# BIOCHEMICAL AND HAEMATOLOGICAL CHANGES DURING AND AFTER LIVER TRANSPLANTATION IN THE PIG: THE EFFECT OF DIFFERENT METHODS OF STORAGE AND FLUSHING SOLUTIONS.

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Thesis submitted to the

UNIVERSITY OF CAPE TOWN

for the degree

MAGISTER CHIRURGIAE

(Ch.M)

April 1991

This thesis

is

dedicated

to

SCHALK WILLEM PIENAAR.

It is as easy to dream a book
as it is hard to write one.

Balzac. 1836.

A critic knows more than the author he criticizes,
or just as much,
or at least somewhat less.

Cardinal Manning. 1892.

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## TABLES.

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1. Changes in plasma calcium and magnesium during and after experimental liver transplantation.

Pienaar H, Hickman R, Wilson G and Belonje. Anesthesia Analgesia In press 1991.

2. Simple cold storage of the porcine liver.

Pienaar H, Schwartz I, Bracher M and Hickman R.

Transplantation. In press 1991.

3. Is heparin the cause of the coagulopathy which may complicate liver grafting?

Bracher M, Hickman R, Pienaar H, Jacobs P, Bird A and Terblanche J.

Surgery, Gynecology & Obstetrics. In press 1990.

4. Is the pig liver a good model for preservation studies.

Pienaar H, Roncone A, Schwartz I and Hickman R.

Proceedings of the South African Transplantation Society Congress.

5. Is pulsatile perfusion preservation of the liver better than simple cold storage?

Pienaar H, Lindell S, van Gulik T, Southard J and Belzer F.

Proceedings of the European Surgical Research Society. May 1989 Brussels, Belgium.

6. Coagulation changes during and after experimental liver transplantation.

South African Surgical Research Society Congress. Oral presentation May 1991.

Pienaar H, Schwartz I, Bracher M and Hickman R.

# ABSTRACT.

Liver transplantation is an accepted form of treatment in advanced liver disease. The procedure qualifies as one of the most severe surgical insults that can be inflicted upon a patient. Despite an ever increasing number of clinical and experimental transplants, a vast number of unanswered questions remains about the effects of storage and transplantation per se, on the functions of this complex organ. The administration of drugs and blood, with the effects of the donor state and preservation damage obscure changes in parameters that are inherently due only to the process of transplantation.

Changes in calcium and other electrolyte homeostasis, liver function assessment, acid base metabolism and coagulation defects that are seen after liver grafting, are of particular interest to transplant physicians. Current clinically employed indices of liver function, such as enzyme levels, are notoriously lacking in specificity and sensitivity.

The aim of the study was to investigate in the experimental situation, the effects of standardised preservation and transplantation, without the added effects of blood transfusion or immunosuppressive drug administration, upon calcium and other electrolyte homeostasis, liver function and coagulation changes.

Furthermore, reliable indicators of liver function and/or damage were looked for. It was not an investigation into preservation methods to determine superiority of one or another of these methods, but an evaluation of changes occurring utilising established and clinically proven methods of preservation.

Since researchers in the J.S. Marais laboratory, as well as their international counterparts, have experienced problems in successful storage of the pig liver for periods longer than 9 hours, a storage duration of six hours was chosen for maximum reproducibility.

A brief overview of liver transplantation history has been given, available literature perused and used in assessment and discussion of data obtained.

Five groups of six animals were used for orthotopic liver transplantation. Two groups were autografted with a non-flushed and Ringers lactate flushed liver respectively. Two groups were allografted with livers stored in Collins and University of Wisconsin solutions respectively. A fifth group was transplanted with a liver stored for six hours by surface cooling alone, without any flushing at all. The latter method has not been described in experimental or clinical liver transplantation before. No immunosuppression was used in any animal, to eliminate

the effects of hepatotoxic drugs. No blood was transfused at any point during or after the transplant. An animal survival rate in excess of 90%, for seven days or longer, was aimed for and obtained.

Blood sampling was done at short intervals in the immediate postoperative period up to six hours and daily for a week. All currently used clinical parameters were determined, as well as indicators which are known, but novel in transplantation.

