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Histologic features of harvested canine kidneys preserved in four different crystalloid solutions

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Abstract
Endless efforts are required in the investigation of the best organ preservative. Normal Saline, 5% dextrose, Darrows and Ringers' Lactate were used as preservatives with the view to investigate the prospect of kidney survival in these solutions post harvest at the Veterinary Teaching Hospital, Ahmadu Bello University-Zaria. Sixteen normal kidneys were harvested, preserved and evaluated from sixteen adult Nigerian indigenous breed of dogs. The dogs were acclimatized and conditioned for 2 weeks while presurgical evaluations were done. Kidney harvesting was performed under general anaesthesia, asepsis was observed strictly in all cases. The left kidneys were harvested for each dog through an 8 cm cranioventral midline approach under general anaesthesia. Post nephrectomy, the kidneys were flushed/perfused through the renal artery and preserved for 96 hours at 4^0C in labeled bowls (A, B C and D) containing these solutions: A-Ringers Lactate, B-Darrows, C-5% Dextroses and D-Normal Saline solutions which was incorporated 15000 iu of heparin, 5mls of 2% xylocaine, 400000 iu penicillin and 75mg streptomycin. Following preservation for 96 hours, histopathology study was undertaken. Mean pre-surgical haematological, serum chemistry and urinalysis values were within normal range. The gross appearances of the harvested kidneys post preservation were normal. The renal parenchyma for all the kidneys preserved for 72 hours were normal. At 96 hours, kidneys preserved in solutions C and D showed no histological changes. The findings revealed that Normal Saline would preserve better followed by 5% Dextrose and then Darrows and Ringers Lactate being the mild preservative.

Keywords: Canine kidney, crystalloid, histologic, preserved, transplantation.

Introduction
Several solutions including: Collins, Modified Collins, Sacks, Hyperosmolar citrate, University of Wisconsin solution (UW), Modified University of Wisconsin, Perfudex, Cryoprecipitated Plasma, Euro-Collins solutions have been used for organ preservation. A successful 72 hours cold storage of kidney with UW solution has been reported (Ploeg *et al*., 1988) and the solution has been recommended as an effective preservative and flush solution for all intra-abdominal organs used for transplantation (Ploeg *et al*., 1990). UW solution has been successfully used to preserve the liver and the pancreas (Lin *et al*., 1995), although the UW solution is considered the standard solution for preserving organ used for transplantation, the presence of the colloid compound; hydroxyethylstarch (HES) makes it controversial (Schlumpf *et al*., 1995) necessitated the addition of retrogde oxygen persufflation to improved preservation time and reduced the effect of HES in UW solution (Yin *et al*., 1996). This will also ameliorate energy loss and improve functional recovery of ischaemically-injured kidneys. Also, to substitute HES in UW solution, Dextran 40 based was added to the solution but there was no overall improvement in graft survival rates (Candinas *et al*., 1996). Rabbit kidneys preserved in Euro-Collins alone did not improve preservation time nor protect vascular endothelium, which regulate function and urine excretion in comparism with Euro-Collins plus Thromboxane A_2 inhibitors (UK 38485) (Kuzu *et al*., 1995). However, Kumada (1997) used Ouabain

containing Euro-Collins solution to preserve kidneys and found that it protected the proximal tubular cell against ischaemic damage. In the past decades, Collins solution was one of the world's widely used kidney preservation Solution (Kreish *et al*., 1978). Therefore several solutions have been used with high optimism to achieve ideal preservation and at very low cost, with less than absolute success. This study therefore aims at contributing towards seeking a low cost, long-term preservative solution for organs intended for transplantation.

Materials and methods

Sixteen kidneys were harvested for evaluation from sixteen adult Nigerian indigenous breed of dogs of varied sex and and were conditioned for 2 weeks. The dogs were judged clinically healthy post physical examination, presurgical laboratory evaluation with haematology, serum chemistry and urinalysis. These parameters were taken two times a week (n=4) for all dogs under study except that temperature values were obtained on daily basis throughout the period of study. All the dogs were screened free of ecto, gastrointestinal and haemo-parasites before commencement of the study. Food and water were withheld for 2 hours before the surgeries. All dogs were aseptically prepared for surgery. The ventral abdomen of all dogs were liberally clipped and scrubbed with 2% chlorhexidine solution.

Anaesthesia

All dogs were anaesthesised with thiopental sodium (20mg/kg intravenously) as the main anaesthetic agent in continuous infusion post premedication with atropine (0.05 mg/kg intravenously) and chlorpromazine (4 mg/kg intravenously). Post anaesthetic induction, all dogs were intubated and placed on a dorsal recumbency and aseptically draped.

