made into sheets and washed thoroughly in running water to remove blood. The capillaries and adhering fat tissues, if any, were removed manually. It was further cleaned by double washing in distilled water.

Crosslinking with glutaraldehyde

The tissue samples thus prepared were treated for three hours with 0.5 per cent (v/v) glutaraldehyde solution (25 per cent) in an aqueous medium containing 0.5 per cent sodium chloride and 0.01 per cent (w/v) sodium acetate. The pH of the solution was adjusted to 6.5 to 7.0 and the material was stirred occasionally.

The sheets thus prepared were aseptically sealed in polythene covers in 98 per cent isopropanol as preservative (Fig.1) and sterilized in gamma irradiation chamber at 2 M rads dose (Sastry, 1989) at Radiotracer Laboratory, Kerala Agricultural University, Vellanikkara.

Fish Collagen sheet

Collection of material

Fish air bladder collected from larger fresh water fishes was washed in running water and sterile normal saline and then stored in sterile bottles containing isotonic saline. The collected samples of fish air bladder were processed at Central Institute of Fisheries Technology, Cochin.

Processing

Fish air bladders were made into sheets and washed thoroughly in running water and dilute salt solutions. The washed air bladders were suspended for 4-6 hours in a pickling solution containing sulfuric acid, sodium chloride and chromium salts (Turley, 1958) to remove all soluble proteins and to obtain pure collagen fibres.

Crosslinking with diacetyl

The collagen fibres of the sheet so obtained were strengthened by crosslinking with diacetyl (Turley, 1958) and dried in air at ambient temperature for 4-6 hours followed by neutralization with dilute ammonia.

The sheets thus prepared were aseptically sealed in sachets containing isopropyl alcohol as preservative (Fig.2) and sterilized in gamma irradiation chamber at 2 M rads dose (Sastry, 1989) at Radiotracer Laboratory, Kerala Agricultural University, Vellanikkara.

EXPERIMENTAL STUDY

Preoperative considerations

All the animals were prepared by withholding feed for 24h and water for 12h prior to the surgery. The animals were secured on dorsal recumbency and the skin on ventral aspect of abdomen, from pubis to umbilicus, was prepared aseptically for surgery.

Anaesthesia

For premedication, atropine sulphate[1], at the rate of 0.04 mg/kg body weight and fifteen minutes later, xylazine hydrochloride[2] at the rate of 5 mg/kg body weight were administered I.M. Ten minutes after xylazine, ketamine hydrochloride[3] at the rate of 50 mg/kg body weight was administered I.M. to effect anaesthesia.

Surgical technique

Cystoplasty

The animal was controlled on dorsal recumbency and the limbs were secured to the side of operation table using tapes. The tongue was pulled out of the oral cavity and kept to one side throughout the period of surgery to keep the airway patent. Surgical site was suitably draped with sterile towels. A4 cm long cutaneous incision was made on the mid ventral region, from 1 cm anterior to pubic symphysis towards the umbilicus. The underlying connective tissue and fascia was freed by blunt dissection from the skin to expose the linea alba. The peritoneal cavity was entered through a small nick incision on the lifted portion of the linea alba. Incision on the linea alba was then extended in either direction and exposed the urinary

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[1] Atropine Sulphate – Atropine sulphate 0.65 mg/0.5ml, Rayan pharma Ltd., Anaparty, India
[2] Xylaxin-Xylazine hydrochloride 20 mg/ml, Indian Immunologicals, Hyderabad, India.
3. Ketmin-Ketamine hydrochloride 50mg/ml, Themis Chemicals, Hyderabad, India

bladder. The bladder was exteriorized and packed off with sterile towel. The distended bladder was punctured at its vertex using a sterile hypodermic needle of size 18 G and urine was completely aspirated by a syringe.

A portion of bladder wall (2 cm^2) close to the vertex, was surgically removed from dorsal aspect of fundus, to create a full thickness defect (Fig.3 and 4). The bleeding points over the incised edges of bladder wall were controlled by gentle mopping with gelatin sponges[1]. The prepared processed graft material was kept ready by washing with three changes of sterile normal saline solution. A final irrigation and immersion in 100 ml of sterile normal saline was carried out before use. The graft material was trimmed to oval shape, sufficient enough to cover the already created defect on bladder. It was placed over the wound edges of bladder and fixed by suturing the edges together using 5-0 braided silk by overlapping continuous horizontal mattress sutures (Fig.5 and 6). The minute bleeding points were again controlled by gelatin sponges and the reconstructed bladder was then replaced into peritoneal cavity. The linea alba along with the peritoneum was approximated in continuos apposition sutures using 1-0 braided silk and skin edges were united in vertical mattress sutures using 1-0 braided silk.

Postsurgical Management

Povidone iodine spray[2] was applied over the suture line and covered with cotton and microporous adhesive tape[3]. The wound was cleaned daily using sterile moist cotton and 70 per cent alcohol and was smeared with povidone iodine ointment[4] till healing was complete.Ampicillin-cloxacillin[5] combination at the rate of 50mg/kg body weight was administered intramuscular daily for five days postoperatively.

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[1] AbGel – Absorbable gelatin sponges I.P. Sri Krishna Laboratories, Bombay, India.
[2] Wokadine Paint Spray – Povidone iodine I.P. 10% w/w Wokhardt Ltd, Mumbai, India.
[3] Microporous adhesive tape- Surgical tape, JMS Co ltd., Japan.
[4] Betadine – Povidone iodine 5% ointment, Wokhardt Ltd, Mumbai, India.
[5] Megapen – Ampicillin Sodium IP 250 mg, Cloxacillin Sodium IP 250 mg Aristo Pharma Pvt Ltd, Mumbai, India.

Animals were fed on normal feed and water postoperatively through out the period of observation.

The skin sutures were removed by 7th postoperative day.

Blood samples were collected preoperatively and on seventh and fifteenth day postoperatively for haematological and biochemical studies. All the animals were kept under observation for a minimum period of one month. Bladder samples were harvested for gross and histomorphological studies at the time of disposal of animals.

Main items of observation

i. General condition

Active, alert, dull, depressed

ii Physiological symptoms

Rectal temperature, pulse rate and respiration rate were recorded preoperatively and daily upto seventh postoperative day

iii Feeding behavior

Normal, reluctant

iv Haematological studies

Blood smears were prepared and venous blood samples were collected from ear vein in EDTA[1] on the pre-operative day and on 7th and 15th day

[1] EDTA – EDTA Disodium salt (Nice Laboratory reagent), New India Chemicals Enterprises, Kochi.

differential leucocyte counts (TLC and DC), erythrocyte sedimentation rate (ESR) and packed cell volume (PCV) (Schalm, 1975). Haemoglobin concentration was estimated by cyan-methaemoglobin method (Jain, 1986) using Digital haemoglobinometer 185.

V. Biochemical studies

Venous blood samples were collected from ear vein on the preoperative day and on 7th and 15th day postoperatively for estimation of serum constituents

a. Blood urea nitrogen level was estimated by urea kit[1] (DAM method) using spectrophotometer model 105

b. Serum creatinine level was estimated by creatinine kit[2] (Alkaline picrate method) using spectrophotometer model 105

c. Total serum protein and albumin content were estimated by total serum protein and albumin kit[3] (Biuret method – Inchiosa, 1964) using spectrophotometer model 5010

d. Serum sodium and potassium concentrations were estimated using flame photometer 128

e. Serum chloride concentration was estimated by chloride kit[4] (Schoenfield colorimetric method) using spectrophotometer model 105

[1] Urea kit – Sigma Diagnostics (India) Pvt Ltd, Baroda, India
[2] Creatinine kit - Sigma Diagnostics (India) Pvt Ltd, Baroda, India
[3] Total protein and albumin kit – Sigma Diagnostics (India) Pvt. Ltd. Baroda, India.
[4] Chloride kit – Biolab Diagnostics, Bombay, India.

VI. Radiological studies

Radiographs of abdomen were recorded on preoperative day and on 7th and 15th day postoperatively. The study included ventrodorsal survey radiography and contrast radiography after injection of organic iodine compound intravenously for intravenous pyelography.

Intravenous Pyelography

Procedure

Under sedation with xylazine[1] and ketamine[2] administered intramuscular at the rate of 2.5mg and 25mg/kg body weight respectively, contrast iodine preparation[3] at a dose of 425mg I/kg body weight was administered intravenously. The calculated quantity of contrast material diluted and made up to 10ml with dextrose saline was injected as bolus after abdominal compression. An immediate ventrodorsal exposure of abdomen was done and then the abdominal compression was released. Another ventro dorsal exposure of lower abdomen was recorded five minutes after the release of compression to assess seepage if any and mucosal details of bladder.

VII. **Gross and histomorphological Studies**

Animals were sacrificed on 30th day for disposal. The urinary bladder was exposed and examined grossly for adhesion with surrounding tissues. The whole urinary bladder was harvested, cleaned and washed in normal saline.

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[1] Xylaxin – Xylazine hydrochloride 20 mg/ml, Indian Immunological, Hyderabad, India
[2] Ketmin - Ketamine hydrochloride 50 mg/ml, Themis Chemicals, Hyderabad, India
[3] Urografin 76% - Diatrizoate Meglumine and Diatrizoate Sodium injection USP, German Remedies Ltd, Mumbai, India

per cent neutral buffered formol saline and the fixed tissues were processed. Paraffin sections of 5-6 μm size were cut and stained using Ehrlich's haematoxylin and eosin (Humason, 1979) for routine observation and van-Gieson's stain for collagen fibres (Bancroft and Cook, 1984)

Statistical analysis

The data obtained in all the groups were analysed using paired 't' test and the means were compared with presurgical values (Snedecor and Cochran, 1967)

CLINICAL STUDY

Clinical studies were conducted in six male dogs weighing 14.83 ± 1.30 kg. bodyweight. Among these cystoplasty using canine collagen sheets was performed in three dogs and using fish collagen sheets in the other three.

Preoperative considerations

All the animals were prepared by withholding food for 24 h and water for 12 h prior to surgery when possible. The animals were given enema one hour before the surgical preparations. After securing the animals on dorsal recumbency the skin on ventral aspect of abdomen from pubis to umbilicus was shaved and prepared aseptically for surgery.

Anaesthesia

The animals were premedicated with triflupromazine hydrochloride[1] at the rate of 1mg/kg. bodyweight given I.M. Fifteen minutes later, thiopentone sodium[2] 5 per cent solution was administered intravenous to effect anaesthesia

[1] Siquil (Vet)- Triflupromazine hydrochloride- 20 mg/ml, Sarabhai Chemicals, Baroda, India.
[2] Intraval Sodium – Thiopentone sodium injection I. P., Rhone – Poulene (India) Ltd., Bombay, India.

Surgical technique

Cystoplasty

Animals were controlled on dorsal recumbency with the limbs secured to the table. The site of surgery was suitably draped. A 6 cm long longitudinal incision was made on skin lateral to the sheath of penis. The subcutaneous fascia was dissected to expose the linea alba. Entered the peritoneal cavity through a nick incision on the linea alba and the incision was extended in either direction to expose the urinary bladder. Exteriorized the urinary bladder through the incision identified the damage on bladder and packed off with sterile towels. Cystoplasty was performed using grafts made of collagen sheets to cover the defect. It was anastomosed to the edges of wound using 3-0 catgut[1] in through and through continuous horizontal mattress sutures (Fig. 7 and 8). The linea alba, peritoneum and skin were approximated in routine manner using 1-0 braided silk and monofilament nylon respectively. A polythene catheter 5 french size was introduced retrograde through the urethra and fixed to the skin just infront of prepucial orifice

Postsurgical Management

The suture line at the mid ventral abdomen was covered by Tr.benzoin seal. The wound was cleaned daily using povidone iodine[2] solution and smeared with povidone iodine ointment.

Ampicillin[3] at the rate of 10 mg./kg body weight was administered intra muscular daily for seven days postoperatively.

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[1] Mersutures : Absorbable Surgical suture (Catgut) USP Ethicon, Johnson and Johnson Ltd, Aurangabad.
[2] Betadine lotion/cream – Povidone iodine IP – 5%, Win – Medicare Ltd, new Delhi
[3] Roscillin – Ampicillin Sodium IP 500 mg, Rambaxy Laboratories Ltd, Dewas, India

Dextrose in normal saline at the rate of 30 ml./kg. body weight was administered intravenous, in divided doses, two times a day for the first 3-4 days postoperatively. The dogs were fed on milk and bread from second day followed by rice as regular diet from 5^{th} day.

The skin sutures were removed by 7^{th} to 10^{th} postoperative day.

Blood samples were collected preoperative and on third, seventh and fifteenth day postoperatively for haematological and biochemical studies.

Urine samples were collected daily for 7 days postoperatively for routine analysis

All the dogs were kept under observation for a maximum period of 15 days.

Main items of observation

I General condition

Active, alert, dull, depressed and dehydrated

II Feeding behaviour

Normal, reluctant

III Urinalysis

Routine examination of urine was conducted after operation daily up to 7 days.

IV Urination

Straining, incontinence, continence

V Haematological studies

Blood smears were prepared and venous blood samples were collected in EDTA[1] on the preoperative day and on 3rd , 7th and 15th day postoperatively for estimation of total erythrocyte count (TEC), total and differential leucocyte counts (TLC and DC), erythrocyte sedimentation rate (ESR) and packed cell volume (PCV) (Schalm,1975). Haemoglobin concentration was estimated by cyan-methaemoglobin method (Jain, 1986) using Digital haemoglobinometer 185

VI Biochemical Studies

Venous blood samples were collected on the preoperative day, and on 3rd, 7th and 15th day postoperatively for estimation of blood urea nitrogen, serum creatinine, total serum protein and albumin, serum sodium, potassium and chloride.

VII Radiological Studies

Plain and contrast radiography of bladder were performed on preoperative day and on 3rd, 7th and 15th day postoperatively. The study included lateral survey radiography and retrograde cystography after injecting contrast medium into the bladder through the urethra considering the feasibility in dogs.

[1] EDTA – EDTA Disodium salt, (Nice Laboratory reagent) New India Chemicals Enterprises, Kochi.

Retrograde Cystography

Procedure

A 2 ml. quantity of contrast iodine preparation[1] was made up to 10 ml with sterile distilled water and the solution was then mixed thoroughly to form uniform distribution. The solution was taken in a syringe and injected slowly to the bladder by connecting nozzle of syringe to the adapter of a polythene catheter of 5 french size introduced transurethral into the bladder. Animal was rolled to and fro for two times and after withdrawal of the catheter a lateral radiograph was taken to assess seepage, if any and mucosal details of bladder.

Statistical analysis

The data obtained in the clinical trials were analyzed using paired 't' test and the means were compared with presurgical values (Snedecor and Cochran, 1967).

[1] Urografin 76% - Diatrizoate Meglumine and Diatrizoate Sodium injection USP, German Remedies Ltd, Mumbai, India

Fig.1 : **Polythene cover containing glutaraldehyde processed canine collagen sheet preserved in isopropanol.**

Fig.2 : **Polythene sachet containing diacetyl processed fish collagen sheet preserved in isopropyl alcohol.**

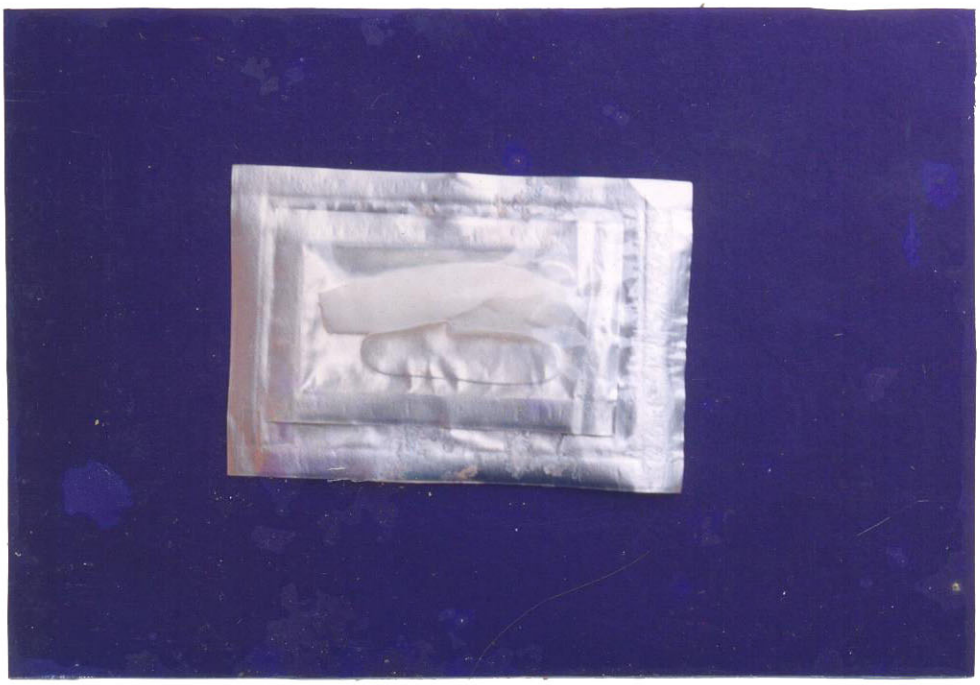
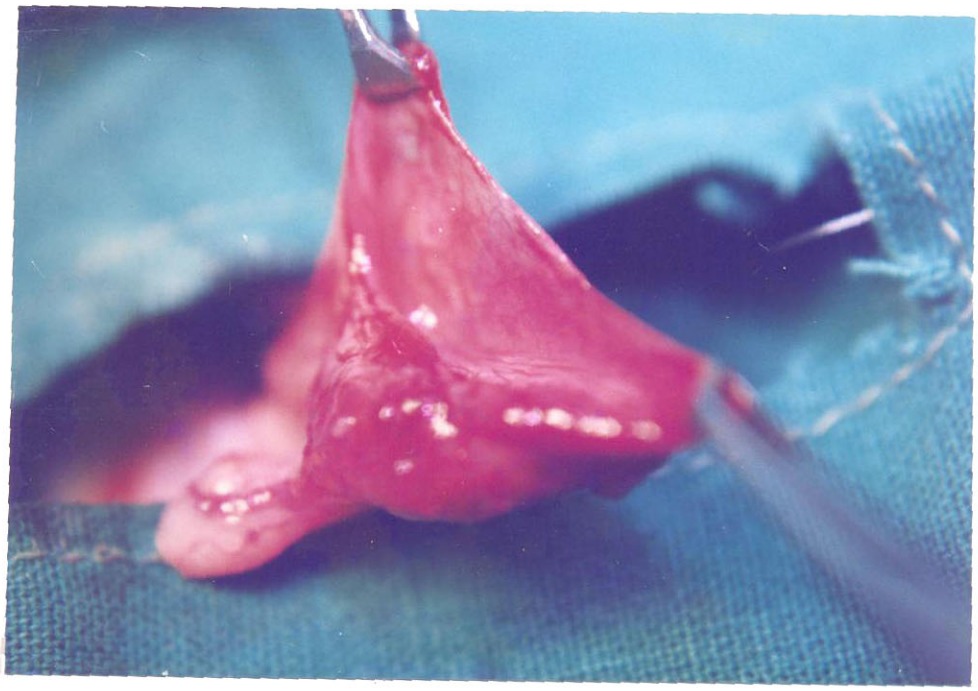


Fig.3 : Urinary bladder of rabbit with experimentally created full thickness defect on the fundus near the vertex.(Gr.I)

Fig.4 : Urinary bladder of rabbit with experimentally created full thickness defect on the fundus near the vertex. (Gr.II)



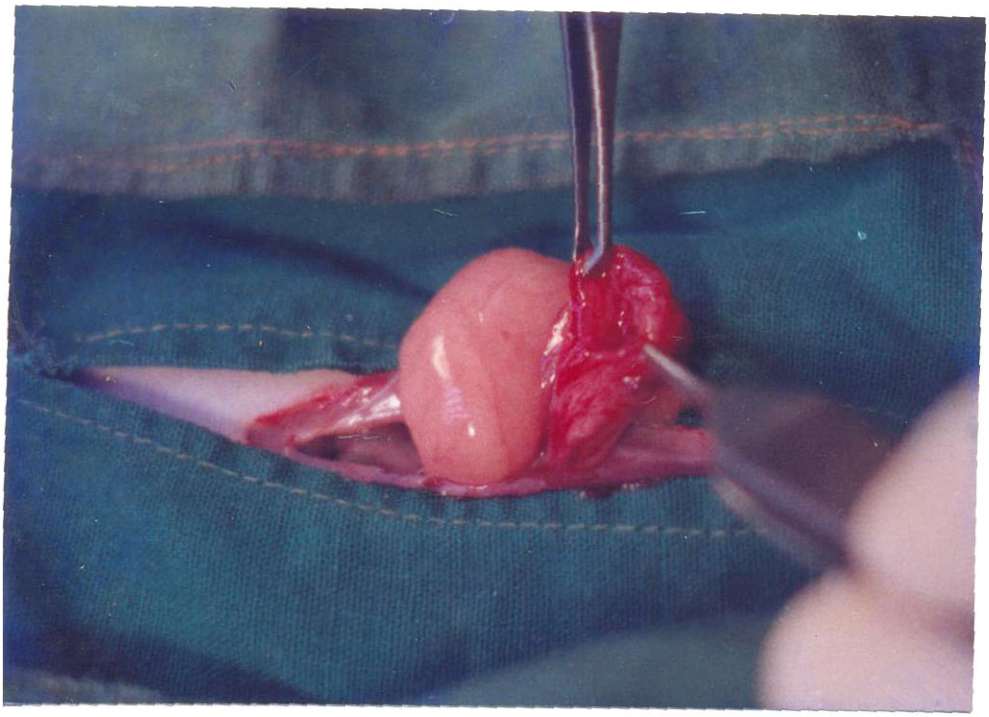
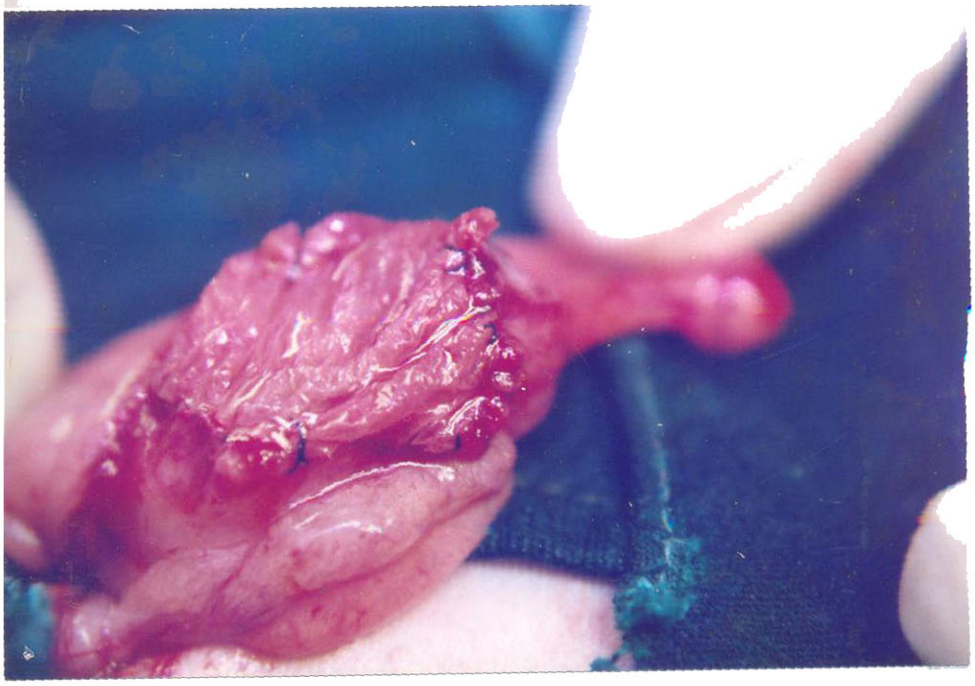