Changes in total and ionised calcium values occurred in all groups and no explanatory mechanism could be identified. There was no correlation in changes between total and ionised calcium, nor any correlation with calcium content of preservation fluids. A reciprocal change in magnesium was identified.

Acid base metabolism was markedly changed during and after the transplant. An increase in serum bicarbonate indicated survival, and a persisting metabolic alkalosis was seen in all survivors. Sodium and potassium values did not show marked changes, except for a temporary hyperkalaemia immediately following reperfusion. Serum values of liver transaminases were not found to be of value to discriminate between groups. Protein metabolism was not affected by transplantation. Glucose metabolism was markedly affected by transplantation and even more so

by poor function. Early return of normal glucose metabolism indicated survival. Lactic acid metabolism was conspicuously altered during transplantation and could also be regarded as an indicator of hepatocyte function.

Coagulation in this series of experiments was affected negligibly and not thought to be influenced by transplantation of a normal liver under ideal circumstances.

Thus, changes in values within groups and variance between groups, if any, were described and possible mechanisms causing variation discussed. New indicators of good liver function post-transplant were identified.

The conclusion was reached that the process of transplantation per se does cause major changes in electrolyte and acid-base metabolism, but that coagulation was not affected by the process of successful preservation and transplantation.

# ACKNOWLEDGEMENTS

I had the privilege and advantage of carrying out the investigations in this study under the supervision of Professor Rosemary Hickman, Head of the Surgical Research Laboratory at the University of Cape Town. Apart from performing five of the transplants for me, her sincere interest and unwavering support from the first day that she introduced me to the intricacies and occasional disappointments of experimental surgery, had been a major impetus in the conduct of this study. Her clear thought processes and inquisitive mind sets a goal for mere mortals to strive to attain.

I would like to thank Professor John Terblanche for providing me the opportunity to work in the Liver Research Unit of the Medical Research Council at the University of Cape Town. His interest and support are appreciated.

Dr Arturo Roncone assisted in all transplants and his cheerful presence made hard work seem like fun.

Mr Hamilton Naki warrants a special word of thanks for his unlimited patience and enthusiasm in teaching and assisting in numerous transplants. His willingness to try and accept new ideas made working with him a pleasure.

Mr George Graham played a major role in maintaining an all important Astrup machine for never ending measurements.

Dr. G. Wilson and his team in the department of Physiology, U.C.T is sincerely thanked for measuring calcium and magnesium in all specimens as well as for constructive advice in interpretation of results.

Mr E. Henry and Mr D. Tango are thanked for their role in supplying and anaesthesing donors and recipients.

Mona Bracher and Zoe Lotz made molehills out of mountains of specimens requiring haematological and biochemical assays respectively.

Dr Joan Fourie determined all the lactic acid values which proved to be an exciting new avenue of investigation.

Joalta Pienaar provided a never ending supply of support, especially when it was needed most.

The wisdom of Jim Southard to insist that I should become familiar with and use a computer can only be appreciated when looking through the "retrospectroscope".

A mingled monster of no mortal kind:
Behind, a dragon's fiery tail was spread,
A goat's rough body bore a lion's head,
Her pitchy nostrils flaky flames expire,
Her gaping throat emits eternal fire.

ILIAD, VI, 180.

# PROLOGUE

Replacement of non-functioning or malfunctioning organs has captured the imagination of surgeons for over two thousand years. The thrust was certainly not the desire to create a monster "breathing eternal fire" but to prolong life and ease suffering.

Legend has it that St. Cosmos and Damian used a cadaver limb to replace same affected by malignancy. Liver transplantation has a much more recent history and although performed clinically since early 1960's, only became a realistic treatment option for end stage liver disease with the advent of cyclosporine in 1976.

Liver transplantation is unique in the sense that the grafted organ has to function immediately, unlike the transplanted kidney or heart, where backup systems such as dialysis and cardiac bypass machines exist to manage primary non-function of grafted organs. There is no machine that can take over the complex functions of the liver.

Changes in biochemical and haemostatic parameters have been associated with liver transplantation since it's inception. These observed phenomena were, and are, due to a multitude of factors existent in both donor and recipient.

