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# DIFFERENTIAL PRESERVATION OF RABBIT KIDNEY AND LIVER TISSUE IN RINGER LACTATE, EURO COLLINS AND UNIVERSITY OF WISCONSIN SOLUTIONS

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**ABSTRACT**
*Formalin, the most widely used preservative, has certain drawbacks. The foremost of them is interference with immunohistochemistry. To evaluate the efficacy of three commonly used organ preservation solutions for tissue preservation this experimental study was carried out on rabbit kidney and liver The tissue slices were stored in Ringer Lactate, Euro Collins and University of Wisconsin solutions (UW solution), for various fixed time intervals. The three solutions were used in their original form as well as with addition of antimicrobial agents. Morphological preservation was assessed by a semi quantitative method. Kidney slices showed better morphological preservation than liver tissue. UW solution demonstrated the highest preservation of the three solutions tried. UW solution was especially effective for liver preservation. Addition of anti microbial agents was more beneficial to kidney preservation than liver. Thus it was concluded that organ preservation solutions may be used for tissue preservation as well.*

***Keywords:*** *Organ preservation, Ringer lactate, Euro Collins solution, University of Wisconsin solution, morphological preservation.*

## INTRODUCTION

Attempts to preserve human tissues and organs from putrefaction and decay began in antiquity with the embalming and mummifying techniques which were developed to a fine art by the Egyptian dynasties. The Spanish Civil War marked the advent of blood banks and hence the clinical application of tissue storage techniques.[1] These attempts continue today whether with the idea of preserving blood, organs, or gametes of human as well as non-human origin, as by conservation biologists.[2-5]

Modern day attempts for tissue and organ preservation have generally been based on immersion in various solutions.[6-8] Initially solutions with electrolyte concentration resembling that of extra cellular fluid were tried.[9] However later it was discovered that solutions with electrolyte concentrations resembling intracellular fluid gave better preservation.[10]

This happens because a cell is deprived of circulation and hence energy, its vital processes gradually come to a halt. These include the Na-K pump, which is responsible for maintaining the high intracellular potassium and the high extracellular sodium levels. As it ceases to function, sodium and potassium drift down the chemical and electrical gradients; and the cell ends up having the same ionic concentration as its environment. Hence, if the environment is adjusted so as to support rather than challenge the intracellular environment, the ionic reversal is minimized. At the same time cell swelling is avoided.[11]

The time honored organ preservation solution Euro Collins solution (EC solution) was based on this principle.[12] This solution proved to be of great help in extending times of successful kidney preservation, but unfortunately it did not preserve hepatic tissue so well.[7]

After extensive research a new solution, University of Wisconsin solution (UW solution), was developed at the University of Wisconsin.[13] This has dramatically increased the effectiveness of liver preservation protocols, and when tried on kidney proved very helpful for this organ too.[3-5]

Tissue preservation is as important to a histopathlogist as to a transplant surgeon, though due to entirely different reasons. For routine histopathology, formalin has established itself as a gold standard.[14] but it has its own disadvantages. One of the most important of these is the masking of antigenic sites that which interfere with subsequent immunohistochemistry. Hence the quest for tissue preservation medium continues which

would satisfactorily preserve both morphology and antigenicity.[15]

In this study an attempt has been made to preserve rabbit kidney and liver tissues in various solutions. Three commonly used solutions,[3] *i.e.* Ringer lactate, EC solution and UW solution were tried in original forms as well as with addition of antimicrobial agents. The morphological preservation of rabbit renal as well as liver tissues stored in these solutions for different periods of time was assessed. The aim was to evaluate the differences in efficacy of these solutions for different tissues.

## MATERIALS AND METHODS

Rabbits were sacrificed and their kidneys and livers were sliced. These slices, 3-4 mm thick, were preserved in six different solutions at room temperature. The solutions were Ringer lactate, EC solution and UW solution. Each solution was used as such as well as with added anti microbial agents (Table 1). Ringer lactate was purchased from Medisol®, while EC solution was prepared after Abebe et al[16] and UW solution was prepared after Baatard et al[17] immediately prior to use. The anti microbial agents employed were Penicillin $10^4$ i.u./ml, Streptomycin 10 mg/ml and Nystatin 2500 i.u./ml.[18]

**Table 1:** *Details of solutions used.*

| Solution | Description |
|---|---|
| A | Ringer lactate |
| B | Ringer lactate with anti microbial agents |
| C | EC solution |
| D | EC solution with anti microbial agents |
| E | UW solution |
| F | UW solution with anti microbial agents |

Tissue slices were removed at various fixed time intervals (0 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, and 96 hr) and processed routinely. six tissue slices were included in each group. Sections were stained with haematoxylin and eosin. Morphological preservation was assessed by a semi quantitative method and expressed in percentage.[14] The 0 hr sections served as a control for each group. Observations were recorded by two independent observers. The results were then compared using paired student's *t* test and slope test.