Fig.5 : Urinary bladder of rabbit after cystoplasty using canine collagen sheet.(Gr.I)

Fig.6 : Urinary bladder of rabbit after cystoplasty using fish collagen sheet. (Gr.II)



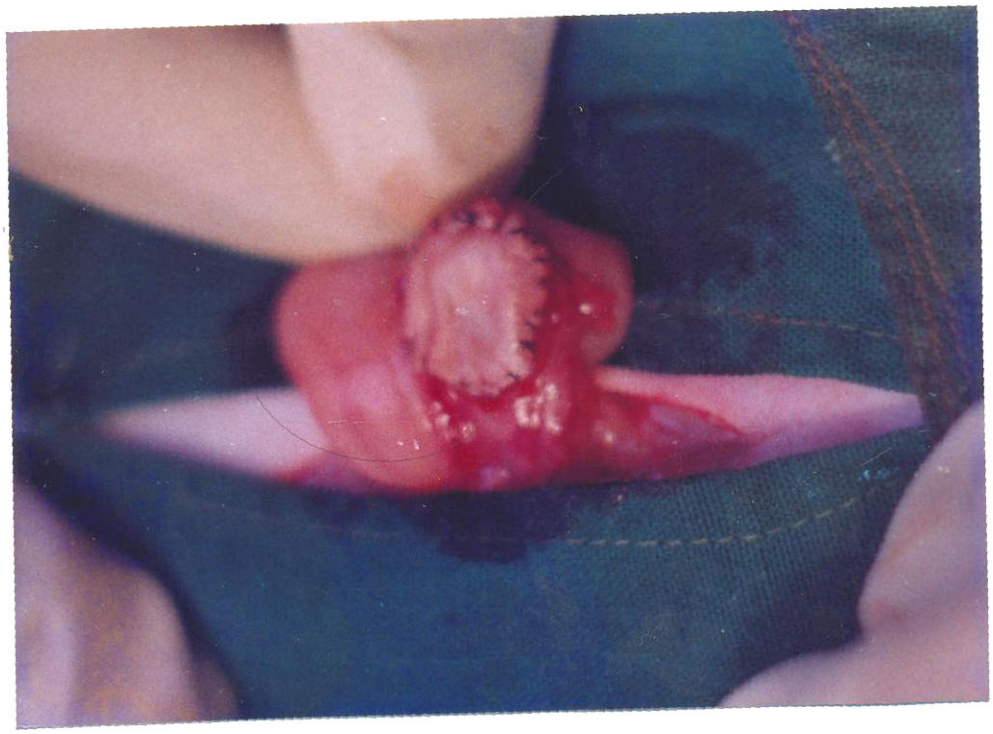
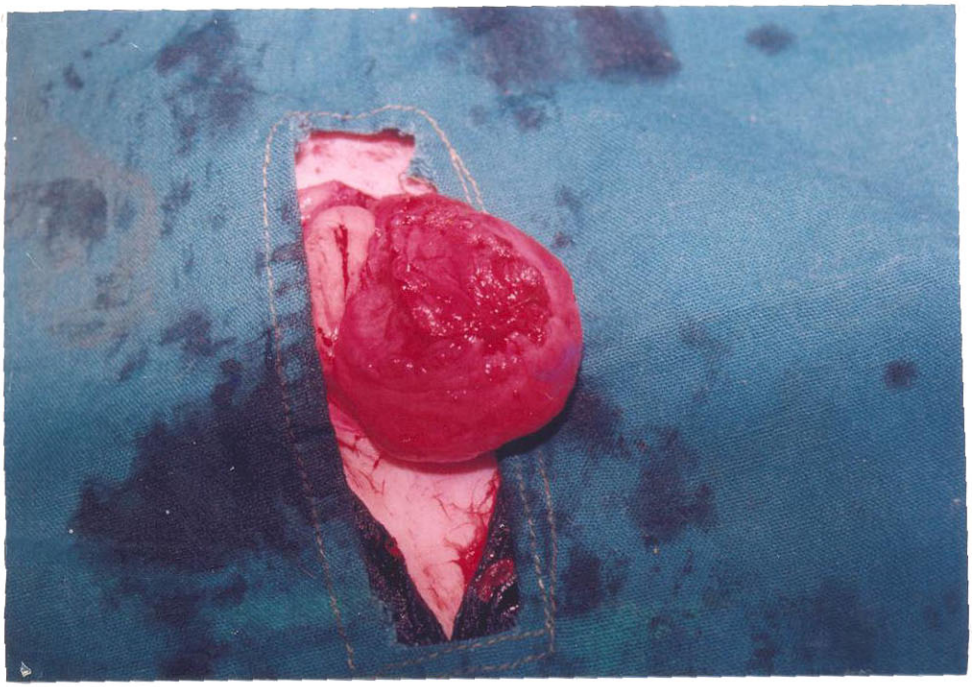
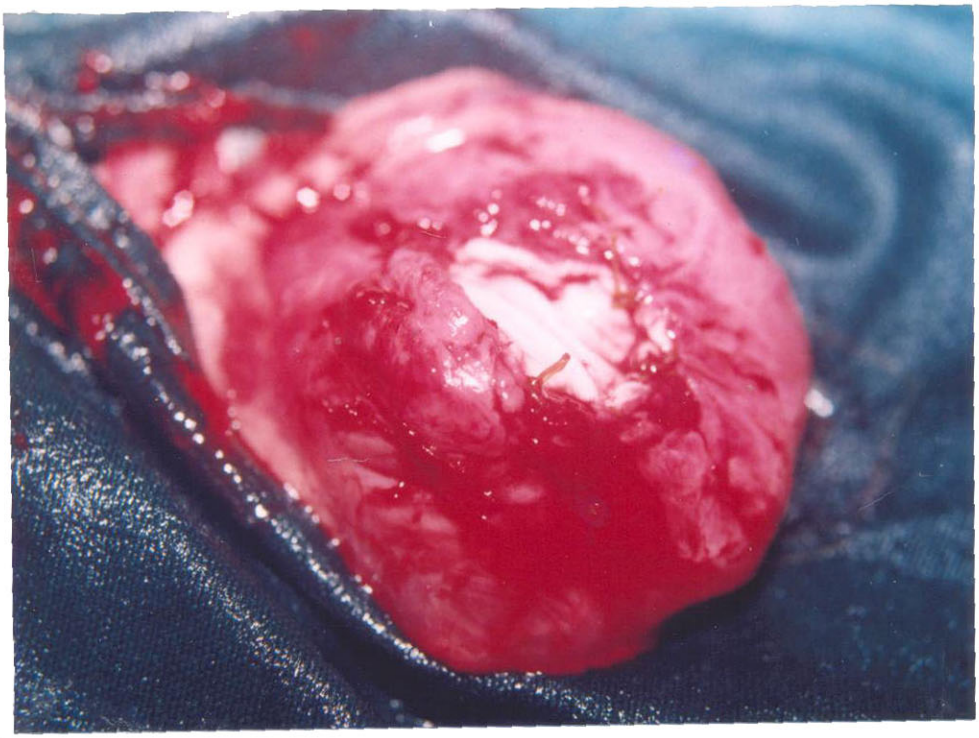


Fig.7 : Urinary bladder of dog after cystoplasty using canine collagen sheet.

Fig.8 : Urinary bladder of dog after cystoplasty using fish collagen sheet.





Results

CHAPTER – IV

RESULTS

Group I

The observations are presented in Tables 1 to 3

Anaesthesia

The average body weight (kilogram) of the animals was 2.55 ± 0.11. The animals were premedicated with atropine sulphate (0.10 ± 0.00mg) and xylazine hydrochloride (12.75 ± 0.53 mg).Ketamine hydrochloride(127.50 ± 5.28 mg) was administered I.M for anaesthesia.

General condition

All the rabbits were lethargic and dull on first postoperative day but were showing resistance on handling for recording routine observations and cleaning of surgical wound. The animals became active and alert by second postoperative day. Covering of wound with microporous bandage could not be continued from second day because of intolerance by the animals. However the wound was kept healthy by daily cleaning and uneventful healing was observed in all animals.

Physiological symptoms

Rectal temperature (^{0}C) was 39.43 ± 0.11 before anaesthesia. After a marginal increase on the first postoperative day the rectal temperature showed reduction from second postoperative day with significant ($P<0.05$) reduction recorded from third to fifth postoperative day. Thereafter marginal and appreciable rise in rectal temperature to near normal was observed by seventh postoperative day.

Pulse rate (per min.) was 168.33 ± 1.91 before anaesthesia. There was marginal increase in pulse rate on the first postoperative day and the rate remained at lower level throughout the observation period. The reduction was significant ($P<0.05$) on third, fourth and fifth postoperative day. The pulse rate showed progressive increase to near normal level from sixth postoperative day.

Respiration rate (per min.) was 70.00 ± 3.20 before anaesthesia. There was marginal increase up to second postoperative day and the respiration rate remained at lower level upto fifth postoperative day. Thereafter the respiration rate showed gradual increase to normal preanaesthetic level.

Feeding behaviour

Reduction in feed and grass intake and water consumption was noticed in all animals on first postoperative day. However the animals resumed normal feeding and water intake from second postoperative day and maintained normal habits throughout the observation period.

Haematological studies

The haemoglobin concentration (g/dl) was 11.18 ± 0.07 before anaesthesia. A significant ($P<0.05$) decrease in Hb value was noticed on seventh postoperative day and this was followed by an increase on the fifteenth postoperative day.

The erythrocyte sedimentation rate (mm/h) was 1.00 ± 0.00 before anaesthesia. Variation in ESR was not noticed during the postoperative period.

The packed cell volume (per cent) was 38.67 ± 0.44 before anaesthsia. There was significant ($P<0.05$) reduction in PCV by seventh postoperative day followed by significant ($P<0.05$) rise afterwards on fifteenth postoperative day.

The total erythrocyte count (10^6/mm^3) was 6.35 ± 0.25 before anaesthesia. A decrease in TEC by seventh postoperative day was noticed followed by increase on fifteenth postoperative day.

The total leucocyte count (10^3/mm^3) was 10.57 ± 0.36 before anaesthesia. There was decrease in TLC by seventh postoperative day. An appreciable increase to near normal level was noticed on fifteenth postoperative day.

The neutrophil count (per cent) was 25.17 ± 0.44 before anaesthesia. Significant ($P<0.05$) rise in neutrophil count was observed on seventh postoperative day followed by significant fall to preanaesthetic level by fifteenth day.

The lymphocyte count (per cent) was 72.17 ± 0.51 before anaesthesia. Reduction in lymphocyte count was observed on seventh postoperative day and the value was returning to normal by fifteenth postoperative day.

The eosinophil count (per cent) was 1.17 ± 0.13 before anaesthesia. It showed marginal and insignificant variation during the period of observation.

The basophil count (per cent) was 0.17 ± 0.07 before anaesthesia. A marginal increase in the count was noticed on seventh postoperative day followed by decrease on fifteenth postoperative day.

The monocyte count (per cent) was 1.33 ± 0.09 before anaesthesia. Reduction in the count on seventh postoperative day and rise on fifteenth postoperative day were marginal.

Biochemical studies

The blood urea nitrogen level (mg/dl) was 35.58 ± 1.07 before anaesthesia. An increase in BUN was noticed on seventh postoperative day followed by gradual decrease to near normal level by fifteenth postoperative day.

The serum creatinine level (mg/dl) was 2.20 ± 0.10 before anaesthesia. There was marginal increase in serum creatinine content on seventh postoperative day and appreciable decrease to normal value on fifteenth postoperative day.

The serum sodium concentration (mEq/l) was 129.40 ± 2.80 before anaesthesia. An insignificant rise in sodium concentration on seventh postoperative day was followed by a fall to near normal value on fifteenth postoperative day.

The serum potassium concentration (mEq/l) was 5.73 ± 0.09 before anaesthesia. There was increase in potassium concentration on seventh postoperative day followed by significant ($P<0.05$) decrease on fifteenth postoperative day.

The serum chloride concentration (mEq/l) was 102.23 ± 0.81 before anaesthesia. A marginal decrease in chloride concentration on seventh postoperative day was followed by an increase to near normal level on fifteenth postoperative day.

The total serum protein content (g/dl) was 7.02 ± 0.14 before anaesthesia. Following a decrease on seventh postoperative day it increased considerably and reached normal value on fifteenth postoperative day.

The serum albumin content (g/dl) was 5.28 ± 0.09 before anaesthesia. There was reduction in serum albumin content on seventh postoperative day followed by increase on fifteenth postoperative day.

Radiographic observations

Intravenous pyelography during postoperative period showed normal production of urine from renal level and filling of reconstructed urinary bladder. Seepage of contents from the bladder, adhesion of bladder with surrounding structures and signs suggestive of cystitis could not be observed on seventh and fifteenth day (Fig. 9, 10, 11 and 12).

The time lapse of five minutes after release of abdominal compression during intravenous pyelography was adequate for filling of the bladder with urine and contrast medium. The concentration of contrast medium was sufficient for consistent opacification of the bladder.

Gross morphological studies

Moderate fibrinous adhesion with adjacent tissues was noticed in all the animals (Fig. 13). The mucosal healing was incomplete (Fig. 14) and in two animals formation of hard denuded cell deposits along the margin of suture was noticed.

Histomorphological studies

The graft material revealed abundant quantity of elastic fibers and collagen fibers with loose arrangement in regular fashion (Fig. 15).

Mucosal proliferation and continuity was almost complete. There was progressive neovascularisation and inflammatory edema. Moderate deposition of collagen fibres and mild fibroplasia adjacent to the graft were observed (Fig. 16, 17 and 18).

Table 1:- Rectal temperature, pulse rate and respiration rate in rabbits before and after cystoplasty using glutaraldehyde processed canine collagen sheets
(Mean ± SE) n=6

Parameters with units	Preoperative	Postoperative interval (days)						
		1	2	3	4	5	6	7
Rectal temperature(^{0}C)	39.43±0.11	39.48±0.09	39.30±0.10	39.18±0.09*	38.70±0.13*	39.08±0.09*	39.30±0.10	39.32±0.08
Pulse rate (per min.)	168.33±1.91	169.17±2.97	161.00±3.67	156.00±3.41*	152.67±2.38*	155.00±1.62*	157.67±4.19	160.83±3.67
Respiration rate(per min.)	70.00±3.20	71.83±2.86	73.00±2.75	70.50±3.17	66.00±3.06	69.83±3.27	71.50±2.97	73.33±2.84

* Significant ($P<0.05$)

Table 2:- Haemogram in rabbits before and after cystoplasty using glutaraldehyde processed canine collagen sheets (Mean± SE)n=6

Parameters with units	Preoperative	Postoperative intervals (days)	
		7	15
Haemoglobin concentration (g/dl)	11.18 ± 0.07	10.22 ± 0.08*	10.63 ± 0.07
Erythrocyte sedimentation rate (mm/h)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Packed cell volume (%)	38.67 ± 0.44	35.83 ± 0.29*	36.83± 0.39*
Total erythrocyte count(10^6/mm^3)	6.35 ± 0.25	5.13 ± 0.14	5.41 ± 0.10
Total leucocyte count(10^3/mm^3)	10.57 ± 0.36	8.95 ± 0.16	9.41 ± 0.19
Neutrophil count (%)	25.17 ± 0.44	27.33 ± 0.44*	26.17± 0.41*
Lymphocyte count(%)	72.17 ± 0.51	70.50 ± 0.40	71.00 ± 0.33
Eosinophil count(%)	1.17 ± 0.13	0.33 ± 0.09	0.50 ± 0.09
Basophil count(%)	0.17 ± 0.07	0.83 ± 0.07	0.50 ± 0.09
Monocyte count(%)	1.33 ± 0.09	1.00 ± 0.15	1.67 ± 0.09

*Significant($P<0.05$)

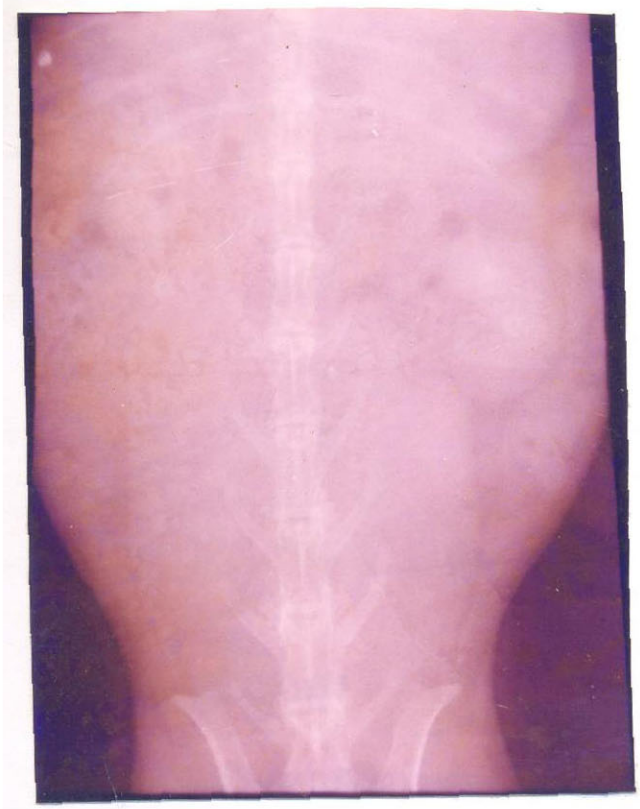
Table 3:- Serum constituents in rabbits before and after cystoplasty using glutaraldehyde processed canine collagen sheets (Mean±SE) n=6

Parameters with units	Preoperative	Postoperative interval (days)	
		7	15
Blood urea nitrogen (mg/dl)	35.58 ± 1.07	44.92 ± 1.76	37.15 ± 1.33
Serum creatinine (mg/dl)	2.20 ± 0.10	2.48 ± 0.12	2.05 ± 0.02
Serum sodium (mEq/l)	129.40 ± 2.80	136.43 ± 3.06	131.64 ± 2.52
Serum potassium (mEq/l)	5.73 ± 0.09	6.87 ± 0.14	6.57 ± 0.17*
Serum chloride (mEq/l)	102.23 ± 0.81	100.86 ± 1.06	106.10 ± 0.92
Total serum protein (g/dl)	7.02 ± 0.14	6.88 ± 0.10	7.05 ± 0.14
Serum albumin (g/dl)	5.28 ± 0.09	4.67 ± 0.12	4.68 ± 0.10

*Significant ($P<0.05$)

Fig.9 : Skiagram-abdomen of rabbit on 7th postoperative day showing kidney and ureter with continuity of contrast contained in urine. (Gr.I)

Fig.10 : Skiagram - lower abdomen of rabbit on 7th postoperative day showing normal filling of urinary bladder. (Gr.I)