A complex interplay of effects of donor status, preservation and hypothermic organ damage, recipient disease, blood transfusion, administered drugs and immunological phenomena makes it difficult to

associate the various changes occurring during and after clinical liver transplantation with precipitating causes of such changes. It is of the utmost importance to detect a non-functioning transplanted liver as early as possible, yet markers of liver damage and function are notoriously lacking in sensitivity and specificity. Tests to determine early abnormalities are necessary to indicate functional status, and should be as uncomplicated and non-interventional as possible for the widest possible application.

In the experimental laboratory, under controlled conditions, it is possible to elicit those changes in biochemical and haemostatic values that are inherently due to the process of transplantation alone and these changes may then be compared to those obtained with current preservation techniques. Such a study could be conducted without the outside influences of drugs and blood administration. Simultaneously, those modalities that are currently used as indicators of liver function after transplantation, could be put to test and possible new indices identified.

A study was set up in which currently accepted storage techniques were used to preserve pig livers for six hours. Furthermore, a novel concept of surface cooling (simple cold storage) alone for six hours was employed in one group of animals. All modalities described are in current practical experimental or clinical use except the

"simple cold storage" method which has not been used in clinical liver transplantation before. The autograft transplant model, without any flushing of the liver, was used as reference. All donor animals were normal; the effect of the donor state was excluded.

Minimal dosage heparin, for spleno-jugular veno-venous bypass, as used in this study, has become standard practice in the J.S. Marais laboratory. Indicators of hepatic function identified in the course of this study are currently being investigated in clinical transplantation, as well as during inflow occlusion, in elective liver resection.

In 33 consecutive porcine liver transplants three animals did not survive for seven days. Each of these came from a different group and it was postulated that non-survival was unrelated to technique or methods used in that particular group.

An understanding of the effects of storage and transplantation per se might be helpful in assessing biochemical and haemostatic changes in the clinical situation, as well as being indicative of function or lack thereof.

The aim of this study was to obtain 100% survival of transplanted animals so that preservation damage did not complicate the observations to be made. The study was not designed to compare, investigate or improve current preservation methods.

CHAPTER 2.

# HISTORY OF LIVER TRANSPLANTATION.

A brief overview of some of the most important aspects of both experimental and clinical liver transplantation is given. The scope of this study makes it impossible to include all the important milestones reached over the last thirty five years, but an attempt has been made to highlight aspects most relevant to this dissertation.

The first report of experimental liver transplantation is usually ascribed to Welch (1955).[36] There is no doubt that Welch's report paved the way for subsequent experimental work leading up to current day clinical liver transplantation. Welch's report was an account of a liver transplanted heterotopically into the right iliac fossa of a dog. The vascular supply of the extra liver was as follows.

1. Donor portal vein to inferior vena cava.
2. Donor hepatic artery to common iliac artery.
3. Donor inferior vena cava to inferior vena cava.
4. Donor cholecyst to duodenum.

Welch and his co-workers subsequently (1956) performed more experimental transplants in dogs, [34] using the orthotopic position. There was then a lapse in reports of experimental liver transplantation until the dedicated pursuit of this branch of surgery was started by Moore in 1959, [39,40] followed by Starzl [41] in 1960 as well as others.[16] Starzl, who pioneered liver transplantation, both in laboratory and the clinical setting, was responsible for establishing this procedure (transplantation) as a viable method of treatment of end stage liver disease.

The other large animal that was used in the study of techniques of liver transplantation was the pig.[23] The first report of experimental orthotopic liver transplantation in the pig was by Gaston Cordier and his co-workers in 1966 in Paris following earlier (1965) preliminary investigations.[1] Their efforts were rewarded with some success after two years. They chose the porcine model because of the "relative similarity of the hepatic anatomy and the bile ducts, to that of man". They used relatively large animals (60 kg.), when compared to current practice (25 kg.), in the laboratory setting. In reporting transplants in 20 animals, they had only six animals surviving more than 24 hours, but this laid the basis for continuing with the essential work.