Surgical Technique and approach

An 8cm cranioventral linea alba incisions were made 3cm from the xiphoid and extending 4cm to the umbilicus, to access the kidneys at the retroperitoneal space for all dogs. Desired exposures and gentility of handling tissues were ensured to minimize or prevent vascular spasm while dissecting perirenal fat especially around the renal vessels. The vessels (renal arteries first and then renal veins) and ureters were adequately identified and ligated with 4-0 chromic catgut and severed. Abdominal closures were routine in 3 layers (Peritoneum, linea alba and subcutis) with 2-0 chromic catgut and the skin with 2-0 nylon. Following harvesting, the kidneys were flushed with a pre-reconstituted flushed/storage heparinised solutions (each 500mls A-Ringers Lactate, B-Darrows, C-5% Dextrose and D-Normal Saline) solutions contained 15000iu of heparin, 5mls of 2% xylocaine, 400000iu penicillin and 75mg streptomycin. Four kidneys were placed into each of the four labeled bowls A, B, C and D containing solutions A, B, C and D after manually flushing using a 20mls sterile syringe and blunt tip needle with their corresponding storage solutions. The kidneys were flushed after 2 minutes of warm ischaemia and maintained at cold ischaemia and preserved at 4ºC in the refrigerator. After every 24 hours the storage solutions are replaced with freshly reconstituted solution and a kidney is removed from each bowl for histopathology study. The same procedure was repeated every 24 hours until the last kidney is taken out of its storage solution at the 96^{th} hour. Slides prepared were viewed under a Dialux 20 microscope and photomicrographs taken.

Results

Presurgery evaluations: haematological values (Table 1), Serum chemistry (Table 2), and urinalysis (Table 3), values were within normal range. Presurgery temperatures were normal.

Table 1: Mean haematological values of all 16 dogs that underwent kidney harvest at Ahmadu Bello University Veterinary Teaching Hospital Zaria

Parameters	PVC %	Hb gm/dl	WBC /L	Neut %	Lymp %	Mon %	Eosin %	Baso %	Tprotein g/mls
N values	37-55	12-28	6-17 x 10^9	60-70	12-30	3-10	2-10	Rare	6-7.5
Dog 1-16	44.5	14.8	10.5	63.3	32.7	2.1	5.4	0.0	6.7

Table 2: Mean BUN and Serum Creatinine values of all 16 dogs that underwent kidney harvest at Ahmadu Bello University Veterinary Teaching Hospital Zaria

Parameters	BUN Mmol/L	Sc Umol/L
Norm values	(3.1-9.2)	50.0
Dog 1-16	3.4	50.1

Table 3:Mean Urinalysis values of all 16 dogs that underwent kidney harvest at Ahmadu Bello University Veterinary Teaching Hospital Zaria

Parameters	Specific Gravity	P^H	Protein g/L	Sugar	Blood	Ketone	Bilirubin	Urobilinogen
Norm values	1.015-1.045	6-7	-ve	-ve	-ve	-ve	-ve	-ve
Dog 1	1.030	7.3	-ve	-ve	-ve	-ve	-ve	-ve

-Ve = Negative

Grossly the preserved kidneys at 72 hours appeared normal in comparison with freshly harvested kidneys. They kidney appeared bright. Histopathology photomicrographs revealed normal sections, well preserved renal architecture but slight distortions were noticed in those preserved at 96 hours in ringers Lactate solution. Cortical and medullary sections showed normal histology for all kidneys preserved for up to 72 hours in solutions A and B (Plates I and II, and Plates III and IV) but were normal throughout preservation up to 96 hours for solutions C and D (Plates V, VI & VII).

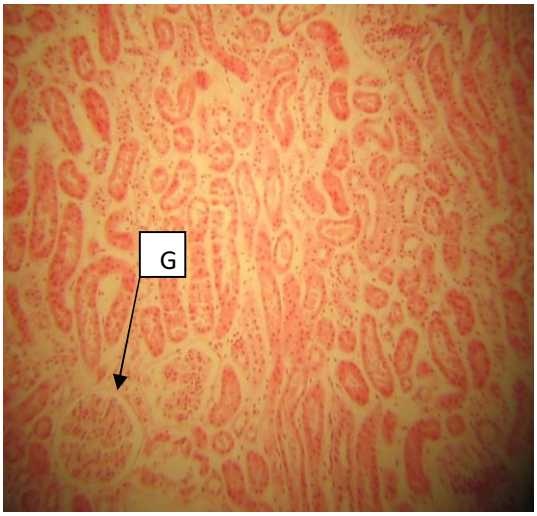

Plate I: Photomicrograph of parenchyma of a kidney stored in solution A for 72 hours showing a well preserved glomerulus (G) with capillary turfs evenly spread, urinary space mildly eosinophilic X500.