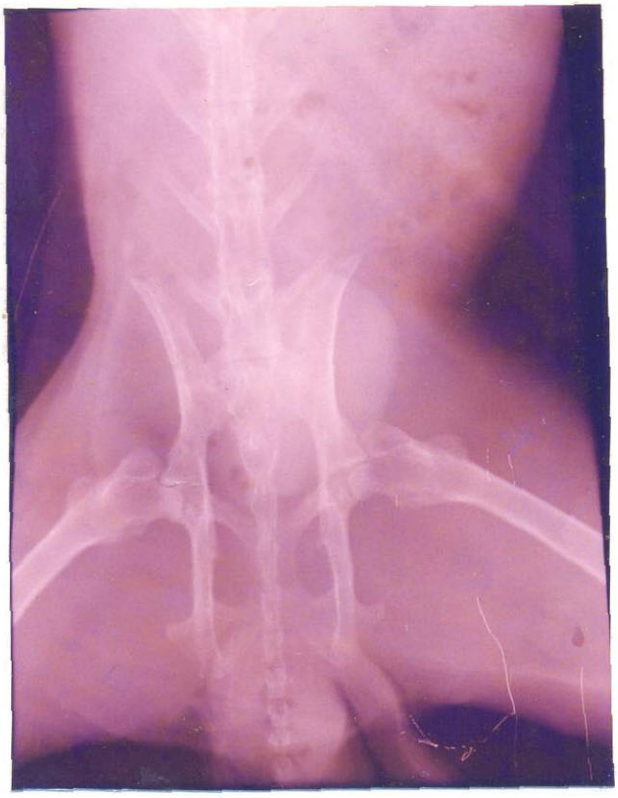
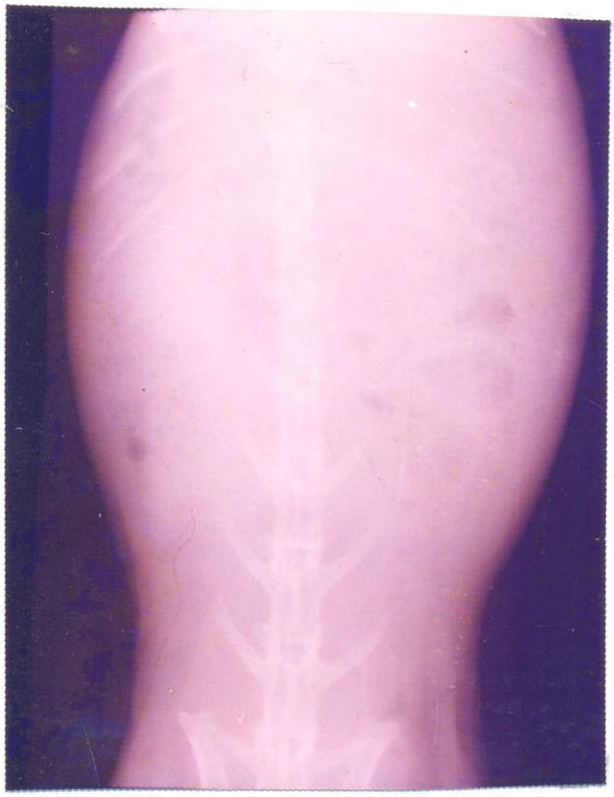


Fig.11 : Skiagram - abdomen of rabbit on15th postoperative day showing kidney and ureter with continuity of contrast contained in urine. (Gr.I)

Fig.12 : Skiagram - lower abdomen of rabbit on 15th postoperative day showing normal filling of urinary bladder. (Gr.I)



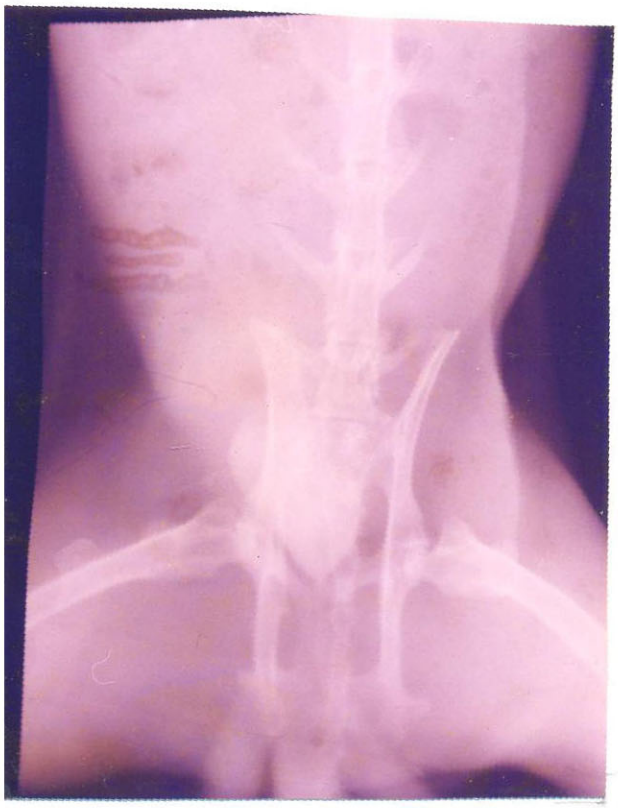
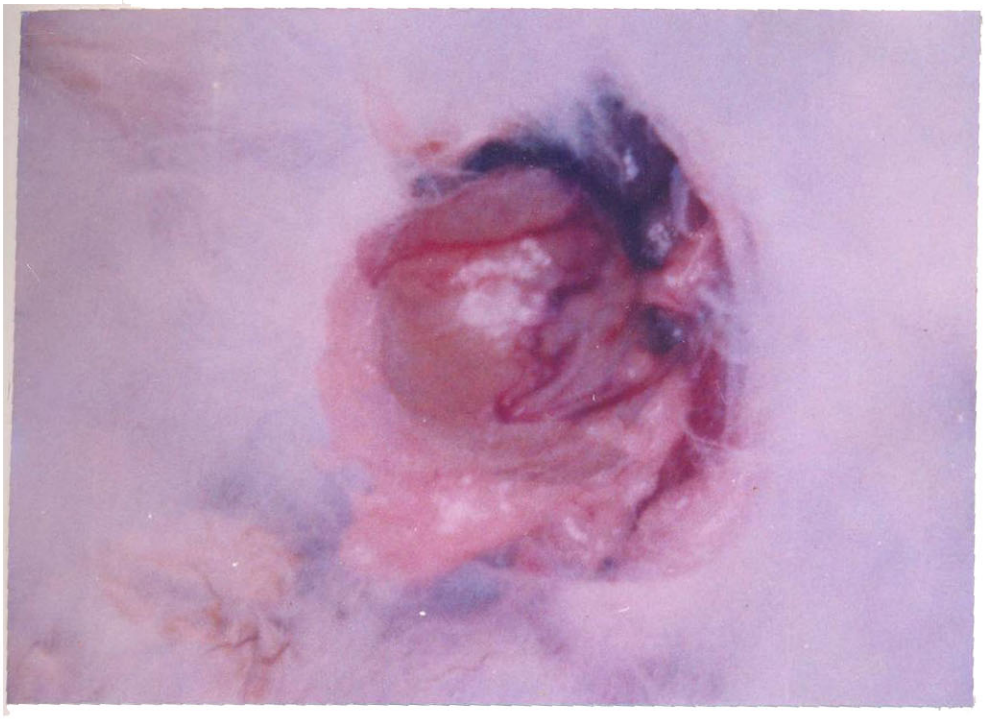


Fig.13 : Urinary bladder *in situ* on 30th postoperative day showing moderate fibrinous adhesion with adjacent tissues. (Gr.I)

Fig.14 : Gross specimen: cystoplasty site on 30th postoperative day showing incomplete mucosal healing at the centre. (Gr.I)



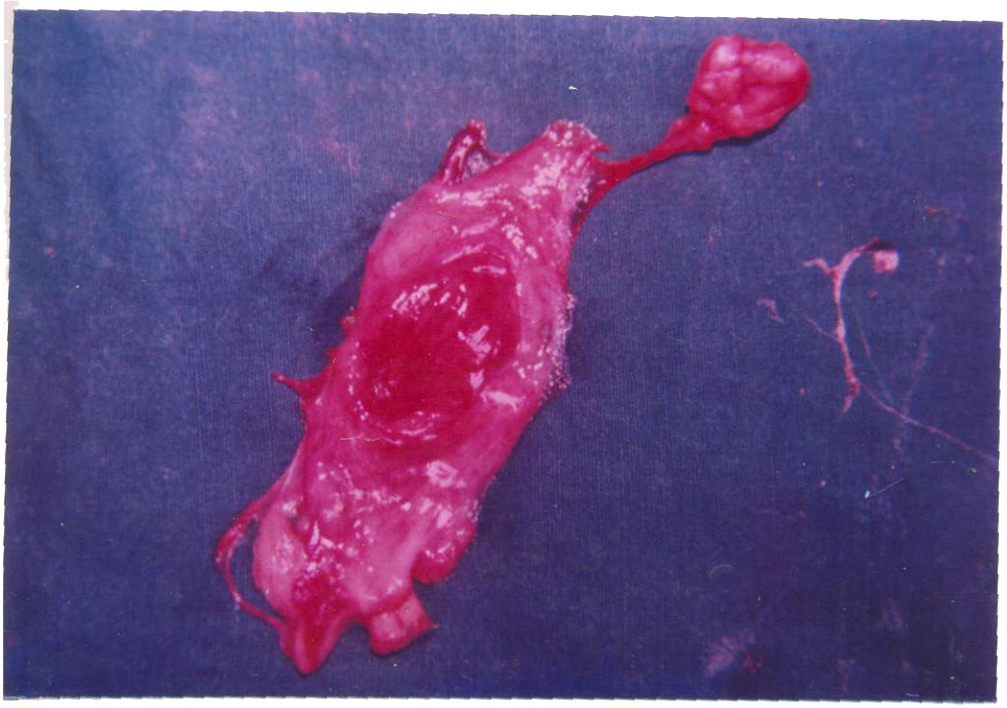
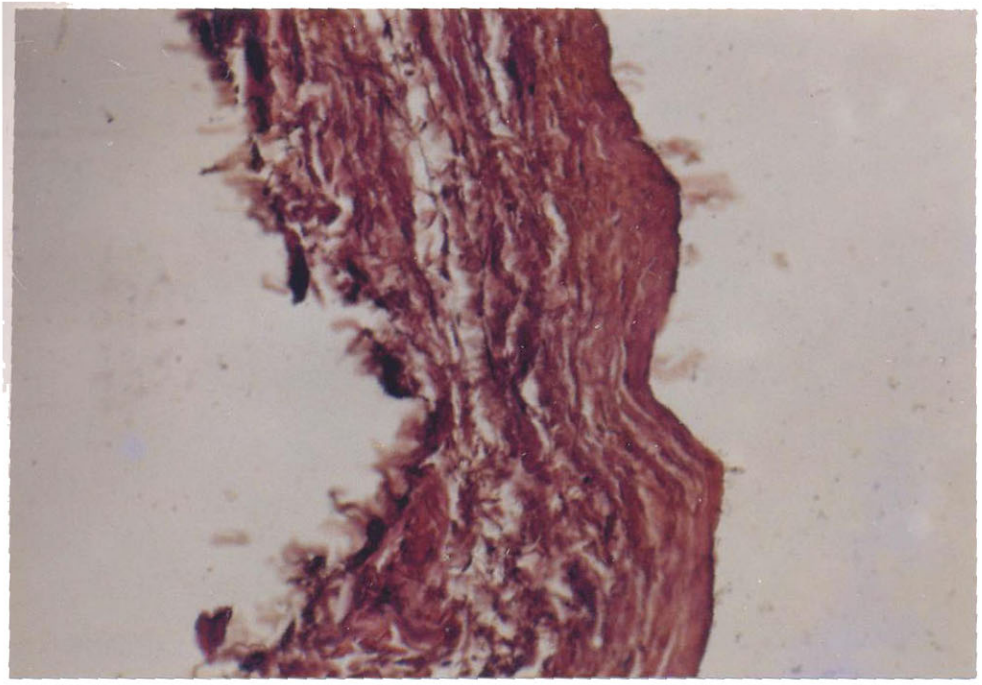


Fig.15 : Photomicrograph - histomorphology of canine collagen sheet with abundant elastic fibres and collagen fibres. (H&E) 250 x 1.25 X.

Fig.16 : Photomicrograph - cystoplasty site on 30th postoperative day showing mucosal proliferation (VAN) 250 x 1.25 X. (Gr.I)



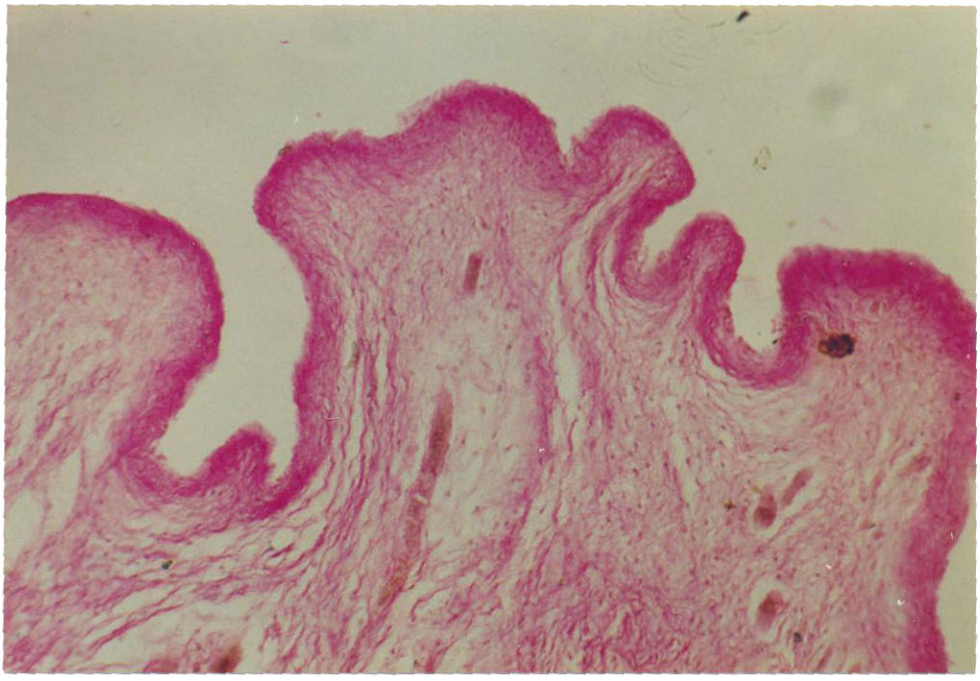
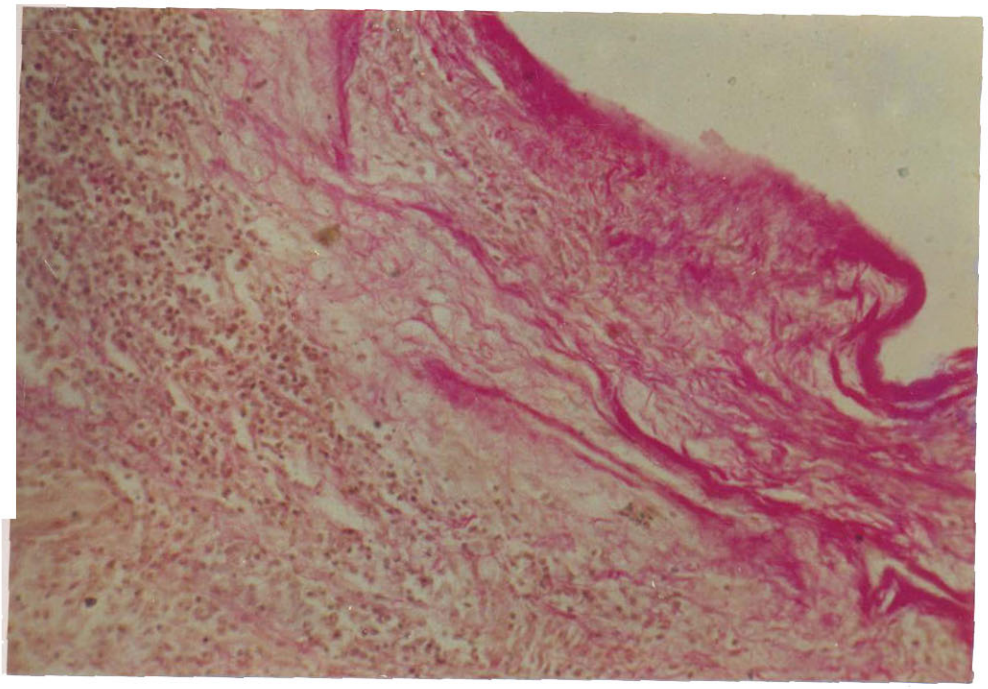
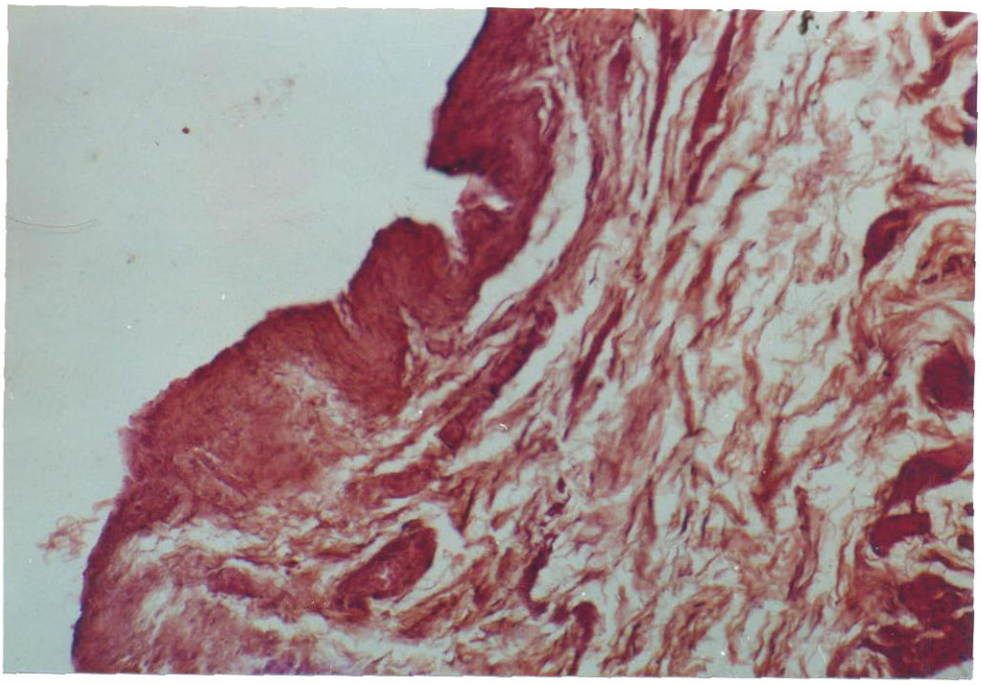


Fig.17 : Photomicrograph - cystoplasty site on 30th postoperative day showing inflammatory edema and deposition of collagen fibres (VAN) 250 x 1.25 X. (Gr.I)

Fig.18 : Photomicrograph - cystoplasty site on 30th postoperative day showing mild fibroplasia (H & E) 400 x 1.25 X. (Gr.I)





Group II

The observations are presented in Tables 4 to 6

Anaesthesia

The average body weight (kilogram) of the animals was 2.32 ± 0.11. The animals were premedicated with atropine sulphate (0.09 ± 0.00mg) and xylazine hydrochloride (11.58 ± 0.52mg).Ketamine hydrochloride (115.83 ± 5.23 mg) was administered I.M. for anaesthesia.

General condition

All the rabbits were lethargic on the first postoperative day. The animals became active and alert by second postoperative day. The operated area on ventral abdomen was clean and healing of wound was uneventful.

Physiological symptoms

Rectal temperature (^{0}C) was 39.22±0.08 before anaesthesia. A marginal increase in temperature was noticed upto second postoperative day and proportionate reduction was recorded from third to fourth postoperative day. From fifth postoperative day it was showing gradual rise to near normal range.

Pulse rate (per min.) was 131.00 ± 2.49 before anaesthesia. A rise in pulse rate was noticed from first postoperative day throughout the observation period with maximum rise on fifth postoperative day, but it was within normal physiological range. From fifth postoperative day it showed marginal fall towards normal level.

Respiration rate (per min.) was 100.33 ± 2.42 before anaesthesia. A fall in respiration rate was recorded from first postoperative day throughout the observation period with least value on third postoperative day, but the values were within normal physiological range.

Feeding behaviour

On the first postoperative day feed and grass intake was slightly reduced. Reduction in water consumption was also noticed. However the animals resumed normal feed intake and water consumption from second postoperative day. Animals maintained their normal habits throughout the observation period.

Haematological studies

The haemoglobin concentration (g/dl) was 11.93 ± 0.21 before anaesthesia. A significant ($P<0.05$) decrease in Hb was observed on seventh postoperative day followed by significant rise to near normal level thereafter on fifteenth postoperative day.

The erythrocyte sedimentation rate (mm/h) was 1.00 ± 0.00 before anaesthesia. Variation in ESR was not noticed during the observation period.

The packed cell volume (per cent) was 39.83 ± 0.51 before anaesthesia. A significant ($P<0.05$) reduction in PCV was observed on seventh postoperative day and this reduction persisted till fifteenth postoperative day.

The total erythrocyte count ($10^6/mm^3$) was 7.39 ± 0.22 before anaesthesia . A significant ($P<0.05$) decrease in TEC by seventh postoperative day was observed and it showed a marginal and proportionate rise on fifteenth postoperative day.

The total leucocyte count ($10^3/mm^3$) was 13.51 $\pm$ 0.45 before anaesthesia. There was significant ($P<0.05$) reduction in TLC by seventh postoperative day, and a significant ($P<0.05$) and progressive rise towards normal value was observed afterwards.

The neutrophil count (per cent) was 26.83 $\pm$ 0.65 before anaesthesia. Significant ($P<0.05$) variations in neutrophil count was observed on seventh and fifteenth postoperative day respectively.

The lymphocyte count (per cent) was 71.50 $\pm$ 0.66 before anaesthesia. Significant ($P<0.05$) reduction in lymphocyte count was noticed on seventh postoperative day and the count showed progressive rise to near normal level on fifteenth postoperative day.

The eosinophil count (per cent) was 0.00 before anaesthesia. It showed a marginal rise on fifteenth postoperative day only.

The basophil count (per cent) was 0.67$\pm$0.09 before anaesthesia. A marginal decrease in the count was noticed on seventh postoperative day followed by gradual increase on fifteenth postoperative day.

The monocyte count (per cent) was 1.00$\pm$0.00 before anaesthesia. Marked variation was not noticed during the observation period.

Biochemical studies

The blood urea nitrogen level (mg/dl) was 37.53$\pm$0.93 before anaesthesia. A marginal increase in BUN was observed on seventh postoperative day and it remained at decreased level even at fifteenth postoperative day but was within the normal range.