Experimental work on liver transplantation in the pig in the United Kingdom was initiated during 1966 by Terblanche[165] and soon followed in 1967 by Calne.[166] Extensive experimental studies on the technique of transplantation naturally progressed into the first hesitant steps in establishing the new science of organ preservation.[1]

The Cape Town group namely Terblanche, Hickman, Spilg and Dent established and refined the orthotopic auto- and allograft in the porcine model.[2,3,4]

Subsequent to these experiments some researchers used a variety of other animal models such as the rat,

rabbit, dog and the calf.[8,9,29,45] The dog, baboon and pig were used in numerous transplants since 1955, but in the current literature (since 1985) only the pig and dog feature as experimental animals[14,18,19], with an emphasis in recent literature on the use of the dog.[6,21,27]

It has been stated that the only real test for assessment of any technique in experimental liver transplantation is the survival of the recipient.[6,42]

A variety of techniques for orthotopic, as well as heterotopic liver transplantation, have since been described.[2,6,32,38] These procedures have been adapted from the first pioneering attempts which were five to six hour procedures with complicated bypass systems, especially in canines, to the latest, [6] where the whole procedure was performed in less than two hours with the use of a passive porto-jugular venous bypass. Experimental transplantation in the pig was extensively described by Calne and others, [7,14,20,22,42] as well as by Terblanche.[2]

A modification of these procedures was described by Dent [4] and this method with slight modifications is used in the JS Marais laboratory (see chapter 5). Hepatic autografting in the pig was described by Hickman in 1977 [3] and this method is extensively used in biochemical studies.

The first attempt at clinical liver transplantation was made by Starzl in 1963 in Denver, followed by single attempts in Boston and Paris.[27]

Initial investigations into the question of hepatic failure treatment, by liver transplantation, centred on heterotopic placement of an auxiliary liver. It was thought that host and adjunct liver would act in concert, but this was soon shown to be incorrect, when it was found that the liver deprived of portal flow would atrophy unless the host liver was almost non-functioning or alternatively removed. The two main theories suggested

a. That the liver was deprived of essential nutrients or hepatotropic factors or alternatively

b that the blood supply was insufficient.[23]

Currently heterotopic placement of livers is only considered when it is thought that the patient will not withstand orthotopic transplantation[44]. Recent literature shows a strong trend towards orthotopic placement of the liver, with recipient hepatectomy, especially in experimental work.[47]

After the establishment of the technique, the advent of cyclosporine A in 1977 represented the next major advance.[48,49] Preservation time of harvested livers (experimentally and clinically ), until very recently, remained at a maximum of 10 hours. Occasional anecdotal reports stated longer preservation periods.[16] A major recent advance in this field has been obtained with the advent of the UW solution. It is now possible to store livers clinically for up to 36 hours with safety, and

experimentally, in the dog and rat, for up to 72 hours.[45,46,47] These extended preservation periods are not possible with the porcine liver, where maximum preservation time with 100% transplant success rate is 6 hours.[Unpublished data(ovs), Shoil R.(personal communication).]

The effect of preservation or transplantation per se or a combination thereof on biochemical homeostasis, as influenced by the liver, has however, not been addressed adequately. Also, changes in haemostasis, without the effects of massive blood transfusions, have not been recorded.[106] It is also important to assess liver function after transplantation.[170]

CHAPTER 3.

# BIOCHEMISTRY OF LIVER TRANSPLANTATION.

3.1. Calcium.

Total calcium.

Ionised calcium.

3.2. Magnesium.

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# BIOCHEMISTRY OF LIVER TRANSPLANTATION.

Liver transplantation is established as an accepted treatment modality.[26,27] The technical aspects in the human have been well documented and few improvements in this field can be expected.[28,48]

Changes in laboratory data values before, during and after liver transplantation are the interactive result of donor state, pre-existing disease in the recipient, iatrogenic manipulations during anaesthesia, the operative procedure, as well as ischaemic and reperfusion damage to the liver.[83]

Products of excretion such as bilirubin have been used as a measure of function both in and ex vivo since the early 1960's.[50] Attempts have been made to predict function from biochemical data in order to prevent or diagnose primary non-function of the grafted organ early. Biochemically measured substances includes absolute values of electrolytes, enzymes, protein fractions and glucose, as well as the metabolism of various substances such as indocyanine green and lignocaine. The absolute measurements of biochemical parameters are to be preferred to tests of metabolism which are invasive and where the substances used might be detrimental to an already compromised liver.[51]

In biochemical evaluation of function or damage the

ideal parameter should be a highly specific and sensitive measurable quantity, and results should be available as soon as possible to be of clinical value.[52]

In order to assess changes in the clinical situation, where multiple variables exist, it is necessary to have knowledge of the effect of the procedure per se, on the basic reference values, that are used.