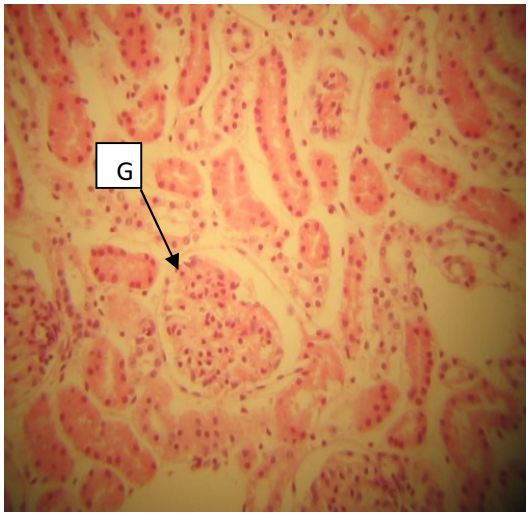

Plate II: Photomicrograph of a kidney preserved in solution A for 96 hours showing mildly preserved glomerulus with capillary turfs evenly spread and urinary space mildly eosinophilic. Basement membrane is intact and interstitium is clear. X800

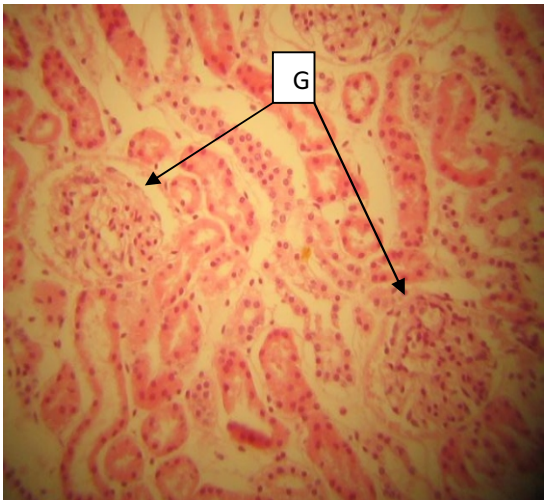

Plate III: Photomicrograph of a kidney stored in solution B for 72 hours showing glomeruli(G) well preserved with afferent and efferent blood vessels are patent, capillary turfs evenly spread with basement membrane intact. X500.

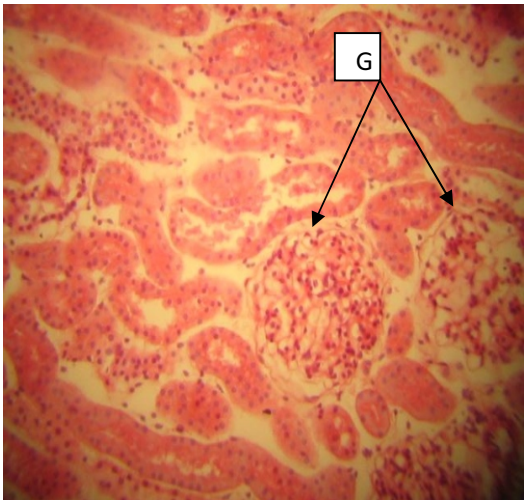

Plate IV: Photomicrograph of a kidney preserved in solution B for 96 hours showing mildly preserved glomeruli(G) with capillary turfs and urinary space evenly spread. X80

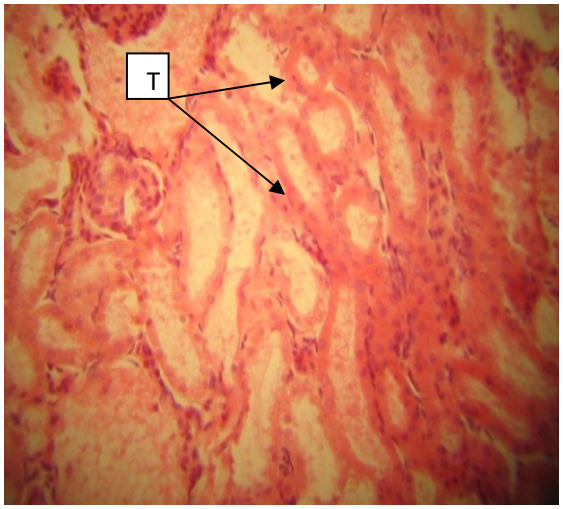

Plate V: Photomicrograph of a kidney stored in solution C for 96 hours showing most of the cells retaining their attachment with the basement membrane. Tubules (T) lie back-to-back with preserved interstitium. X500