The serum creatinine level (mg/dl) was 2.25 ± 0.11 before anaesthesia. Variation in creatinine content was not noticed during the observation period.

The serum sodium concentration (mEq/l) was 130.84±1.71 before anaesthesia. An increase in sodium concentration on seventh postoperative day followed by a proportionate fall on fifteenth postoperative day were observed.

The serum potassium concentration (mEq/l) was 6.33±0.12 before anaesthesia. There was marginal reduction in potassium concentration on seventh postoperative day and the reduction was persisting on fifteenth postoperative day within the normal range.

The serum chloride concentration (mEq/l) was 107.85±0.94 before anaesthesia. It showed marginal decrease on seventh postoperative day and the value remained at lowered level even on fifteenth postoperative day.

The total serum protein content (g/dl) was 6.92 ± 0.10 before anaesthesia. A marginal decrease was observed on seventh postoperative day followed by remarkable rise in the content towards normal value on fifteenth postoperative day.

The serum albumin content (g/dl) was 4.15 ± 0.18 before anaesthesia. There was a marginal reduction on seventh postoperative day followed by proportionate rise in value on fifteenth postoperative day.

Radiographic observations

Normal production of urine from renal level and filling of reconstructed bladder were observed. The reconstructed bladder appeared to have normal filling capacity at seventh and fifteen postoperative day. Seepage of contents from the bladder, adhesion of bladder with surrounding structures and signs suggestive of cystitis could not be observed on seventh and fifteenth postoperative day. The

shadow of kidney, ureter and bladder with contrast medium was recognizable (Fig. 19,20,21 and 22)

Gross morphological studies

Moderate fibrinous adhesion of the reconstructed area with the adjacent structures were noticed (Fig. 23). The area of reconstruction was not distinguishable. The mucosal healing was incomplete (Fig. 24).

Histomorphological studies

The graft material showed dense and uniform arrangement of longitudinal, circular and oblique fibres arranged in layers (Fig. 25).

Mucosal proliferation and continuity was almost complete. Diffuse and nodular aggregation of lymphocytes below the mucous membrane were noticed at the site of healing. Progressive vascularisation and inflammatory reaction was much greater at the grafted area with deposition of collagen fibres and mild fibroplasia (Fig. 26, 27 and 28).

Table 4:- Rectal temperature, pulse rate and respiration rate in rabbits before and after cystoplasty using diacetyl processed fish collagen sheets (Mean ± SE) n=6

Parameters with units	Preoperative	Postoperative interval (days)						
		1	2	3	4	5	6	7
Rectal temperature (^{0}C)	39.22±0.08	39.27±0.08	39.27±0.09	39.07±0.05	39.00±0.05	39.07±0.07	39.13±0.06	39.15±0.05
Pulse rate (per min.)	131.00±2.49	134.67±2.15	135.83±2.00	137.00±2.07	137.33±1.82	138.00±2.14	137.00±1.83	136.33±1.77
Respiration rate(per min.)	100.33±2.42	94.50±1.62	93.50±1.83	92.00±1.91	95.83±1.65	92.67±1.59	94.33±1.40	93.83±1.40

Table 5:- Haemogram in rabbits before and after cystoplasty using diacetyl processed fish collagen sheets (Mean +-SE) n=6

Parameter with units	Preoperative	Postoperative interval (days)	
		7	15
Haemoglobin concentration(g/dl)	11.93 ± 0.21	10.80 ± 0.19*	11.07 ± 0.18*
Erythrocyte sedimentation rate(mm/h)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Packed cell volume(%)	39.83 ± 0.51	37.50 ± 0.53*	37.50 ± 0.31
Total erythrocyte count(10^6/mm^3)	7.39 ± 0.22	6.70± 0.19*	6.75 ± 0.11
Total leucocyte count (10^3/mm^3)	13.51 ± 0.45	11.78 ± 0.41	12.54 ± 0.35*
Neutrophil count(%)	26.83 ± 0.65	29.00 ± 0.67*	28.33 ± 0.67*
Lymphocyte count (%)	71.50 ± 0.66	69.50 ± 0.70*	70.00 ± 0.57
Eosiophil count(%)	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.09
Basophil count(%)	0.67 ± 0.09	0.33 ± 0.09	0.50 ± 0.09
Monocyte count(%)	1.00 ± 0.00	1.17 ± 0.07	1.00 ± 0.15

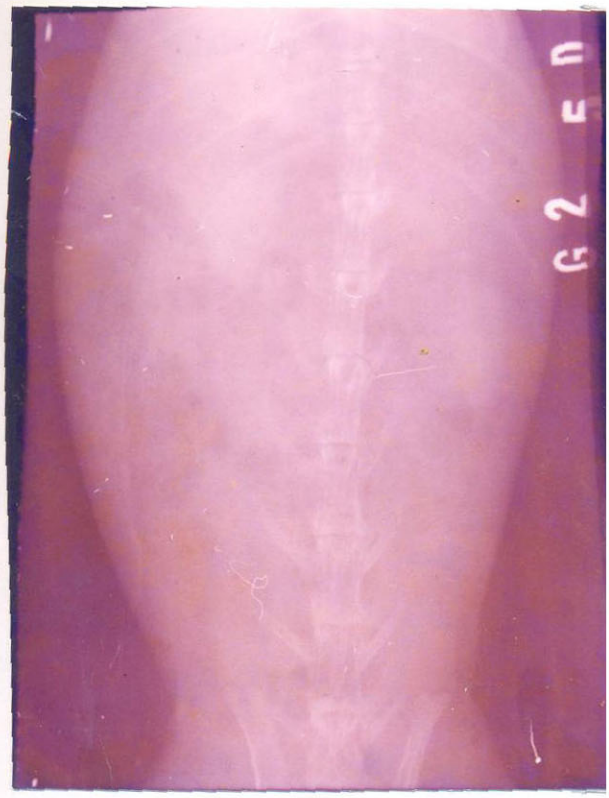
*Significant (P<0.05)

Table 6:- Serum constituents in rabbits before and after cystoplasty using diacetyl processd fish collagen sheets (Mean± SE)n=6

Parameters with units	Preoperative	Postoperative interval (days)	
		7	15
Blood urea nitrogen(mg/dl)	37.53 ± 0.93	39.77 ±1.24	31.15 ± 1.10
Serum creatinine (mg/dl)	2.25 ± 0.11	2.25 ± 0.14	2.30 ± 0.10
Serum sodium(mEq/l)	130.84 ± 1.71	139.25 ± 1.91	135.74 ± 1.87
Serum potassium (mEq/l)	6.33 ± 0.12	6.29 ± 0.05	5.98 ± 0.07
Serum chloride (mEq/l)	107.85 ± 0.94	106.33 ± 0.83	104.73 ± 1.05
Total serum protein (g/dl)	6.92 ± 0.10	6.63 ± 0.14	6.87 ± 0.11
Serum albumin (g/dl)	4.15 ± 0.18	3.95 ± 0.11	4.23 ± 0.12

Fig.19 : Skiagram - abdomen of rabbit on 7th postoperative day showing kidney and ureter with continuity of contrast contained in urine. (Gr.II)

Fig.20 : Skiagram - lower abdomen of rabbit on 7th postoperative day showing normal filling of urinary bladder. (Gr.II)


G2 E D

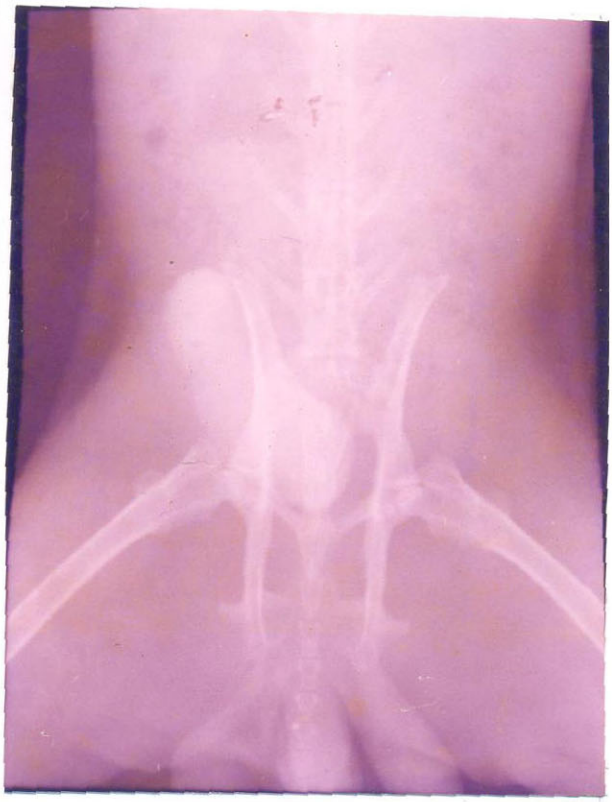
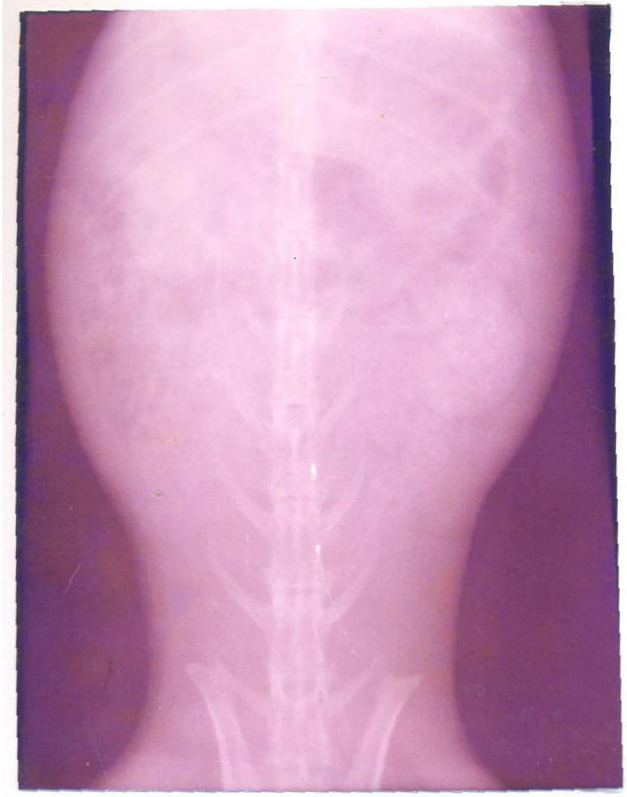


Fig.21 : Skiagram-abdomen of rabbit on 15th postoperative day showing kidney and ureter with continuity of contrast contained in urine. (Gr.II)

Fig.22 : Skiagram-lower abdomen of rabbit on 15th postoperative day showing normal filling of urinary bladder. (Gr.II)



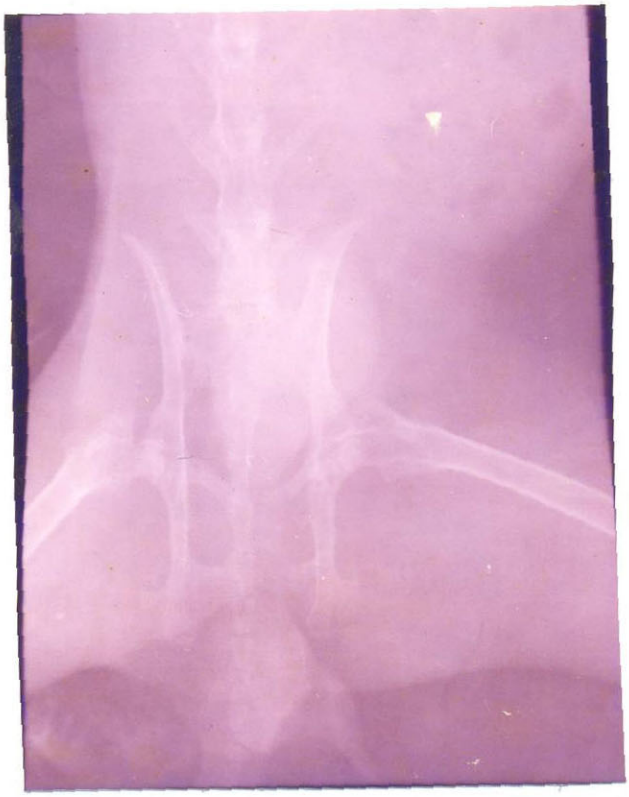
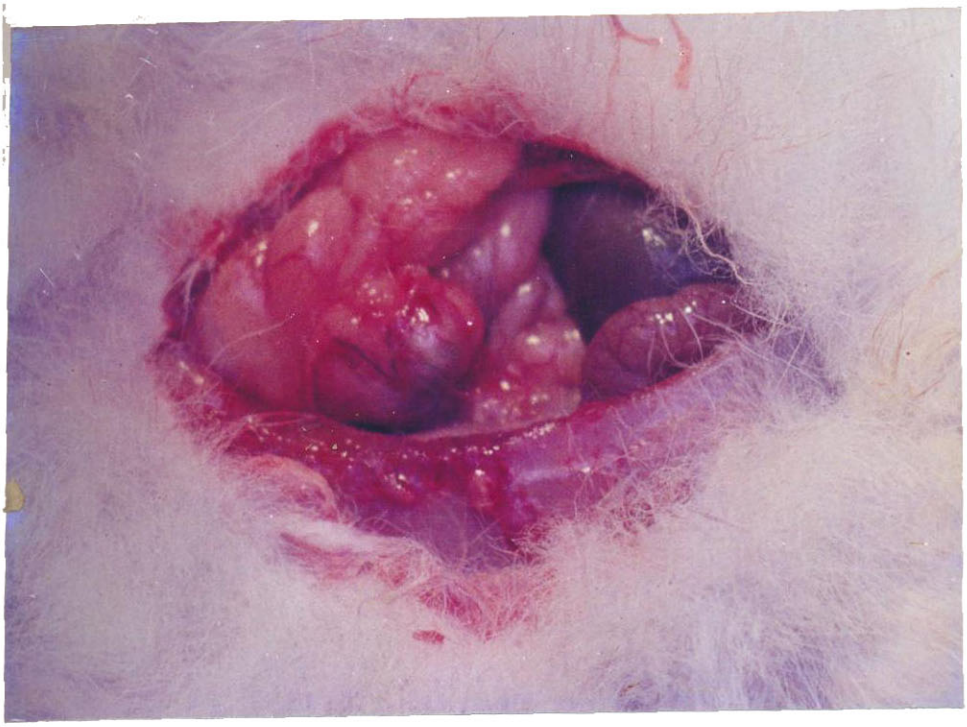


Fig.23 : Urinary bladder *in situ* on 30th postoperative day showing moderate fibrinous adhesion with adjacent tissue. (Gr.II)

Fig.24 : Gross specimen: cystoplasty site on 30th postoperative day showing incomplete mucosal healing at the centre. (Gr.II)



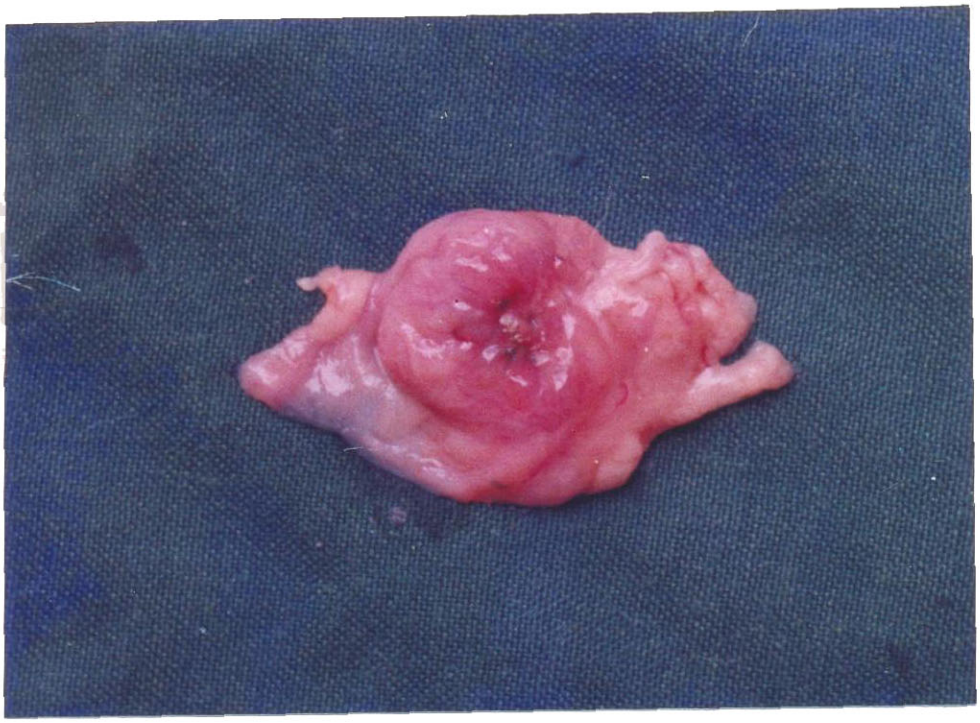
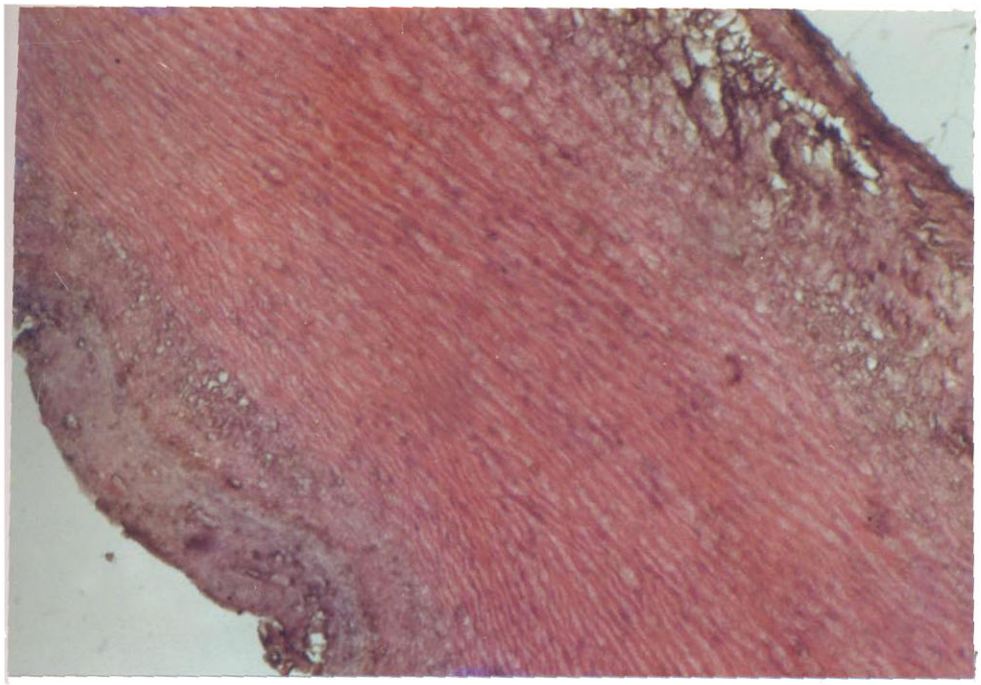


Fig.25 : Photomicrograph - histomorphology of fish collagen sheet with uniform arrangement of longitudinal, circular and oblique fibres. (VAN) 250 x 1.25 X.