## CALCIUM.

Ionised calcium concentration in extra-cellular fluid under normal circumstances varies between 0.9 - 1.2 mMol/L. Intra-cellular free ionized calcium is maintained at 0.05-0.2 microMol/L.

It is clear that the processes responsible for maintaining this $10^4$ gradient can only exist in viable cells, due to the high energy required for maintenance of this gradient. The systems available for calcium transport across the plasma membrane differ and are dependent upon the tissue type. In the hepatocyte a $Na^+/Ca^{++}$ exchange system and a $Ca^{++}$-ATP-ase pump are the most important factors in the plasma membrane maintaining $Ca^{++}$ efflux and thus intra-cellular calcium homeostasis. Evidence has been presented for a vasopressin responsive $Ca^{++}$ transport system.[89]

In the endoplasmic reticulum and mitochondria, $Ca^{++}$ is used in the formation and breakdown of ATP. It is thought that most intra-cellular calcium is bound in these two systems and that they play the most important part in the dynamic regulation of intra-cellular $Ca^{++}$.[90]

The presence of calcium binding proteins in the cytosol is well known. The first identified was calmodulin, an activator of cyclic nucleotide phospho-diesterase. Calmodulin regulates plasma membrane $Ca^{++}$pump activity as well as glycogen breakdown through mitochondrial function.[89]

Ionised hypocalcaemia in liver transplantation is seen upon reperfusion in association with lowering of pH, hyperkalaemia and hypotension and can be prevented by administration of calcium chloride.[83,87] It is impossible to predict how much of calcium given as chloride salt will disassociate.[86] An increased level of $Ca^{++}$ in the cytosol has been shown to be toxic.[88] It is therefore necessary to be able to measure the ionised calcium available, before and after administration of calcium chloride, in order to rationalise doses given. Measurement of ionised calcium is performed by an ion-selective electrode which method is subject to instrument failure. Nomograms exist whereby estimated ionised calcium values can be determined from total serum calcium values. The accuracy of calculated values for ionised calcium is completely unreliable, not only under normal circumstances, but especially in the case of liver disease where changes may occur in pH as well as albumen and globulin ratios.[87,104]

## TOTAL MAGNESIUM.

It is accepted that magnesium metabolism is regulated on the same basis as calcium and that these divalent ions compete for the same binding sites.[110] This competition also accounts for difficulties in measuring ionised magnesium, due to the higher affinity of ion-selective electrodes for calcium. In a medium containing both ions, under ideal circumstances, errors in measuring of one of the ions will be compounded by errors in measurement of the other.[110]

# ACID–BASE METABOLISM

The concept of acid- base metabolism consists of a complex buffer system and the ability to excrete acid or base through the kidney or lungs. The status of this system is described by pH. Disturbances in acid- base metabolism are described as metabolic or respiratory in origin, depending on the relative quantities of $CO_2$, $HCO_3^-$ and organic acids.[58]

Significant derangements in acid- base metabolism are frequent in clinical liver transplantation. The occurrence of acidosis is ascribed to pre-existing disease, anaesthetic management, massive blood transfusion and lactic acid load from the grafted liver.[84]

## pH

Normal hydrogen ion concentration ($[H^+]$)in blood or serum is 0.00004mEq/L. $[H^+]$ is by convention, due to the small numbers, relative to other ions in body fluids, expressed as pH. It depicts the antilogarithm of $[H^+]$.[54] This conceptual bypass makes statistical interpretation of pH values difficult because the mean, standard deviation and error are not arithmetic functions. Although some authors use pH values directly in statistical applications, $[H^+]$ should be calculated and used in this respect.[55] The measurement of pH is part of the "Astrup" and performed repetitively as part of routine medical care, both intra- and postoperative.

pH is intimately related to $pCO_2$, $HCO_3^-$; therefore it is influenced by changes in respiratory and renal function.