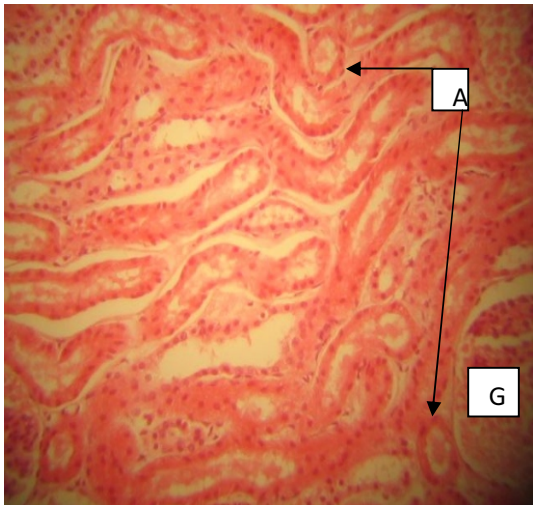

Plate VI: Photomicrographs of a kidney stored in solution D for 96 hours with well-demarcated arteries showing elastic fibre endothelium. Glomerulus (G) well preserved, capillary turfs evenly spread out. Urinary space is adequate and empty. Vascular pole is patent. Mesengial cells appear neat with granular cytoplasm. Cells are generally lying back-to-back on their basement membrane. X500

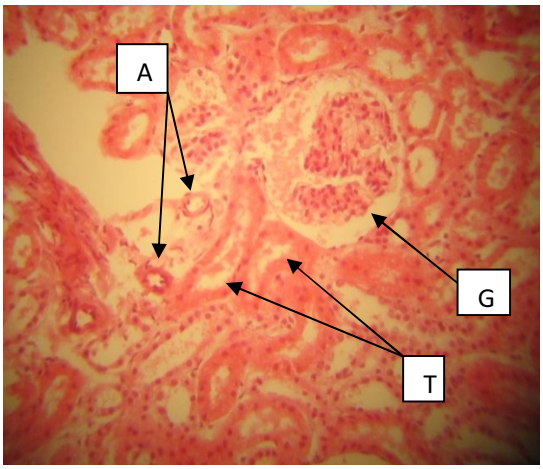

Plate VII: Photomicrograph of a kidney preserved in solution D showing a preserved glomerulus (G) well preserved with capillary turfs evenly spread, urinary space spread and tubules lying back-to-back. X80

The glomeruli in all appeared well preserved, capillary turfs evenly spread, urinary space adequate and empty. Tubular lumina appear regular in sizes lying back-to-back. The vascular pole was patent showing mesengial cells with granular cytoplasm and the cells generally lying back-to-back on their basement membranes. Arcuate arteries appear well preserved, with normal walls and patent lumen. At 96 hours a few clumped glomeruli, shrunken capillary turfs, urinary space with mild eosinophilic casts and moderate loss of cellular details. The basement membrane appeared dissolving with interstitial cloudiness and cellular inclusions noticed in kidneys preserved in Ringers Lactate and Darrows solutions (Plate II). No apparent distortions were noticed with kidneys preserved up to 96hours in Normal Saline and 5% Dextrose solutions.

Discussion

In this study, kidney harvesting and preservation using the four heparinised solutions was a success. Preservability of harvested kidneys in the studied crystalloid solutions at 4°C for up to 96 hours is a significant contribution towards attaining the desired long-term preservation. All preserved kidneys were kept and maintained at 4°C. The histology of the kidneys were normal with blood vessels, glomeruli, tubular and parenchymal features appearing normal. The minimal changes noticed were slightly clumped glomeruli insignificant to distort physiologic functioning of the kidney as previously reported (Barry *et al.,* 1981). The few mildly clumped glomeruli were noticed with kidneys preserved in solutions A and B for 96 hours. This was probably due to the high electrolyte concentrations in these solutions. They solutions gave a successful preservation compared to other solutions as previously reported (Collins *et al*., 1969; Collste *et al.,* 1970). Slight oedema noticed with kidneys preserved in solutions A and B was insignificant in relation to efficiency of preservatives (Bishop & Ross, 1975), thus the kidneys preserved by the studied crystalloid solutions showed only slight gross and microscopic signs of oedema. This was noticed with some of the kidneys preserved for 96 hours in solutions A and B. However, the renal tissues were histologically well preserved with no unusual microscopic distortion of the renal architecture. Mild increases in cellularity in preserved kidneys at 96 hour preservation in most cases were histologically inconsequential.

The results showed that it is possible to harvest kidneys and preserved them in any of the studied crystalloid solutions at 4°C for up to 96 hours. This will meet the basic standards of preservation as described by other researchers (Collins, Bravo-Shugarman and Tasaki, 1969; Barry *et al*., 1981; Ploeg *et al.,* 1988). The fundamental objective of preservation, is to keep the organ viable outside their natural environment without catabolic or tissue damage, it is conceivable that all the preservatives studied are compatible with normal physiological functions and could be recommended without reservation.

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