Fig.26 : Photomicrograph - cystoplasty site on 30th postoperative day showing mucosal proliferation (H & E) 250 x 1.25 X. (Gr.II)



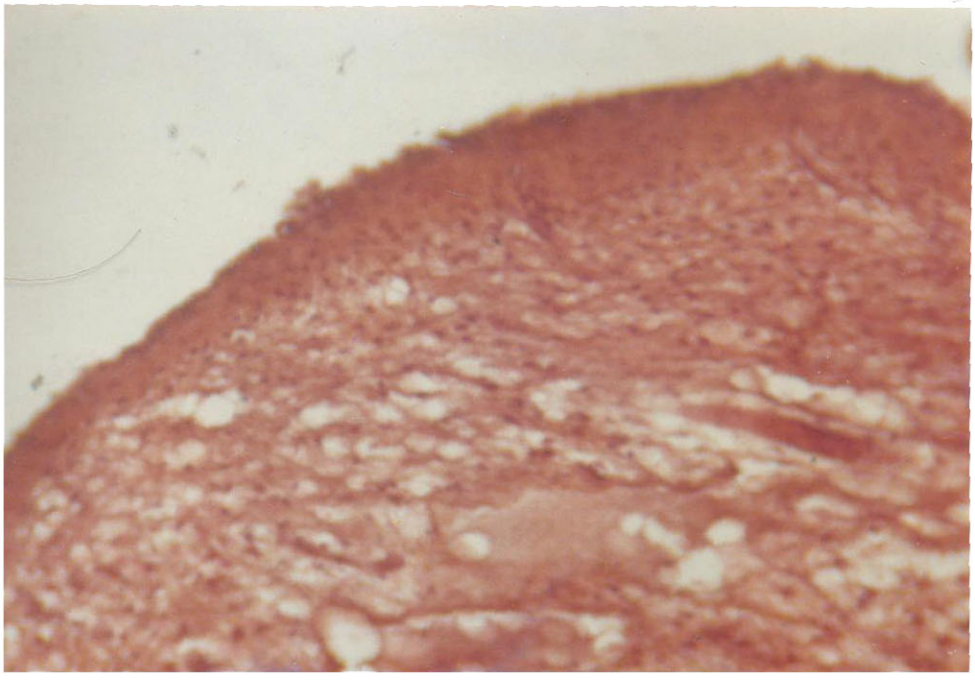
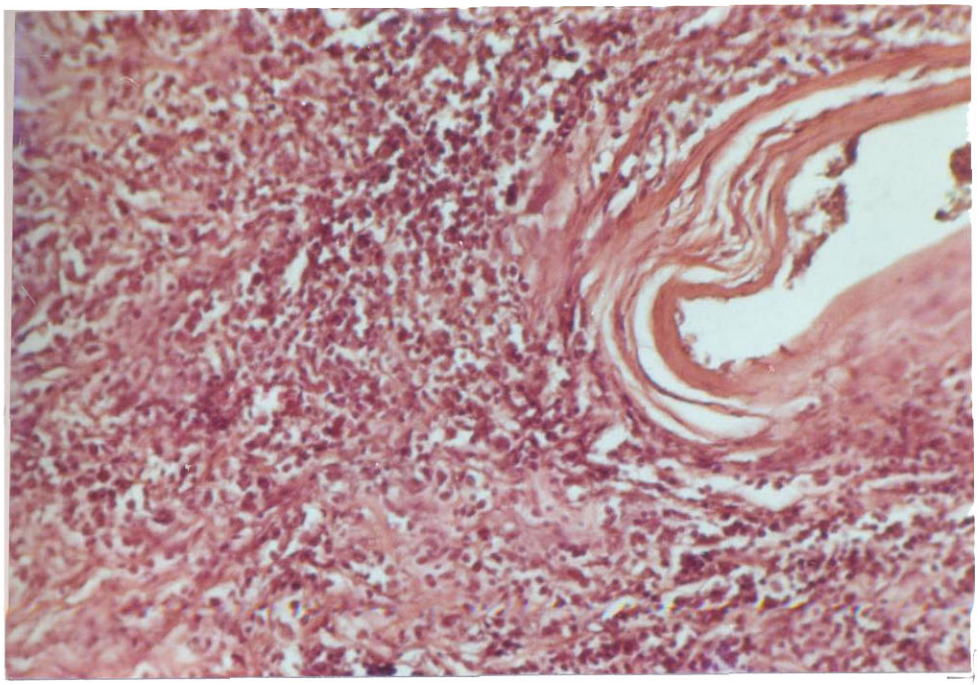
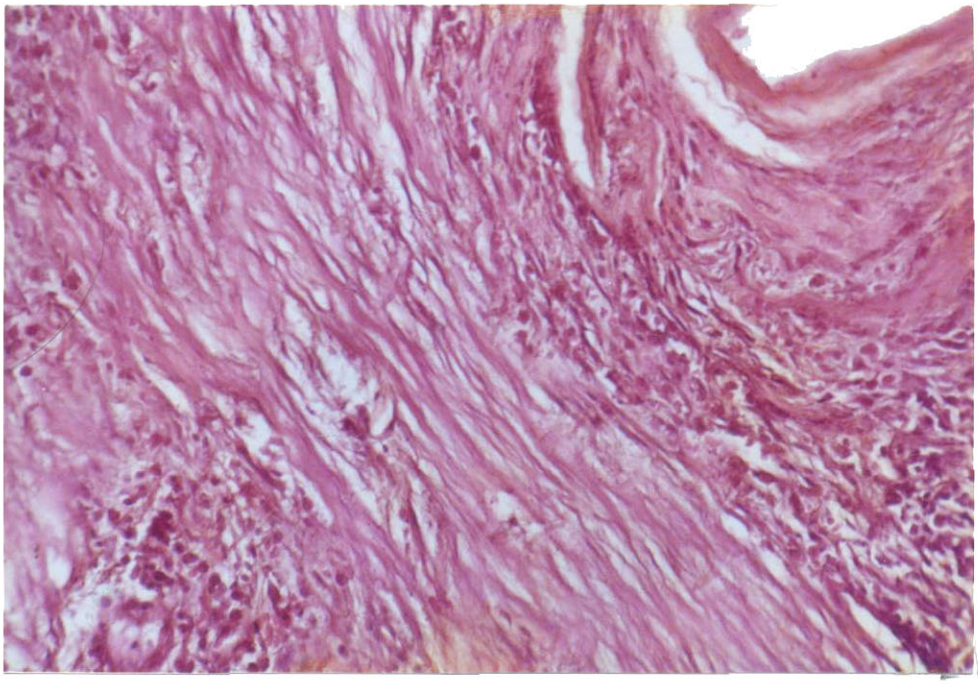


Fig.27 : Photomicrograph - cystoplasty site on 30th postoperative day showing diffuse and nodular aggregation of lypophocytes below the mucous membrane (VAN) 400 x1.25 X. (Gr.II)

Fig.28 : Photomicrograph - cystoplasty site on 30th postoperative day showing deposition of collagen fibres and fibroplasia (VAN) 400 x 1.25 X. (Gr.II)





Group III

The observations are presented in tables 7 to 15

Anaesthesia

The average bodyweight (kilogram) of the dogs were 14.83 ± 1.30. The animals were premedicated with triflupromazine hydrochloride (14.83 ± 1.30 mg) and anaesthetized using thiopentone sodium (450.00 ± 25.82 mg) to effect.

General Condition

All the dogs were dull and depressed on the first postoperative day and most of the time remained on recumbent position. Animals gradually regained their activity from second postoperative day and became alert and normal in habits from fourth postoperative day. The cutaneous wound was clean during the postoperative period and healing of the wound was uneventful.

Feeding behaviour

All the dogs started feeding on a little quantity of milk from first postoperative day and gradually they attained normal appetite by fourth postoperative day. Animals started feeding on rice gruel thereafter and maintained normal feeding habits throughout the observation period.

Urinalysis

Colour

Animals voided blood tinged urine for two to three days after surgery and urine colour returned to normal by third postoperative day.

pH

The pH of urine was slightly alkaline for first two days after surgery and became normal thereafter.

Blood

Urine samples in all dogs were positive for occult blood for first two days after surgery and negative afterwards during the observation period.

Protein

Urine samples were positive for protein for first two days after surgery and thereafter became negative during the observation period.

Microscopic examination

Presence of granular casts, phosphate crystals, epithelial cells, red blood cells and white blood cells in excess than normal was noticed for 2-3 days after surgery. Thereafter their presence was limited to normal levels in the urine.

Urination

All the animals were straining for urination on the first postoperative day. The catheter was not in position on the very first day after surgery and straining during urination was not observed from second postoperative day.

Physiological symptoms

Rectal temperature (^{0}C) was 39.07$\pm$0.07 before anaesthesia. A marginal increase was noticed on first postoperative day and thereafter the rectal temperature

showed reduction throughout the observation period with significant ($P<0.05$) low value on fourth postoperative day. From fifth postoperative day a gradual and proportionate increase to normal level was noted.

Pulse rate (per min.) was 87.50 ± 1.40 before anaesthesia. There was significant ($P<0.05$) increase in pulse rate from the first postoperative day throughout the observation period and the values showed gradual and progressive decrease to normal level upto seventh postoperative day.

Respiration rate (per min.) was 26.67 ± 0.40 before anaesthesia. There was marginal increase on first postoperative day and thereafter it attained normal preanaesthetic level early by second postoperative day.

Haematological studies

The haemoglobin concentration (g/dl) was 13.97 ± 0.17 before anaesthesia. A decrease in Hb was noticed on third postoperative day and it showed persistent decrease during the observation period.

The erythrocyte sedimentation rate (mm/h) was 1.00 ± 0.00 before anaesthesia. Variation in ESR was not noticed during the postoperative period.

The packed cell volume (per cent) was 42.67 ± 0.98 before anaesthesia. There was marginal increase in PCV on third postoperative day and thereafter gradual decrease was noticed upto fifteenth postoperative day.

The total erythrocyte count ($10^6/mm^3$) was 6.73 ± 0.21 before anaesthesia. An increase in TEC was noticed on third postoperative day and the increased level was maintained upto seventh postoperative day followed by a proportionate decrease on fifteenth postoperative day.

The total leucocyte count ($10^3/mm^2$) was 12.68 $\pm$ 0.61 before anaesthesia. There was significant ($P< 0.05$) increase in TLC on third postoperative day and thereafter it showed gradual and progressive decrease. TEC returned to near normal on fifteenth postoperative day.

The neutrophil count (per cent) was 53.83$\pm$0.50 before anaesthesia. A significant ($P<0.05$) rise in neutrophil count was observed on third postoperative day followed by the value returning to near normal on fifteenth postoperative day. The fall at seventh postoperative day was significant.

The lymphocyte count (per cent) was 43.83 $\pm$ 0.52 before anaestheia. A significant ($P< 0.05$) reduction in lymphocyte count was observed on third postoperative day and thereafter the lymphocyte count showed significant rise at seventh day followed by gradual and proportionate rise to normal by fifteenth postoperative day.

The eosinophil count (per cent) was 1.17$\pm$0.07 before anaesthesia. It showed insignificant variation during the postoperative period in the normal range.

The basophil count (per cent) was 0.33 $\pm$ 0.09 before anaesthesia. Marked variation in basophil count was not observed during the postoperative period.

The monocyte count (per cent) was 0.83 $\pm$ 0.13 before anaestheseia. A marginal decrease was observed on third postoperative day followed by the count returning to normal on seventh postoperative day.

Biochemical studies

The blood urea nitrogen level (mg/dl) was 19.40±0.64 before anaesthesia. A significant ($P< 0.05$) increase in BUN was noticed on third postoperative day and the level showed significant decrease at seventh day followed by gradual and remarkable decrease upto fifteenth postoperative day.

The serum creatinine level (mg/dl) was 2.35 ± 0.08 before anaesthesia. There was marginal decrease in serum creatinine content from seventh to fifteenth postoperative day.

The serum sodium concentration (mEq/l) was 126.05±0.72 before anaesthesia. Significant ($P< 0.05$) increase in sodium concentration was observed on third and seventh postoperative day followed by proportionate decrease by fifteenth postoperative day.

The serum potassium concentration (mEq/l) was 7.06 ± 0.13 before anaesthesia. There was increase in potassium concentration upto seventh postoperative day which was significant ($P< 0.05$) on seventh postoperative day and the level showed gradual decrease to attain near normal by fifteenth postoperative day.

The serum chloride concentration (mEq/l) was 111.85 ± 1.65 before anaesthesia. It showed significant ($P< 0.05$) decrease on third postoperative day followed by significant increase from seventh postoperative day, returning to near normal value by fifteenth postoperative day.

The total serum protein content (g/dl) was 6.60±0.04 before anaesthesia. Significant ($P< 0.05$) reduction in serum protein content was observed upto seventh postoperative day and it showed gradual increase by fifteenth postoperative day.

The serum albumin content (g/dl) was 3.58±0.04 before anaesthesia. There was rise in serum albumin content upto seventh postoperative day and it showed gradual fall on fifteenth postoperative day.

Radiographic observations

On retrograde cystography the reconstructed urinary bladder appeared to have normal contour and filling capacity at third, seventh and fifteenth day postoperatively. The mucosal lining of bladder appeared to be complete. No seepage of contents and signs of cystitis with clinical significance could be observed during the postoperative period (Fig.29, 30, 31, 32, 33 and 34).

Table 7:- Rectal temperature, pulse rate and respiration rate in dogs before and after cystoplasty using collagen sheets (Mean ± SE) n=6

Parameters with units	Preoperative	Postoperative interval (days)						
		1	2	3	4	5	6	7
Rectal temperature (^{0}c)	39.07 ± 0.07	39.93 ± 0.05	38.97 ± 0.04	38.70 ± 0.05	38.35 ± 0.06*	38.40 ±0.05	38.63 ± 0.05	38.83 ± 0.03
Pulse rate (per min.)	87.50 ± 1.40	99.67 ± 1.96*	98.00 ± 2.08*	97.67 ± 1.35*	96.33 ± 1.29*	95.67 ±1.02*	93.83 ± 1.19*	92.67 ± 0.83*
Respiration rate (per min.)	26.67 ± 0.40	27.50 ±0.43	26.67 ± 0.34	26.67 ± 0.44	26.50 ± 0.72	26.67 ±1.22	26.50 ± 0.16	26.67 ± 0.34

*Significant ($P<0.05$)

Table 8:- Haemogram in dogs before and after cystoplasty using collagen sheets (Mean ± SE) n=6

Parameter with units	Preoperative	Postoperative interval (days)		
		3	7	15
Haemoglobin concentration (g/dl)	13.97 ± 0.17	13.62 ± 0.30	13.45±0.16	13.25 ± 0.17
Erythrocyte sedimentation rate (mm/h)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Packed cell volume(%)	42.67 ± 0.98	43.00 ± 1.55	41.83 ± 1.14	41.67 ± 0.83
Total erythrocyte count(10^6/mm^3)	6.73 ± 0.21	7.28 ± 0.06	7.42 ± 0.11	6.90 ± 0.13
Total leucocyte count (10^3/mm^3)	12.68 ± 0.61	14.72 ± 0.50*	13.41 ± 0.29	12.72 ± 0.38
Neutrophil count (%)	53.83 ± 0.50	63.17 ± 0.29*	57.67± 0.71*	54.83 ± 0.51
Lymphocyte count (%)	43.83 ± 0.52	35.50 ± 0.43*	40.17 ± 0.57*	43.50 ± 0.46
Eosinophil count (%)	1.17 ± 0.07	0.33 ± 0 .09	1.00 ± 0.21	0.67 ± 0.09
Basophil count (%)	0.33 ± 0.09	0.33 ± 0.09	0.33 ± 0.09	0.83 ± 0.13
Monocyte count (%)	0.83 ± 0.13	0.67 ± 0.09	0.83 ± 0.13	0.17 ± 0.07

*Significant (P<0.05)

Table 9:- Serum constituents in dogs before and after cystoplasty using collagen sheets (Mean ± SE) n= 6

Parameters with units	Preoperative	Postoperative interval (days)		
		3	7	15
Blood urea nitrogen (mg/dl)	19.40 ± 0.64	24.23 ± 0.76*	22.95 ± 0.68*	21.70 ± 0.64
Serum creatinine (mg/dl)	2.35 ± 0.08	2.32 ± 0.04	2.23 ± 0.07	2.25 ± 0.04
Serum sodium (mEq/l)	126.05 ± 0.72	129.58 ± 0.40*	129.05 ± 0.70*	127.86 ± 0.68*
Serum potassium (mEq/l)	7.06 ± 0.13	8.10 ± 0.13	8.17 ± 0.11*	7.24 ± 0.13
Serum chloride (mEql)	111.85 ± 1.65	98.58 ± 1.76*	104.33 ± 1.52*	107.60 ± 1.37
Total serum protein (g/dl)	6.60 ± 0.04	6.38± 0.05*	6.28 ± 0.04*	6.35 ± 0.05
Serum albumin (g/dl)	3.58 ± 0.04	3.82 ± 0.17	4.17 ± 0.15	3.92 ± 0.06

*Significant (P<0.05)

Table 10. Observations on urinalysis in dogs

Dog No:1

Parameters	Postoperative interval (Days)						
	1	2	3	4	5	6	7
Colour	Blood tinged	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow
pH	7.5	6.5	6.5	6.5	6.5	6.5	6.5
Blood	(+)	(+)	(-)	(-)	(-)	(-)	(-)
Protein	(+)	(+)	(-)	(-)	(-)	(-)	(-)
Microscopic examination							
Epithelial Cells	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Casts	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Red Blood Cells	(+)	(+)	(-)	(-)	(-)	(-)	(-)
White Blood Cells	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Crystals	(+)	(+)	(+)	(+)	(+)	(+)	(+)

(+) Positive
(-) Negative

Table No. 11: Observations on urinalysis in dogs

Dog No. 2

Parameters	Postoperative intervals (Days)						
	1	2	3	4	5	6	7
Colour	Brown	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow
pH	7	7	6.5	6.5	6.5	6.5	6.5
Blood	(+)	(+)	(-)	(-)	(-)	(-)	(-)
Protein	(+)	(+)	(+)	(-)	(-)	(-)	(-)
Microscopic examination							
Epithelial Cells	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Casts	(+)	(+)	(+)	(+)	(+)	(+)	(+)
RBC	(+)	(+)	(-)	(-)	(-)	(-)	(-)
WBC	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Crystals	(+)	(+)	(+)	(+)	(+)	(+)	(+)

(+) Positive
(-) Negative

Table No. 12 : Observations on urinalysis in dogs

Dog No. 3

Parameters	Postoperative interval (Days)						
	1	2	3	4	5	6	7
Colour	Blood tinged	Blood tinged	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow
pH	7	7	6.5	6	6	6	6
Blood	(+)	(+)	(-)	(-)	(-)	(-)	(-)
Protein	(+)	(+)	(+)	(-)	(-)	(-)	(-)
Microscopic examination							
Epithelial Cells	(+)	(+)	(+)	(+)	(+)	(+)	(+)
RBC	(+)	(+)	(-)	(-)	(-)	(-)	(-)
Cast	(+)	(+)	(+)	(+)	(+)	(+)	(+)
WBC	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Crystals	(+)	(+)	(+)	(+)	(+)	(+)	(+)

(+) Positive
(-) Negative

***Table 13* : Observations on urinalysis in dogs**

Dog No. 4

Parameters	Postoperative interval (Days)						
	1	2	3	4	5	6	7
Colour	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow
pH	6.5	6	6	6	6	6	6
Blood	(+)	(-)	(-)	(-)	(-)	(-)	(-)
Protein	(+)	(-)	(-)	(-)	(-)	(-)	(-)
Microscopic examination							
Epithelial Cells	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Cast	(+)	(+)	(+)	(+)	(+)	(+)	(+)
RBC	(+)	(-)	(-)	(-)	(-)	(-)	(-)
WBC	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Crystals	(+)	(+)	(+)	(+)	(+)	(+)	(+)

(+) Positive
(-) Negative

Table No. 14 : Observations on urinalysis in dogs

Dog No.5

Parameters	Postoperative interval (Days)						
	1	2	3	4	5	6	7
Colour	Blood tinged	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow
pH	7	6.5	6.5	6.5	6.5	6.5	6.5
Blood	(+)	(+)	(-)	(-)	(-)	(-)	(-)
Protein	(+)	(+)	(-)	(-)	(-)	(-)	(-)
Microscopic examination							
Epithelial Cells	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Cast	(+)	(+)	(+)	(+)	(+)	(+)	(+)
RBC	(+)	(+)	(-)	(-)	(-)	(-)	(-)
WBC	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Crystals	(+)	(+)	(+)	(+)	(+)	(+)	(+)

(+) Positive
(-) Negative

Table 15: Observations on urinalysis in dogs

Dog No. 6

Parameters	Postoperative intervals (Days)						
	1	2	3	4	5	6	7
Colour	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow
pH	7	6.5	6.5	6.5	6.5	6.5	6.5
Blood	(+) (+)	(-)	(-)	(-)	(-)	(-)	(-)
Protein	(+) (+)	(-)	(-)	(-)	(-)	(-)	(-)
Microscopic examination							
Epithelial Cells	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Casts	(+)	(+)	(+)	(+)	(+)	(+)	(+)
RBC	(+)	(-)	(-)	(-)	(-)	(-)	(-)
WBC	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Crystals	(+)	(+)	(+)	(+)	(+)	(+)	(+)

(+) Positive
(-) Negative

Fig.29 : Skiagram - pelvis of dog on 3rd postoperative day after cystoplasty using canine collagen sheet showing normal filling of bladder. (Gr.III)

Fig.30 : Skiagram - pelvis of dog on 7th postoperative day after cystoplasty using canine collagen sheet showing normal filling of bladder. (Gr.III)

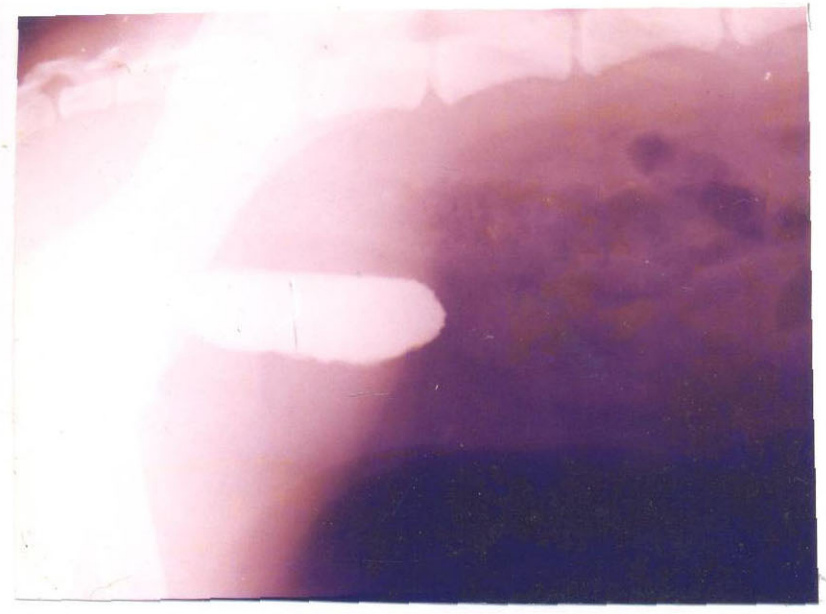
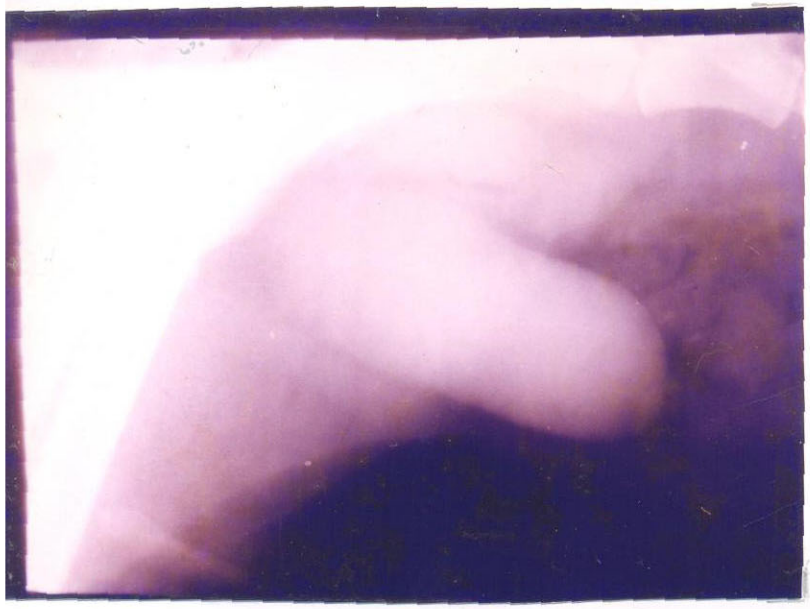