## RESULTS

Kidney was found to show better preservation than liver. This was seen in all fluids. This difference was statistically significant in case of Ringer lactate and EC solution with added anti microbial agents. Liver tissue revealed better preservation in UW solution, with and without anti microbial agents, in early stages. When time passed kidney preservation surpassed liver preservation in these solutions as well (Table 2).

**Table 2:** *Comparison of preservation of rabbit renal and liver tissues in different solutions*

| Solution | Tissue | Percentage Preservation | | | Slope | P value |
|---|---|---|---|---|---|---|
| | | At 24 hrs | At 48 hrs | At 96 hrs | | |
| A | Kidney | 65 | 45 | 19 | -0.83 | p= 0.32 |
| | Liver | 83 | 40 | 15 | -0.97 | |
| B | Kidney | 94 | 70 | 50 | -0.55 | P= 0.01* |
| | Liver | 90 | 65 | 28 | -0.81 | |
| C | Kidney | 95 | 53 | 18 | -0.96 | p= 0.98 |
| | Liver | 85 | 40 | 15 | -0.96 | |
| D | Kidney | 90 | 73 | 50 | -0.52 | p=0.03* |
| | Liver | 85 | 65 | 40 | -0.65 | |
| E | Kidney | 94 | 70 | 64 | -0.39 | p= 0.45 |
| | Liver | 96 | 75 | 40 | -0.59 | |
| F | Kidney | 98 | 87 | 80 | -0.19 | p=0.30 |
| | Liver | 99 | 85 | 70 | -0.30 | |

* = p value statistically significant

## DISCUSSION

Tissue preservation is at a stage of rapid advancement.[19] Current research in many centers is targeted at evaluating the efficacy of the numerous components of UW solution and improving it further. An ongoing challenge is to increase the efficiency of the preservation solutions while minimizing the cost.[4-8] Histopathologists are trying to incorporate these developments into their own domain so as to avail the benefits of these solutions while avoiding the drawbacks of formalin.[20] The results showed that renal tissue was more tolerant to ischaemia than liver tissue (Table 2). This conforms to findings in other studies.[21] This happens because the higher metabolic rate of heptocytes makes them more sensitive to the deleterious

effects of anoxia. Ischaemia cuts off the supply of oxygen and nutrients. Metabolism becomes anaerobic; lactic acid is produced causing progressive intracellular acidosis, that activates lytic enzymes leading to autolysis.[8,21]

Another reason for this lowered resilience of hepatocytes is that it is more permeable to glucose and mannitol. This leads to reduced osmotic control and increased acidosis due to anaerobic glycollysis.[8]

The results suggest that UW solution is better liver tissue than EC solution. The difference is so marked that for the early stages of preservation in UW solution, liver tissue reveals better preservation than renal tissue. However on prolonged storage, renal tissue regained its supremacy as the more resilient tissue. This dramatic fall of morphological preservation of liver with the passage of time has also been reported earlier.[21]

Another finding was the protection conferred on renal tissue by anti microbial agents (Table 2). Although renal tissue revealed better preservation almost throughout, the addition of anti microbial agents rendered statistical significance to this difference. Tissue damage during preservation occurs via two mechanisms. One is invasion by microbial agents; the other involves the metabolic changes already described, triggering autolysis.[21-23] The second mechanism is more operative in the liver tissue, while in the renal tissue deterioration is caused mainly by microbial agents. Hence, the addition of anti microbial agents is more beneficial to renal tissue. This benefit was obvious in all solutions. As already stated, the addition of anti microbial agents to Ringer lactate and EC solution made them solutions significantly more potent. This finding has also been reported earlier.[24-25] With UW solution the scenario is different. While plain UW solution revealed better preservation of liver tissue in early stages, the addition of antimicrobial agents improved the preservation of renal tissue to a degree that it came close to that of liver tissue.

In **conclusion**, organ preservation solutions can be employed for tissue preservation as well. Satisfactory preservation of renal tissue can be obtained with the cheaper and more readily available EC solution, while the more vulnerable liver tissue would require a more sophisticated solution like UW solution.

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