Fig.31 : Skiagram - pelvis of dog on 15th postoperative day after cystoplasty using canine collagen sheet showing normal filling of bladder. (Gr.III)

Fig.32 : Skiagram - pelvis of dog on 3rd postoperative day after cystoplasty using fish collagen sheet showing normal filling of bladder. (Gr.III)



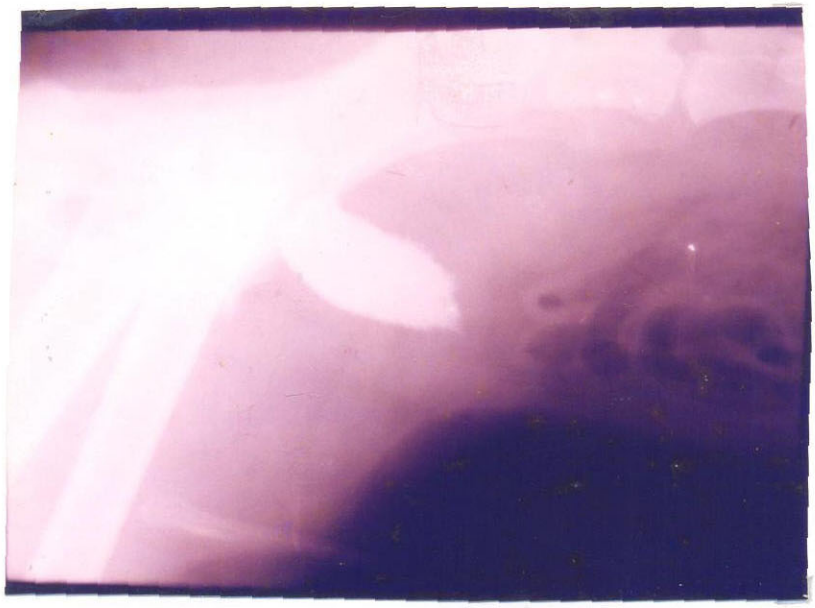
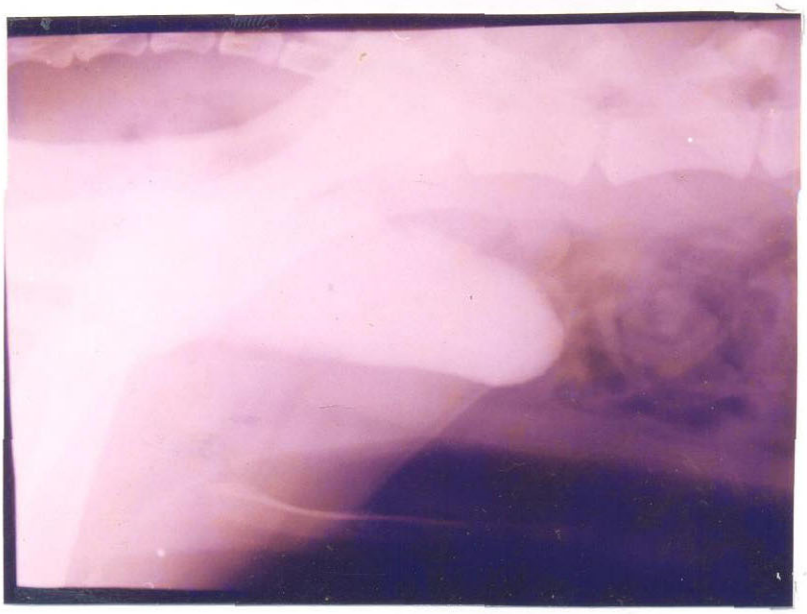


Fig.33 : Skiagram - pelvis of dog on 7th postoperative day after cystoplasty using fish collagen sheet showing normal filling of bladder. (Gr.III)

Fig.34 : Skiagram - pelvis of dog on 15th postoperative day after cystoplasty using fish collagen sheet showing normal filling of bladder. (Gr.III)



Discussion

CHAPTER – V
DISCUSSION

Urinary bladder is an organ with remarkable powers of regeneration. However, in many disease conditions reconstruction of urinary bladder becomes necessary for anatomical and functional re-establishment of the organ. Being an organ coming in contact with umpteen number of waste products of the body constantly, the materials used for reconstruction of bladder must tolerate such an environment. Ever since the concept of cystoplasty techniques were conceived and performed in animals, autogenous grafts were the more acceptable material.

Collagens, a family of proteins, are widely distributed in vertebrates and invertebrates and are abundant in animals. These macromolecular assemblies of collagen fibres, support and hold the body together.

India has emerged as one of the leading fish producing countries in the world, handling substantial quantities of fresh water fishes as well as marine fishes. Fish air bladder is rich in collagen which forms 90 per cent of the available protein in it. These air bladders are thrown out when fish is used for edible purpose. This can be used as a relatively cheap biological material and at the same time ensure better utilization of the presently wasted aquatic tissue.

Homologous viable functional substitute or heterologous tissues as graft materials for reconstructive surgery of hollow organs were the choice in the earlier days. But disadvantages such as additional trauma, stress on healing, difficulty in the availability or harvesting of suitable and sizable tissue, problem of tissue rejection with associated functional disturbances and complication of long term nature may co-exist. Hence the necessity of a readily available prosthesis is the choice for immediate restoration of continuity and functional capacity of hollow organs. Several synthetic, biological and functional biografts had been used in surgical reconstruction of urinary bladder. Autogenous fascia (Tsuji *et al,* 1967), skin (Nair *et al,* 1988a) and peritoneum (Nair *et al,* 1988b) have been used with least

complications. Allogenic graft materials such as preserved urinary bladder wall (Shivaprakash *et al,* 1991a) and duramater (Prasad and Tyagi, 1980) showed variable results with regard to regeneration of the host bladder tissue.

Xenogenic graft material comprising of OMS (membranous chromic catgut made in Japan) membrane (Tsuji *et al,* 1967) and chromicised duramater (Sambandam, 1992) are associated with severe tissue reaction at the site of cystoplasty.

Synthetic grafts made up of polytetrafloroethylene (Shivaprakash *et al,*1991b) and terylene lined hollow plastic balls (Sharma,1997a) had been tried and obtained varied results.

Biological grafts made up of collagen sheets have qualities which make them suitable substitutes for hollow organ reconstruction. Collagens are natural macromolecules which offer many of the desirable characteristics consistently important for a biomaterial (Sastry, 1989). This material has better biocompatibility with minimal cellular reaction. It is physiologically biodegradable and has positive wound healing effect. Moreover it can be chemically modified to impart better mechanical properties and shelf life. Collagen is being used frequently as an important prosthetic material in clinical surgery. Manipulation and alteration of physical properties of collagen allow a wide range of use. Decreased antigenicity allows the use of collagen prosthesis with minimal host inflammatory response. Control of solubility, strength and resorption can be accomplished through change of composition and structure of collagen. Minimal tissue reaction after implantation is a significant factor favouring the use of collagen as a biomaterial (Simpson, 1983)

Biological grafts made up of collagen has been used in medical field in urology for satisfactory control of incontinence and to reconstruct bladder wall defects (Simpson, 1983). Collagen has important role in blood coagulation by promoting contact activation of coagulation mechanism and platelet activation and aggregation (Gentry *et al,* 1981)

In the present study tissue samples of urinary bladder harvested from recently died dogs were processed and made into sheets, crosslinked with glutaraldehyde and preserved for use as graft material. Air bladder collected from fresh water fishes were also made into sheets, processed, cross linked with diacetyl and preserved for use as graft material in urinary bladder reconstruction in rabbits.

Processing of the animal and aquatic tissues does not require complicated procedures and the cost involved is marginal. The processed collagen sheets have good handling qualities and shelf life. Gamma irradiation of collagen was satisfactory for sterilization of the collagen products for medical use with slight denaturation (Chvapil, 1973).

Cross linking of collagen tissue using glutaraldehyde is a widely accepted method for preparation of biomaterial (Nimni and Cheung, 1994; Ruijgrok *et al*, 1994). Jayakrishnan and Jameela (1996) reported that glutaraldehyde was a versatile agent used in the preparation of vascular graft, making them biocompatible, non thrombogenic and non-antigenic. It reduced the biodegradation of the graft, preserving its anatomic integrity, leaflet strength and flexibility. Cross linking of collagen also impeded the activity of bacterial collagenase to provide extra stability (Simpson, 1983). Roe *et al,* (1990) reported that cross linked collagen bioprosthesis were designed to be inert and non resorbable, resulting in fatigue and wear failure in high stress environments. *In vitro* assays revealed that they were bacterial collagenase resistant with slow rate of resorption.

Glutaraldehyde crosslinked bovine artery (Shetty *et al,* 1982, and Sawyer *et al,*1987), dermal collagen (Frankland, 1986), human amniotic membrane (Rameshkumar, 1993), pericardium (Santillan *et al,* 1995) urinary bladder (Sreenu *et al,*1998) and aorta (Balagopalan, 1998) were reported to be satisfactory as implants for experimental reconstruction of hollow organs in bovines and dogs. *In vivo* evaluation of acetylated collagen was tried by Srivastava *et al,* (1990) after implantation as film and sponge into rat lumbar muscles. Mukundan (1992) reported the use of collagen fibres of fish intestines cross linked with diacetyl for preparation

of surgical sutures and opined that diacetyl treatment increased the strength of collagen fibres of the suture making them strong, durable, fully absorbable and free from tissue reactions.

EXPERIMENTAL STUDY

Cystoplasty in rabbits

A portion of urinary bladder ($2cm^2$) close to the vertex was surgically removed from the dorsal aspect of the fundus in all experimental animals. The site in the bladder chosen for cystoplasty was the fundus as recommended by Kudale and Hattangady (1971).

Cystoplasty was performed using glutaraldehyde processed canine collagen sheet in rabbits of group I and, diacetyl processed fish collagen sheet in rabbits of group II. The beneficial haemostatic effect of collagen could be observed while placing the material over the defect for fixing to the edges. The topical haemostatic effect observed during this study is in agreement with the reports of Gentry *et al,*(1981), Simpson (1983), Sastry (1989) and Al - Khateeb *et al*; (1996). The graft material was fixed to the edges of defect using 5-0 braided silk by overlapping continuous horizontal mattress sutures, and the healing was satisfactory. Sharma (1997) and Sreenu *et al,* (1998) reported use of silk for experimental cystoprosthesis in animals. Shivaprakash *et al,* (1991a) recommended overlapping mattress suture for experimental bladder reconstruction in goats.

Postsurgical management

The animals were kept under observation after surgery for a minimum period of one month. Normal feed and water were offered postoperatively and also during observation. Antibiotics were administered for 5 days postoperatively.

Routine cleaning and dressing of cutaneous wound was carried out and the sutures were removed by 7^{th} postoperative day after complete healing.

Observations on general condition, physiological symptoms, feeding behaviour, haemogram, serum constituents, and radiological studies were performed during the post observation period and, gross and histomorphological changes were noted on disposal of (30^{th} day) animals.

Systemic evaluation

The rabbits in both the groups were lethargic and dull on first postoperative day. The animals became alert and active by second postoperative day. The changes noticed in their general condition might have been due to stress from anaesthesia, surgical trauma and handling prior to surgery. The healing of skin wound was uneventful in all the groups.

A marginal increase in rectal temperature was observed in rabbits of group I on the first postoperative day and in group II upto second postoperative day. The rectal temperature gradually decreased from second to fifth postoperative day in group I and third to fourth day in group II and thereafter it became near normal (Fig.35). The values from third to fifth day in group II were statistically significant ($P<0.05$). The increase in rectal temperature observed initially could be attributed to the presence of inflammation following surgical trauma. The observations are in agreement with Rameshkumar (1993) on experimental cystoplasty in buffalo calves.

There was increase in pulse rate on the first day postoperatively in both the groups and thereafter it remained at lowered level in group I, but it persisted at elevated level throughout the observation period in group II (Fig.35). However the elevated levels were within normal range. The observations on pulse rate are in agreement with Shivaprakash *et al,* (1991a) on experimental urinary bladder

reconstruction in goats and Rameshkumar (1993) on experimental cystoplasty in buffalo calves.

After a marginal increase upto second postoperative day gradual decrease in respiration rate was observed in animals of group I and later it returned to preoperative level. In group II the respiration rate was seen decreased throughout the postoperative observation period (Fig.35). However the rate was within normal range. Rameshkumar (1993) noticed elevation of respiration rate in buffalo calves on second postoperative day after cystoplasty and it agrees with the observation in group I.

In all the animals, reduction in feed intake and, water consumption was noticed on the first postoperative day. They resumed normal feeding and water intake from second postoperative day.

The change in feeding behaviour noted on the very next day of the surgical procedure might have been due to the stress from anaesthesia, surgical trauma and handling prior to surgery.

In all the experimental animals, haemoglobin concentration showed a significant ($P<0.05$) decrease on seventh day postoperatively followed by a progressive increase on fifteenth day which was significant in group II animals (Fig.36).

The erythrocyte sedimentation rate was not seen altered in both the groups (Fig.36).

A significant ($P<0.05$) reduction in PCV was noticed in both the groups on seventh day postoperatively which showed gradual but significant ($P<0.05$) rise on fifteenth day in group I, but in group II it persisted at a lower level (Fig.36). However the values were within normal range.

A marginal decrease in TEC was observed in both the groups on seventh day postoperatively and it was significant ($P<0.05$) in group II. This was followed by gradual and proportionate rise on fifteenth day (Fig.37).

A reduction in TLC was observed in both the groups on seventh postoperative day followed by gradual and proportionate rise on fifteenth day (Fig.37). The reduction and rise in TLC on seventh and fifteenth day respectively were significant ($P<0.05$) in group II.

A significant ($P<0.05$) rise in neutrophil count was noticed in both groups on seventh day postoperatively, followed by steady fall to near normal values on fifteenth day (Fig.37).

Animals of both the groups showed reduction in lymphocyte count on seventh day postoperatively and it was significant ($P<0.05$) in group II. The lymphocyte count returned to near normal level in both the groups on fifteenth day (Fig.37).

The eosinophil, basophil and monocyte counts showed marginal variation during the period of observation in animals of both the groups (Fig.37).

The variations observed in the haemogram represent the cellular reaction consequent to surgical trauma during the healing process (Gourley and Vasseur, 1985)

An increase in blood urea nitrogen level was observed on seventh day postoperatively and it showed gradual decrease by fifteenth day in animals of both the groups (Fig.38). However the values observed during the postoperative observation period were within normal range. This is in agreement with the observations in experimental cystoplasty observed by Sharma and Khan (1978 and 1980), Gera *et al;* (1980), Rameshkumar (1993) and Sharma (1995 and 1997) in buffalo calves, Nair *et al,* (1988 a and b) in dogs and Shivaprakash *et al,* (1991 a) in

goats. The transient rise in blood urea nitrogen level in all the groups was probably due to urinary reflux following trauma to the bladder.

There was no significant variation in serum creatinine level during the postoperative period in animals of both the groups (Fig.38). Similar observations were reported by Shivaprakash *et al,* (1991 a) in goats. These observations suggest normal renal function in all the animals.

A marginal increase in serum sodium concentration was observed by seventh post operative day in animals of both the groups. The values then decreased to reach near normal by 15^{th} day (Fig.38).

A marginal increase in serum potassium concentration was noticed in animals of group I on seventh postoperative day followed by significant ($P<0.05$) decrease on 15th day. In group II the potassium level remained at lower level throughout the observation period (Fig.38). However the levels were within normal range.

Serum chloride concentration was observed at a decreased level on seventh postoperative day in animals of both the groups. Thereafter the level increased on 15th day in group I and remained at a lower level in group II (Fig.38). However the lower levels were within normal range.

The marginal variation observed in serum electrolytes during the postoperative period might be attributed to surgical stress and these observations were in agreement with the observations of Prasad *et al,* (1973) and Rameshkumar (1993) in buffalo calves and, Shivaprakash *et al,* (1991a) in goats.

A decrease in total serum protein content by seventh day postoperatively followed by an increase on 15^{th} day was noticed in animals of group I and group II (Fig.38). The initial decrease observed in total serum protein content might be

attributed to the relative protein deficit associated with surgical stress (Carlson, 1997)

The serum albumin content was decreased by seventh day postoperatively followed by an increase to reach near normal value by 15th day in animals of both the groups (Fig.38). The reduction in serum albumin content is in agreement with the systemic changes following trauma in general (Zaslow, 1984)

The observations on general condition, feeding behaviour, physiological symptoms, haematological studies and biochemical evaluation during the postoperative period suggest that both the graft materials and the surgical technique adopted in the present study for cystoplasty produce minimal systemic effects and the clincial symptoms manifested were not alarming or untoward.

Structural and Functional evaluation

Intravenous pylographic studies during postoperative period showed normal passage of urine from renal level and normal filling of reconstructed bladder in the experimental animals. Signs of cystitis, seepage of contents and adhesion to surrounding structures and clinically significant alterations like dilatation and ampulla formation were not observed in any of the animals, in the present study.

In both the groups the time lapse of five minutes after release of abdominal compression during intravenous pyelography was adequate for filling of urinary bladder with urine and contrast medium. The concentration of contrast medium used was sufficient for consistant opacification of urinary bladder.

Moderate fibrinous adhesions were noticed between grafted site and adjacent tissues like omental folds, omental fat and mensentry in animals of both the groups. The area of reconstructed bladder was indistinguishable in group II animals. The mucosal continuity was not complete at the centre portion as evidenced by the

presence of relative thinness of the tissue. Mild corded thickening of the mucosa bordering the central portion of grafted area was also observed. Mild fibrinous adhesion around the serosal aspect of bladder on 30th day have been reported in experimental cystoplasty in buffalo calves (Sharma, 1995 and1997a).

On 30th day the mucosal proliferation was almost complete in both the groups. Progressive neovascularisation and inflammatory reaction were noticed around the grafted area and these reactions were much greater in group II. The implanted collagen material was not evident in both the groups. Acetylated collagen films were reported to evoke much greater inflammatory cell response (Srivastava *et.al.,* 1990).

Moderate deposition of collagen and mild fibroplasia were also noticed in the present experiment. Nandi *et al,* (1995) and Sharma (1997a) noticed cellular reactions and healing pattern following cystoplasty in goats and buffalo calves respectively similar to these observations in the present study.

The structural and functional evaluation of the experimentally grafted material in the present study suggest that,

i) it is readily accepted,

ii) it causes very little adverse tissue reaction, and

iii) healing is satisfactory and anatomical and functional integrity of urinary bladder could be well established by the procedure.

CLINICAL STUDY

Cystoplasty in dogs

Cystoplasty using processed canine collagen sheets (in three dogs) and fish collagen sheets (in three dogs) were performed for urinary bladder reconstruction. The rent on the bladder was reconstructed after fixing the collagen sheets to the edges of bladder using 3-0 cat gut in through and through horizontal mattress sutures. An indwelling polythene catheter (5-french) was placed into the bladder to facilitate urine outflow. The abdominal wound was closed in routine manner. The presence of catheter in the bladder was helpful for drainage of urine for the first day after surgery. Thereafter the dogs did not tolerate indwelling catheter.

The dogs were observed for a period of 15 days after operation and maintained on intravenous fluids, milk and bread upto fourth day followed by regular diet from fifth day. Antibiotics were administered as postoperative therapy. The skin sutures were removed by 7-10 day postoperatively after complete healing of abdominal wound. Observation on general condition, feeding behaviour, urinalysis, urination, physiological symptoms, haemogram, serum constituents, and radiological studies were conducted in all the dogs.

Systemic evaluation

The dogs were dull and depressed on the first postoperative day and most of the time, the animals were assuming a recumbent position. Animals gradually became active from second postoperative day. Depression was reported following "cup patch" ileocystoplasty for urinary bladder reconstruction in a dog. (Schwarz *et al.*,1991) and in goats (Nandi *et al.*, 1995).

The dogs started feeding on milk from first postoperative day and gradually attained normal appetite by fourth day. Thereafter the dogs started feeding on rice gruel and maintained normal feeding habits throughout the period of observation.

The dogs voided blood tinged urine for two to three days following bladder reconstruction and the urine colour returned to normal by third - fourth day. Similar observations were made by Gera *et al;* (1980) and Sharma and Khan (1978) in buffalo calves and Shivaprakash et al. (1991) in goats.

The pH of urine was slightly alkaline for first two days after surgery in all the dogs and the pH became normal thereafter. The urine samples in all the dogs were positive for presence of occult blood for the first two days after surgery and thereafter the samples became normal during the observation period. Urine was positive for protein in all the dogs for first two days and negative during the remaining period of observation. Microscopic examination of urine in all dogs revealed granular casts, phosphate crystals, epithelial cells, red blood cells and white blood cells during the postoperative period.

Straining while urination was noticed on first day in all the dogs after the surgery as the catheter was not in position in most of the dogs. Straining ceased gradually by second postoperative day. This is in contrast with the studies in man where intermittent catheterization has been a necessary postoperative measure (Taguchi et al, 1977). However animals took more time to urinate during the immediate postoperative days. This is in agreement with observations made by Shivaprakash *et al*, (1991) and Nandi *et al*, (1995) in goats. Diminished tone of bladder in the early postsurgical period and lack of stretching ability in the grafted segment of bladder must have been responsible for this (Goldwasser and Webster, 1986).

A marginal increase in rectal temperature was noticed on first postoperative day and there was gradual reduction to reach near normal level in all the dogs by seventh day.

There was significant ($P<0.05$) increase in pulse rate from first postoperative day throughout the observation period and the rate showed gradual and progressive decrease to near normal by seventh day. However the variations were within normal range.

A marginal increase in respiration rate was noticed on first postoperative day and it attained preanaesthetic level by second postoperative day and maintained at this level during the observation period.

In all the dogs a reduction in haemoglobin concentration was observed during the post surgical observation period .

The erythrocyte sedimentation rate did not show any variation in dogs.

The packed cell volume was elevated marginally in all the animals on third day postoperatively and it became near normal by 15th day.

There was marginal rise in TEC, upto seventh postoperative day in all the animals and the value became near normal by fifteenth day.

Animals showed a significant increase in TLC on third postoperative day and it gradually returned to normal value by fifteenth day

The neutrophil count showed a significant increase on third postoperative day followed by significant ($P<0.05$) decrease on seventh day. Thereafter the value decreased gradually to normal by fifteenth day.

The lymphocyte count showed significant reduction on third postoperative day followed by significant ($P<0.05$) rise on seventh day and it became near normal by fifteenth day.

The eosinophil, basophil and monocyte counts showed marginal and insignificant variations during the post surgical observation period in all the dogs.

The marginal variations observed in haemogram might be due to cellular reaction to surgical trauma during the healing process(Gourley and Vasseur ,1985) and the initial reaction to the implant(Mohanty,1995)

A significant ($P<0.05$) increase in blood urea nitrogen was observed on third postoperative day in all the dogs followed by significant ($P<0.05$) decrease on seventh day. The value gradually returned to near normal by fifteenth day. However the elevated BUN levels were within normal range. Schwarz *et.al*(1991) had also observed elevated but normal level of blood urea nitrogen following "cup patch" ileocystoplasty in a dog.

There was no significant change in serum creatinine levels during the postoperative period.

Serum sodium concentration showed significant ($P<0.05$) elevation during the postoperative observation period and the values became normal level by fifteenth day.

A marginal increase in serum potassium concentration was noticed in all the dogs during the early postoperative period with significant ($P<0.05$) increase on seventh day and the level reached normal by fifteenth day.

The serum chloride concentration showed significant ($P<0.05$) fall on third postoperative day and the levels were returning to near normal by fifteenth day and the rise in chloride concentration was significant at seventh day.

The marginal variation in serum electrolytes during the postoperative period could be attributed to surgical stress and restricted water intake during the early

postoperative period. The persistent elevation in serum sodium and potassium did not appear alarming as the values remained well within normal range.

A significant ($P<0.05$) decrease in serum protein content was observed in third and seventh day postoperatively and the level was returning to normal by fifteenth day. The initial decrease observed in total serum protein content might be attributed to relative protein deficit associated with surgical stress (Carlson, 1997)

A marginal increase in serum albumin content was noticed up to seventh postoperative day and the albumin content returned to near normal level by fifteenth day.

The observations on general condition, feeding behaviour, urinalysis, urination physiological symptoms, haematological studies and biochemical studies during postoperative period reveal that both the collagen sheets did not evoke any serious systemic response in dogs.

Functional evaluation

Observations on retrograde cystography during postoperative period revealed normal contour and moderate filling capacity of reconstructed bladder. The countour of the bladder was squared off at its apex and the bladder wall thickness was normal with smooth mucosal surface.

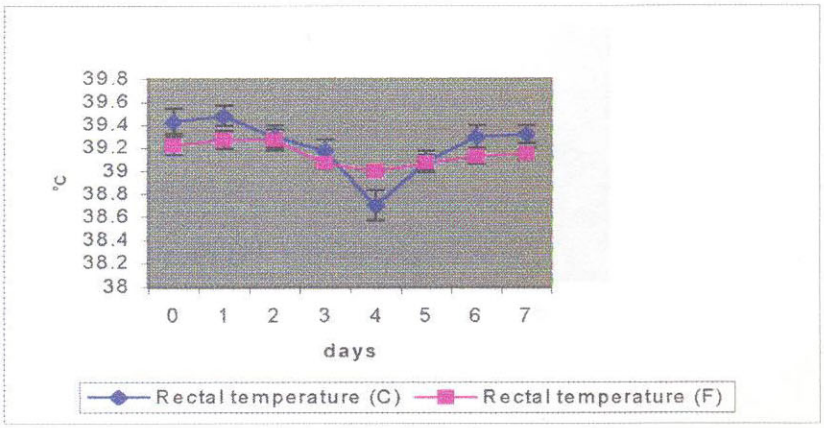
Both the collagen sheets were well tolerated and accepted in clinical studies, considering its normal alteration in physiological, haematological, biochemical and radiological observations.

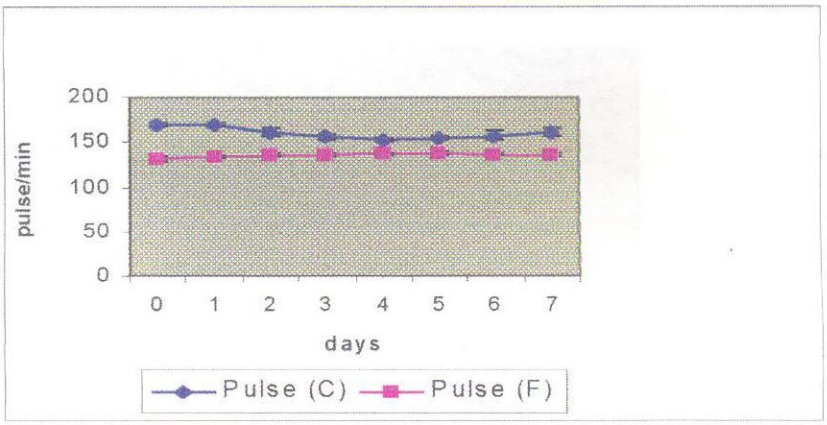
These observations establish and substantiate the properties of collagen as an excellent scaffold for promoting cellular infiltration, new vascularization, tissue integration and fibrinogenesis. Cross linking of collagen by gluteraldehyde or

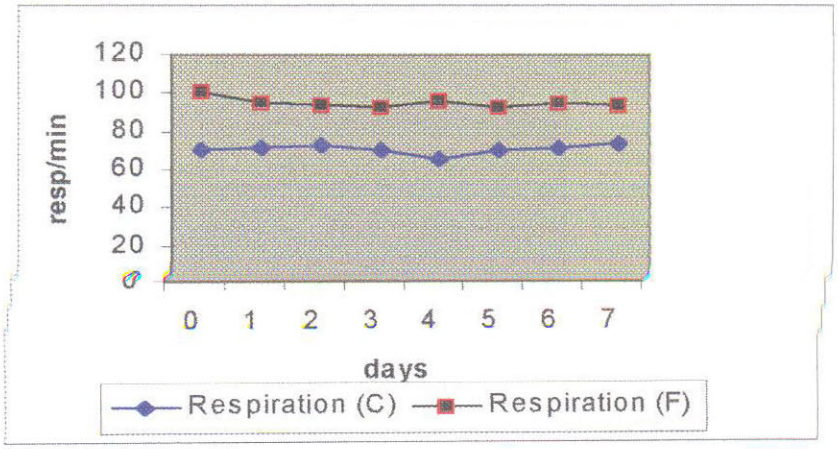
diacetyl produced strengthening of aminoacid bonds and reduced its antigenicity and, provided an ideal, biomaterial for reconstruction of bladder. These observations on experimental and clinical evaluation indicated that it satisfied all the qualities of biomaterial as suggested by Taylor (1982)

The qualities of collagen sheets and their compatibility as a graft or substitute in a versatile environment of body system in this study open up new avenues for their wide application in clinical condition of animals. This can provide a cheap, easily available material over the synthetic ones and it ensures better utilization of the presently wasted animal tissues and fish air bladder which are rich in collagen.

Figure 35: Comparison of rectal temperature, pulse rate and respiration rate after cystoplasty using collagen sheets in rabbits.

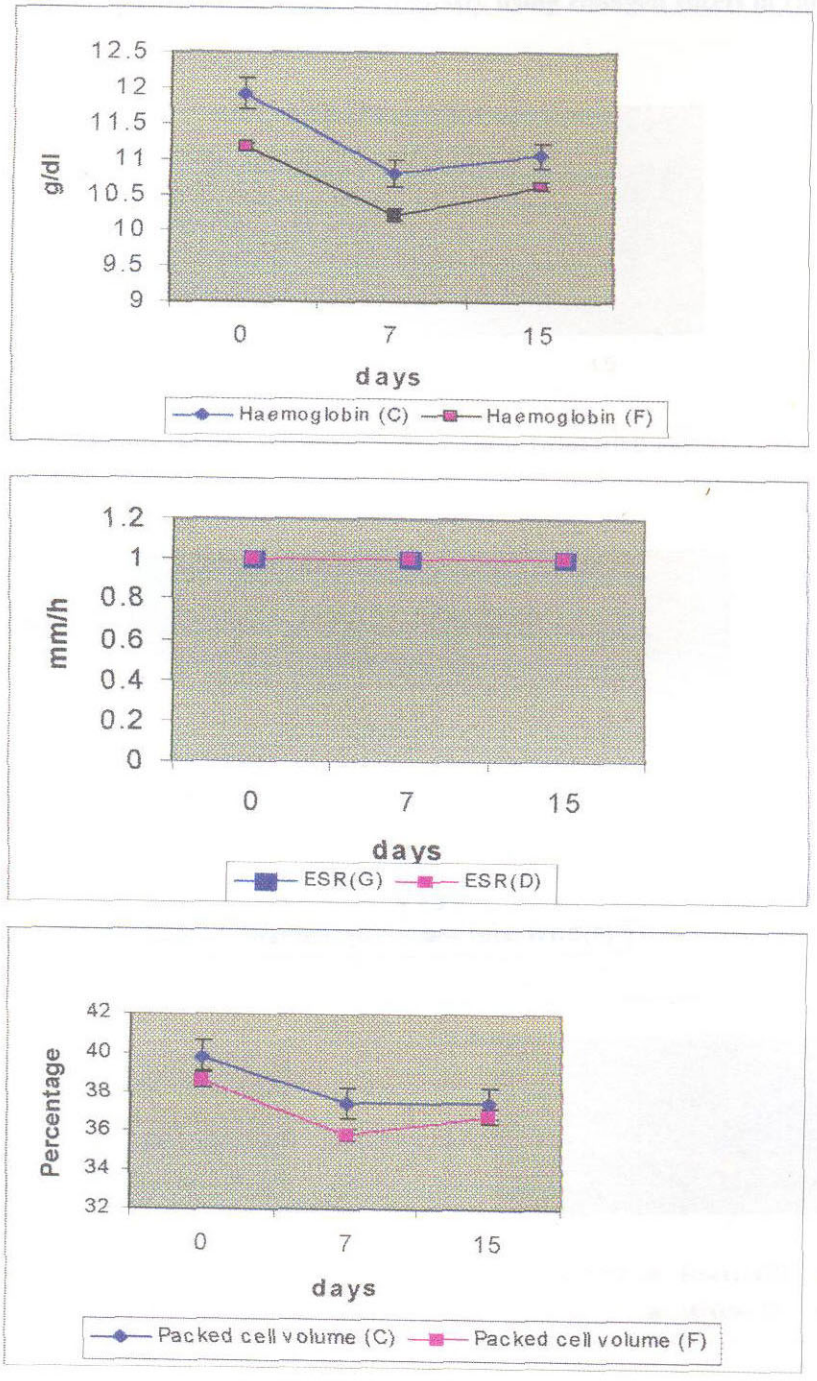






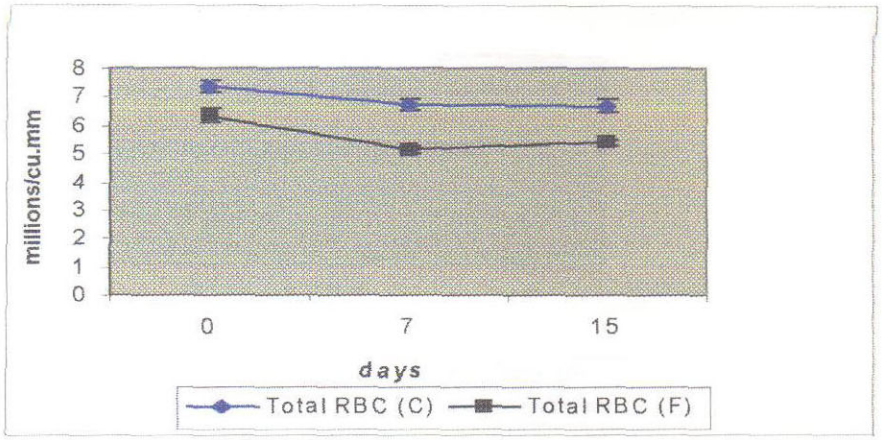
C= Canine collagen; F= Fish collagen

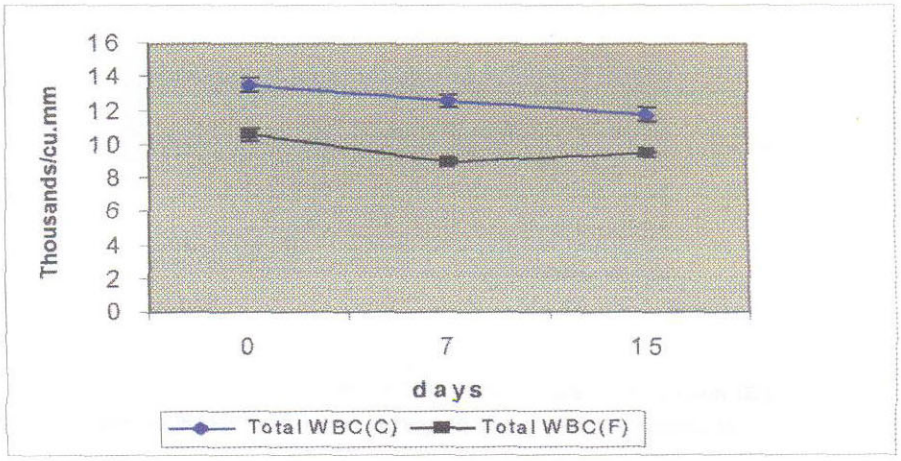
Figure 36 :Comparison of haemoglobin erythrocyte sedimentation rate and packed cell volume after cystoplasty using collagen sheets in rabbits.

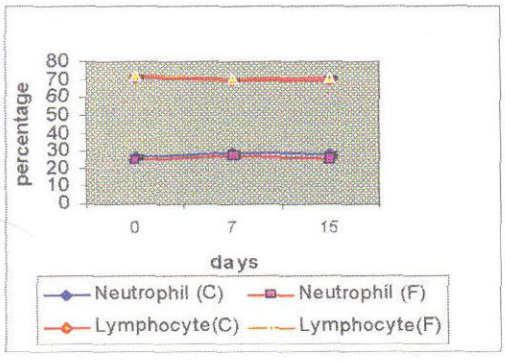


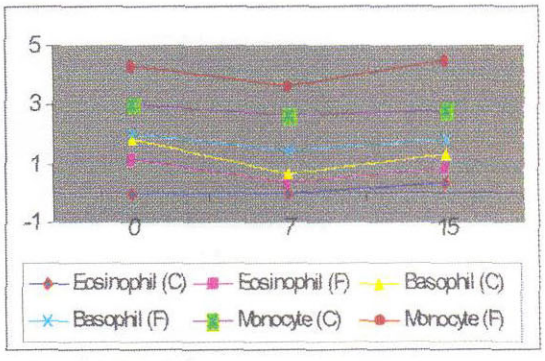
C= Canine collagen; F= Fish collagen

Figure 37 : Comparison of total erythrocyte count, total leucocyte count and differential leucocyte count after cystoplasty using collagen sheets in rabbits.



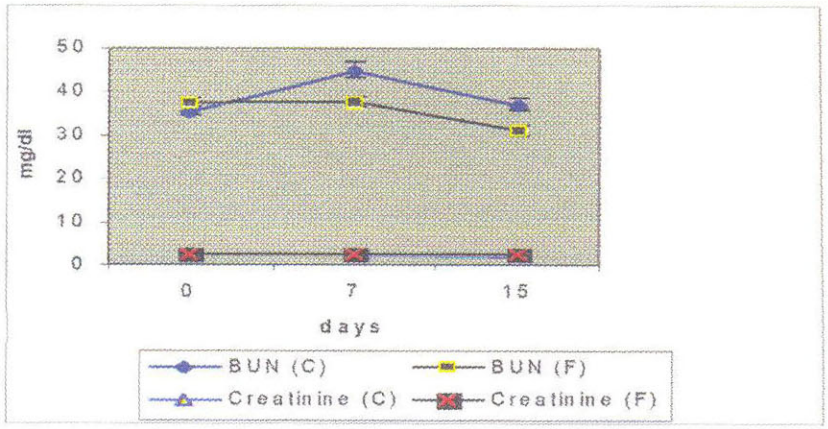


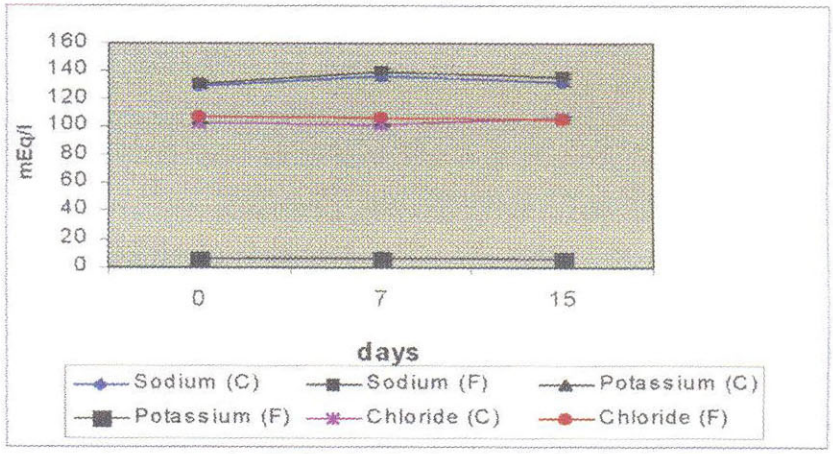


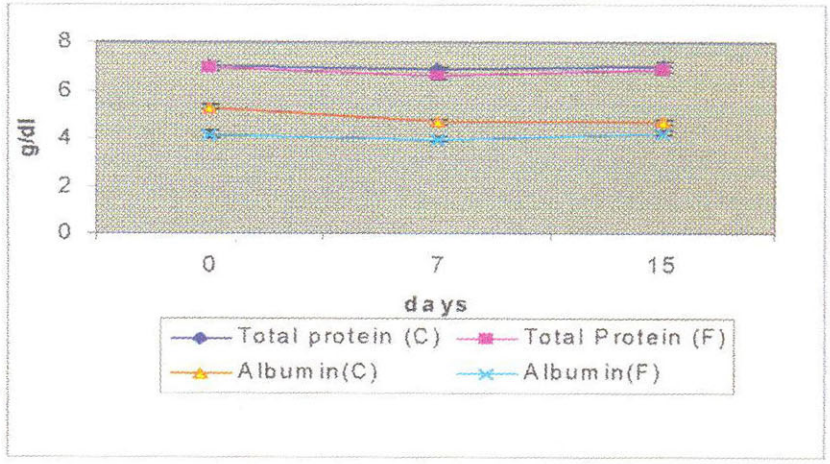


C= Canine collagen; F= Fish collagen

Figure 38 : Comparison of serum constituents after cystoplasty using collagen sheets in rabbits.







C= Canine collagen; F= Fish collagen

Summary

CHAPTER – VI

SUMMARY

The experimental study was conducted in twelve apparently healthy adult New Zealand White rabbits of either sex weighing 2.1 – 3 kg, divided into two groups I and II., with six animals in each group.

Animals were subjected to partial cystectomy and in group I cystoplasty was done using glutaraldehyde processed canine collagen sheet and in group II cystoplasty was done using diacetyl processed fish collagen sheet.

The clinical study (group III) was conducted in clinical cases in six male dogs in which bladder defects were corrected by cystoplasty using processed canine collagen sheet and fish collagen sheet.

Tissue samples of urinary bladder harvested from died dogs were made into sheets, processed and crosslinked with glutaraldehyde at the Central Leather Research Institute, Adayar, Chennai. Fish air bladders collected from larger fresh water fishes were made into sheets, processed and crosslinked with diacetyl at Central Institute of Fisheries Technology, Cochin.They were sterilised by gamma irradiation.

All the rabbits were premedicated with atropine sulphate and xylazine hydrochloride and anaesthetized using ketamine hydrochloride.

A portion of bladder wall (2 cm^2) close to the vertex was removed from dorsal aspect of fundus and the full thickness defect was covered by cystoplasty using collagen sheet. The graft material was fixed by 5-0 braided silk with overlapping continuous horizontal mattress sutures. The collagen sheet was beneficial for effective haemostasis at the surgically created defects.

The rabbits were kept under observation for a minimum period of one month.

The animals of both the groups were lethargic and dull on the first postoperative day and active and alert from second day.

A marginal increase in rectal temperature, pulse rate and gradual decrease in respiration rate were observed during early postoperative period in all experimental animals.

A reduction in feed and grass intake, and water consumption was noticed only on the first postoperative day in the animals.

Marginal reduction in haemoglobin concentration, packed cell volume, total erythrocyte count, and total leukocyte count were observed on seventh postoperative day in haemogram.

Differential leukocyte count showed neutrophilia in both the groups and decrease in lymphocyte count on seventh postoperative day. Eosinophil, basophil and monocyte counts showed marginal variations during the period of observation.

During the early period of observation an increase in blood urea nitrogen level was noticed in both the groups. Serum creatinine level did not show any variation. A marginal increase in serum sodium and potassium and decrease in chloride concentration, total protein and albumin were noticed during the period of observation in both the groups.

Intravenous pyelogram during postoperative period showed normal passage of urine from renal level and normal filling of reconstructed bladder in all the animals. Signs of cystitis, seepage and adhesions were not noticed.

Gross morphological changes were not observed externally on the bladder or on the mucosa. Moderate fibrinous adhesions were noticed between grafted site and adjacent tissues, but mild corded thickening of mucosa bordering the central portion of grafted area was observed.

Histomorphological studies by 30th day revealed almost complete proliferation of mucosa with progressive neovascularisation and inflammatory reaction in both the groups. The proliferation of blood vessels and inflammatory reactions were much greater in group II.

In clinical study in the dogs, cystoplasty was done using canine and fish collagen sheets. The fish collagen sheets were comparatively thicker than canine collagen sheet.

The dogs were kept under observation for a period of 15 days postoperatively.

The dogs started feeding on milk from first postoperative day and gradually attained normal appetite by fourth day. Thereafter the dogs began feeding on regular diet and maintained normal habits throughout the observation period.

The dogs passed blood tinged urine for two to three days postoperatively. Urine pH was slightly alkaline for first two days after surgery in all the dogs. The urine samples were positive for the presence of protein for first two days. Microscopically urine sample revealed granular casts, phosphate crystals, epithelial cells, red blood cells and white blood cells during the observation period.

Straining while urination was observed for the first day in all the dogs but it ceased from second day.

A marginal increase in rectal temperature, pulse rate and respiration rate were observed during early postoperative period in all the dogs.

A marginal decrease in haemoglobin concentration and increase in packed cell volume, total erythrocyte count, total lymphocyte count were the salient features of haemogram during early postoperative period.

Differential leukocyte count showed mild neutrophilia in all the dogs. A decrease in lymphocyte count was noticed during the early period of observation. The eosinophil, basophil and monocyte count showed marginal variation during the period of observation in all the dogs.

During the early period of observation there was significant increase in blood urea nitrogen but variation in serum creatinine level was not significant in all the dogs.

Marginal elevation in serum sodium and potassium concentration and marginal fall in chloride concentration during the early period of observation was noticed in all the dogs.

A marginal decrease in total serum protein and increase in serum albumin was noticed in early period of observation. All the dogs regained near normalcy by 15^{th} day postoperatively.

Retrograde cystogram during the postoperative period revealed normal contour and moderate filling capacity of reconstructed bladder.

The following conclusions could be drawn from this study:

(1) The collagen sheets were proved beneficial for haemostatic effect on diffuse bleeding during the surgical implantation

(2) The glutaraldehyde crosslinked canine collagen sheets and diacetyl cross linked fish collagen sheets satisfied the requirement as a graft material for cystoplasty in rabbits and dogs

(3) The proliferative changes observed was minimum in the vicinity of the graft material.

(4) The graft material acted as a better scaffold for triggering cellular and tissue ingrowth similar to that of normal first intention healing.

(5) The time taken for acceptance of graft material was minimum and similar to that of a viable graft as evidenced by the physiological, haematological and biochemical changes.

(6) The healing at the cystoplasty site with either of the collagen sheet did not vary considerably and the graft materials were indistinguishable on gross examinations, when healing was complete.

(7) Survival of the graft material was not affected in the versatile environment of urinary bladder, and hence both the materials could be recommended for cystoplasty in dogs.

References

REFERENCES

Al- Khateeb, T.L., H.A. Marouf and M.M. Anwar. (1996). Modified bovine type I collagen membrane as a wound graft material in oral surgery (an experimental study). *J. Ir. Dent. Assoc.* **42** (3) : 46-50

Arconti, E.N. (1962). Cited by Kudale, M.L. and S.R. Hattangady. (1971). An intestinal pedicle for correcting defects of the wall of urinary bladder in dogs. *Indian Vet.J.* **48** (10): 1064-1069.

Balagoplan T.P. (1998). Processed aortic allografts for oesophagoplasty in dogs. PhD Thesis submitted to Kerala Agricultural University. pp 100-126

Bancroft, J.D. and H.C. Cook. (1984). Manual of Histological Techniques. Churchil Livingstone, Edinburgh, London. 1st ed. pp. 18-20, 39-41, 56-58

Barness, R.W., W.M. Wilson, R.T. Bergman, S.E. Farley and H.L. Hadley (1953). Cited by Kudale, M.L. and S.R. Hattangady. (1971). An intestinal pedicle for correcting defects of the wall of urinary bladder in dogs. *Indian Vet.J.* **48** (10) : 1065-1069

Blumenthal, N.M. (1988). The use of collgen membrane to guide regeneration of new connective tissue attachment in dogs. *J. Periodontol.* **59** (12) : 830-836

Carlson, G.P.(1997). Fluid, electrolyte and acid-base balance. In Clinical Biochemistry of Domestic Animals. Ed. J.J. Kaneko., J.M. Harvey and M.L. Bruss. Academic Press, London. 5th ed. pp. 124-132

Chvapil, M., R.Kronenthal and Van Wrinkle. (1973). Medical and surgical application of collagen. *Int. Rev. Connect. Tissue. Res.* **6**(1): 61. Cited by

Simpson, R.L. (1983). Biomaterials in Reconstructive Surgery. The C.V. Mosby Company, London. 2nd ed. p.110

Chvapil, M., T.A. Chvapil and J.A. Owen. (1987). Cited by St.Jean, K.Y., E. Gaughan., G. St. Jean and R.Frank. (1995). Evaluation of porous bovine collagen membrane bandage for management of wounds in horses. *Am. J. Vet. Res.* **56** (12): 1663-1667

Chvapil, M., H.Holubec and T.A. Chvapil. (1991). Cited by St.Jean, K.Y., E. Gaughan., G. St.Jean and R.Frank. (1995). Evaluation of porous bovine collagen membrane bandage for management of wounds in horses. *Am. J. Vet. Res.* **56** (12): 1663-1667

Feinberg, S.E. and E.J. Mc Donnell. (1995). The use of a collagen sheet as a disc replacement in the rabbit. *J.Oral Maxillo fac. Surg.* **53** (5) : 535-542

Frankl and A.L.(1986). Use of porcine dermal collgen in the repair of perineal hernia in dogs- a preliminary report. *Vet. Rec.* **119**(1):13-14

Gao, Z.R., Z. Q.Hao and Y.Li (1992). Cited by St.Jean, K.Y., E. Gaughan., G. St.Jean and R.Frank. (1995). Evaluation of porous bovine collagen membrane bandage for management of wounds in horses. *Am. J. Vet. Res.* **56** (12): 1663-1667

Gentry, P.A., M.D. Schneider and J.K. Miller. (1981). Plasmaclot – Promoting effect of collagen in gelatin to collagen- platelet interaction . *Am. J. Vet . Res.* **42**:708-715

Gera, K.L., J.M. Nigam and R.P.S. Tyagi. (1980). Biochemical changes following transplantation of preserved bladder allografts in buffalo calves. *Indian Vet. J.* **57** (1): 67-72

Goldwasser,B. and G.D. Webster. (1986). Augmentation and substitution entero-cystoplasty. Review article. *J. Urol* **135**: 215-224.

Gourley, I.M. and P.B. Vasseur. (1985). General Small Animal Surgery. J.B. Lippincott Co., Philadelphia. pp. 241-251

Gupta, R.L., R.K. Jain, M. Kumar, V.K. Prathap, K.J . Scaria and M. Snatappa. (1978). Fate of collagen sheet cover for artificially treated raw areas : An experimental study . *Indian .J .Surg.* **40**:641-645

Hammer, J.M., P.H. Seay, E.J. Hill and F.W. Prust (1953). Cited by Kudale, M.L. and S.R. Hattangady. (1971). An intestinal pedicle for correcting defects of the wall of urinary bladder in dogs. *Indian Vet.J.* **48** (10): 1064-1069

Humason, G. L. (1979). Animal tissue techniques. WH Freeman & Co. Sanfancisco. 4th ed. p. 129

Inchiosa, M.A. (1964). Direct biuret determination of total protein in tissue homogenates. *J. Lab. Clino. Med.* **63**: 319-324

Jain,N.C. (1986). Schalm's Veterinary Haematology, Lea and Febiger, Philadelphia. 4th ed. pp. 35-44, 53-56

Jayakrishnan, A. and S.R. Jameela. (1996). Glutaraldehyde as a fixative in bioprosthesis and drug delivery matrices. *Biomaterials*. **17** (5): 471 – 584

Kudale, M.L. and S.R. Hattangady. (1971). An intestinal pedicle for correcting defects of the wall of urinary bladder in dogs. *Indian Vet.J* . **48**(10): 1065-1069

Marcowitz, J., J.Archibald and H.G .Downie. (1959). Experimental Surgery. Williams & Wilkins Co. Baltimore. 4th ed. pp.180-181

Mian, M., R. Aloisi, and D.Bernetti. (1992). Cited by St.Jean, K.Y., E. Gaughan., G. St.Jean and R.Frank. (1995). Evaluation of porous bovine collagen membrane bandage for management of wounds in horses. *Am. J. Vet. Res.* **56** (12): 1663-1667

Mohanty, M. (1995). Biological evaluation of materials for medical devices. *Trends in Biomaterials and Artificial organs* .**9**(1): 2-8

Mukundan, M.K. (1992). Surgical sutures from fish gut collagen- Popular Science Lecture Series, Central Institute of Fisheries Technology, Kochi. pp. 1-7

Nair, N.R., M.R. Patel and R.P. Awadhiya. (1988a). Cystoplasty with peritoneal grafts in dogs. *Indian J. Vet. Surg.* **9**(2):82-85

Nair, N.R. M.R Patel and R.P. Awadhiya. (1988b). Cystoplasty with skin grafts in dogs. *Indian J. Vet. Surg.* 9(2):86-91

Nandi, S.K., P.K. Bose., D.K. Das.,J.Sengupta and A.K. Dutta. (1995). Preserved homogenous pericardial grafts for bladder reconstruction in goats. *Indian J.Vet. Surg.* **16** (1):50-51

Nimni, M.E. and D.T. Cheung. (1994). Pure collagen network preparation from animal tissue. Cited in Derwent Biotechnology Abstracts No: 95-02642

Portis, A.J., A.M. Elbahnasy, A.L. Shathav, A. Brewer, P. Humphrey, E.M. Mc Dougall and R.V. Claymen. (1998). Laparoscopic augmentation cystoplasty with different biodegradable grafts in an animal model. *J. Urol.* **159** (3) : 998-1005

Prasad, B. and R.P.S. Tyagi. (1980). Experimental cystoplasty in dogs using formalin preserved buffalo duramatter. *Zol. Vet. Med.* **27A** (6):479-484

Prasad, B., R.P.S. Tyagi and V. Ramakumar. (1973). Caecocystoplasty in buffalo calves . *Indian Vet .J.* **50** :1046

Rameshkumar, B. (1993). Comparative studies on the use of different grafts for experimental cystoplasty in buffalo calves. Thesis abstract cited in *Indian J.Vet. Surg.* **16** (1): 73.

Roe, S.C., B.K. Milthorpe and K.Schindhelm. (1990). Collagen cross-linking and resorption: effect of glutaraldehyde concentration. *Artif. Organs.* **14** (6): 443 – 448

Ruijgrok, J.M.,J.R.Dewijn and M.E. Boon. (1994). Optimizing glutaraldehyde crosslinking of collagen – effects of time, temperature and concentration as measured by shrinkage temperature. *J.mater. Sci. Mater. Med.* **5**:80-87

Sambandam, C.H.T. (1992). Studies on cystoplasty using chromicised collagen sheet and chromicised calf duramater in dogs. M.V.Sc. thesis submitted to Tamil Nadu Veterinary & Animal Sciences University. pp 70-81

Santillan, D.P., V.R. Jasso, V.A. Sotress, R.Olmes, J.L.Arreola, D.Garcia, B. Vanda and M. Gaxiola. (1995). Repair of thoraco abdominal wall defects in dogs using a bovine pericardium prosthesis. *Rev. Invest. Clin.* **47** (6) : 439-446

Sarmah,B.D., and R.T.J. Holl –Allen. (1984). Porcine dermal collagen repair of incisional herniae. *Br.J. Surg.* **71** (7): 524-525

Sastry, T.P. (1989). Development and evaluation of collagen based biomaterials. Ph.D. Thesis submitted to Madras University. pp 97-109

Sawyer, P.N., J. Fitzgerald and M.J. Kaplitt. (1987). Ten year experience with the negatively charged glutaraldehyde tanned vascular graft in peripheral vascular surgery. Initial multicenter trial.*Am.J.Surg.* **154** : 533-537

Schalm, C.W. (1975). Veterinary Haematology, Lea and Febiger, Philadelphia, 3rd ed. pp. 39-40, 52-66

Schwarz, P.D., E.L. Egger and S.E. Klause. (1991). Modified "cup patch" ileocystoplasty for urinary bladder reconstruction in a dog. *J. Am. Vet.Med. Ass.* **198** (2): 273 – 277

Sharma, S.P. (1995). Cystoplasty using formalin preserved urinary bladder grafts in buffalo calves. *Indian J. Vet. Surg.* **16** (1): 44-46

Sharma, S.P. (1997a). Urinary bladder reconstruction using the intact caecum : An experimental study. *Indian Vet.J.* **74** (1): 51-53

Sharma, S.P. (1997 b). Subtotal cystectomy and prosthetic bladder replacement in buffalo calves. *Indian Vet.J.* **74** (1): 65-67

Sharma, S.P. and A.A. Khan. (1978). Studies on cystoplasty for partial substitution of the urinary bladder in buffalo calves. *Indian Vet .J.* **55**:326-332

Sharma, S.P. and A.A. Khan. (1980). Studies on caecocystoplasty with "sheet graft" technique in buffalo calves. *Indian . J. Anim. Hth* **19**: 35-38

Shetty, B.R., G.R. Singh, J.M. Nigam and A.P. Singh. (1982). Histopathological observations following transplantation of fresh and treated bovine arterial xenografts in canine. *Indian J. Anim. Sci.* **52** (6): 422-428

Shivaprakash, B.V., G.R. Singh, C.S. Celly and D. Swarup. (1991a). Experimental evaluation of bladder reconstruction using polytetrafluoroethylene, caecal pedicle, fresh autogenous and preserved allogenic bladder grafts. *Indian J. Vet. Surg.* **12** (1): 12 – 16

Shivaprakash, B.V., G.R. Singh and Kali-Charan. (1991b). Pathomorphological changes following cystoplasty using polytetrafluoroethylene, caecal

pedicle, fresh autogenous and frozen allogenic bladder grafts in goats. *Indian J.Anim. Sci.* **61** (12): 1297-1299

Simpson, R.L. (1983). Collagen as a biomaterial. Cited by Rubin, L.R. (1983). Biomaterials in reconstructive surgery. C.V. Mosby Company, London. pp.109-117

Snedecor, G.W. and W.G. Cochran (1967). Statistical methods. Oxford and IBH Publishing Co., Calcutta, 6th ed. p. 59

Sreenu,M., T.P. Sastry, O.Ramakrishna, G.V. Lakshmipathi and T.S. Rao. (1998). Cystoplasty using chromic sulphate and glutaraldehyde crosslinked allogenic bladder grafts in dogs: gross and histopathologic changes. *Indian J.Vet. Surg.* **19** (1): 35-36

Srivastava, S., S.D. Gorham, D.A. French, A.A. Shivas and J.M. Courtney. (1990). In vivo evaluation and comparison of collagen, acetylated collagen and collagen/ glycosaminoglycan composite films and sponges as candidate biomaterials. *Biomaterials.* **11** (3):155-161

St.Jean, K.Y., E. Gaughan., G. St.Jean and R.Frank. (1995). Evaluation of porous bovine collagen membrane bandage for management of wounds in horses. *Am. J. Vet. Res.* **56** (12): 1663-1667

Taguchi, H., E. Schizuka and K. Saito. (1977). Cystoplasty by regeneration of the bladder *J. Urol.* **52**: 752-756

Taylor, D . E . M. (1982). Biomaterials for reconstructive surgery. In Reconstructive procedures in surgery. Ed. Bevan, P.G.. Blackwell Scientific Publications , London . pp.43-63

Tizzoni, G. and A. Foggi. (1988). Cited by Sharma , S.P. and A.A. Khan. (1980). Studies on caecocystoplasty with "sheet graft" technique in buffalo calves. *Indian . J. Anim. Hth* **19**: 35-38

Tsuji, I., K. Kuroda, J. Fujieda, Y.Shiaishi, K. Kunishima and S.Orikasa. (1967). Cited by Sharma S.P. (1997b). Subtotal cystectomy and prosthetic bladder replacement in buffalo calves. *Indian Vet. J.* **74** (1): 65-67

Turley, H.G. (1958). Cited by O' Flaherty, F., W.T.Roddy and R.M. Lollar. (1958). In Chemistry and Technology of Leather. Vol.I, Rehind Publishing Corporation New York, pp. 388-425.

Zaslow, I.M.(1984). Veterinary Trauma and Critical Care. Lea and Febiger, Philadelphia. 1st ed. pp. 33-37

PROCESSED CANINE AND FISH COLLAGEN SHEETS FOR CYSTOPLASTY IN RABBITS AND DOGS

By
C.B. DEVANAND

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

Doctor of Philosophy

FACULTY OF VETERINARY AND ANIMAL SCIENCES
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2002

ABSTRACT

The study was conducted with the objective of evaluating the host acceptability and healing of processed collagen sheets of animal and aquatic tissue for reconstruction of experimentally created urinary bladder defects in rabbits and for reconstruction of urinary bladder in clinical cases in dogs.

The study was conducted in twelve apparently healthy adult New Zealand White rabbits of either sex weighing 2.1 – 3 kg, divided into two groups, I and II., of six animals each and in six male dogs (group III).

The rabbits were subjected to partial cystectomy followed by cystoplasty using glutaraldehyde processed canine collagen sheet in group I, diacetyl processed fish collagen sheet in group II and in clinical cases in dogs, cystoplasty using both the materials.

Tissue samples of urinary bladder harvested from died dogs were made into sheets, processed and crosslinked with glutaraldehyde at Central Leather Research Institute, Adayar, Chennai. Fish air bladder collected from larger fresh water fishes were made into sheets, processed and crosslinked with diacetyl at Central Institute of Fisheries Technology, Cochin. The collagen sheets were sterilised with gamma irradiation. The fish collagen sheet was thicker than canine collagen sheet.

All the rabbits were premedicated with atropine sulphate and xylazine hydrochloride and were anaesthetized using ketamine hydrochloride.

In the rabbits a portion of bladder wall (2 cm^2) was removed from dorsal aspect of fundus close to the vertex. The graft material was fixed at the defects, using 5-0 braided silk in overlapping continuous horizontal mattress sutures. The collagen sheet effected haemostasis at the bleeding points of surgically created defects.

The rabbits of both the groups were kept under observation for a minimum period of one month.

The animals of both the groups were lethargic and dull on the first postoperative day and active and alert from second day.

A marginal increase in rectal temperature, pulse rate and gradual decrease in respiration rate were observed during early postoperative period in all the animals.

A reduction in feed and grass intake, and water consumption was noticed on the first postoperative day in all the animals and normal feeding and water intake were resumed from second postoperative day.

Marginal reduction in haeomoglobin concentration, packed cell volume, total erythrocyte count, and total leukocyte count were observed on seventh postoperative day in haemogram.

Differential leukocyte count showed neutrophilia in both the groups and decrease in lymphocyte count on seventh postoperative day. Eosinophil, basophil and monocyte counts showed marginal variations.

During the early period of observation an increase in blood urea nitrogen was noticed in both the groups, but serum creatinine level did not show any variation. A marginal increase in serum sodium, potassium and decrease in chloride concentration, total protein and albumin were noticed during the period of observation in both the groups.

Intravenous pyelogram during postoperative period showed normal passage of urine from renal level and normal filling of reconstructed bladder in all the animals.

Gross morphological changes were not observed externally on the bladder or on the mucosa. Moderate fibrinous adhesions were noticed between grafted site and adjacent tissues, but mild corded thickening of the mucosa bordering the central portion of grafted area was observed .

Histomorphological studies by 30th day revealed almost complete proliferation of mucosa with progressive neovascularisation and inflammatory reaction in both the groups. The proliferation of blood vessels and inflammatory reactions were much greater in group II.

In clinical study in the dogs cystoplasty was done using canine and fish collagen sheets.

All the dogs were premedicated with triflupromazine hydrochloride and anaesthetized using thiopentone sodium 'to effect'. The rent on the bladder was reconstructed by fixing the graft material to the edges of the bladder using 3-0 catgut in through and through continuous horizontal mattress sutures.

The dogs were kept under observation for 15 days postoperatively.

The animals started feeding on milk from first postoperative day and gradually attained normal appetite by fourth day.

Animals passed blood tinged urine for two to three days postoperatively. Urine pH was slightly alkaline for first two days after surgery. The urine samples were positive for occult blood and protein for first two days. Microscopically urine sample revealed granular casts, phosphate crystals, epithelial cells, red blood cells and white blood cells during the observation period.

Straining while urination was observed for the first day in all the dogs but it ceased from second day.

A marginal increase in rectal temperature, pulse rate and respiration rate were observed during early postoperative period in all the dogs.

A marginal decrease in haemoglobin concentration and increase in packed cell volume, total erythrocyte count, total lymphocyte count were the salient features of haemogram during early postoperative period.

Differential leukocyte count showed mild neutrophilia in all the dogs. A decrease in lymphocyte count was noticed during the early period of observation. The eosinophil, basophil and monocyte count showed marginal variation during the period of observation.

During the early period of observation an increase in blood urea nitrogen with no variation in serum creatinine level were noticed in all the dogs.

Marginal elevation in serum sodium and potassium concentration and marginal fall in chloride concentration during the early period of observation was noticed in all the dogs.

A marginal decrease in total serum protein and increase in serum albumin was noticed in early period of observation. All the dogs regained near normalcy by 15^{th} day postoperatively.

Retrograde cystogram during the postoperative period revealed normal contour and moderate filling capacity of reconstructed bladder.