In practice clinical acid-base disturbances are referred to as of metabolic or respiratory origin depending on the relative values of $CO_2$ and $HCO_3^-$. This description is expressed in the Henderson-Hasselbalch equation, which deals with three variables namely $CO_2$, $HCO_3^-$ and pH.

$$pH = pK(H_2CO_3) + \log[HCO_3^-] \cdot [H_2CO_3]^{-1}$$

In order to assess the value of one of these variables properly, the other two must be known.[56] Attempts at defining the temporal and spatial occurrence of these changes, with a view to preventive treatment have been made.[84]

In clinical liver transplantation variations in acid-base status usually exist before the start of surgery. The difficulties in management are compounded by the magnitude of surgery, effect of anaesthesia per se and management of fluid and blood loss. During the anhepatic phase metabolic acidosis is a common occurrence due to increased lactic acid production (hypoperfusion of peripheral tissue) as well as decreased removal (anhepatic phase). These effects are demonstrated by a decline in pH but it is important that this should be seen as part of a complex metabolic situation.[75]

The infusion of massive volume of blood during liver transplantation has been suggested as one of the causes of

the decline in pH. Banked blood contains high levels of lactic and pyruvic acid.[74]

An important cause of acidosis, which has not been evaluated, might be reduced tissue (other than liver) perfusion. It was shown in 1959 that in extra-corporeal circulation, low tissue perfusion was the main cause for development of acidosis and resultant low pH.[133]

Postoperative metabolic alkalosis is often seen after clinical liver transplantation, especially early in the recovery phase. It has been ascribed to various causes such as citrate toxicity, nasogastric drainage of acid gastric contents, lactate metabolism and excess administration of sodium bicarbonate.[85,74]

There is controversy in the literature about the use of sodium bicarbonate on a routine or prophylactic basis in the management of liver transplanted, as well as routine surgical, patients.[85,86]

BICARBONATE.

Bicarbonate plays an extremely important role in acid-base equilibrium through bicarbonate-carbonic acid buffering.

$$H_2O + CO_2 = HCO_3^- + H^+$$

With pH this anion is used in assessment of acid base balance, in determining metabolic or respiratory origin or existence of alkalosis and acidosis. It is necessary to calculate or directly measure bicarbonate to make correct deductions regarding acid base status.[53]

The administration of sodium bicarbonate was thought to be an integral and essential part of the intraoperative management of hepatic transplantation for the prevention of metabolic acidosis.[69]

The metabolic alkalosis seen after liver transplantation is thought to arise from the metabolism of citrate administered with transfused blood or blood products.see Chapter 3: Lactic acid.

SODIUM.

Sodium, with associated anions, including chloride, is the most important electrolyte in maintaining plasma osmolality. An intracellular shift of sodium or absolute loss of sodium will cause major changes in extracellular fluid volume.

Hyponatraemia is more significant in the surgical setting than hypernatraemia. The rate of development of hyponatraemia is more important than absolute values when assessing presenting symptomatology.[81]

Disturbances in sodium metabolism in liver transplantation relate to retention and increase in total body sodium with expansion of the total body water component in hepatic cirrhosis. This derangement in volume homeostasis, relates to adequate fluid management of the recipient in the postoperative period. In the transplanted liver hepatic volume sensing mechanisms[82], will not be functioning, due to destruction of afferent pathways during the harvesting by transection of relevant vagal nerve branches.

Sodium homeostasis in liver transplantation does not appear to present problems in relation to intra- or postoperative management of recipients.

POTASSIUM.

Potassium, with sodium, is an essential electrolyte in normal homeostasis. Normal osmolality of body fluids depends on both ions, which is in constant flux. Any change in one of these will cause reciprocal changes in other ions to maintain osmotic as well as ionic balance. Potassium is mainly an intra-cellular cation. Hydrogen ion and potassium compete in the renal tubule and therefore direct correlation between them exists. Hyperkalaemia in the extra-cellular fluid will increase metabolic acidosis and hypokalaemia metabolic alkalosis.[73]

Hypokalaemia might present late in liver transplanted patients in association with metabolic alkalosis following massive blood transfusion.[74]

Hyperkalaemia is a significant event in liver transplantation and is most often seen immediately following revascularisation; it is thought to be caused by a combination of washout of metabolic by-products from the ischaemic liver and residual preservation solution. This period is the most unstable phase of transplantation characterised by hypotension, bradycardia and dysrythmia.[86]

Existing hyperkalaemia in the hepato-renal syndrome, with reduced capacity of potassium excretion, will be further aggravated by metabolic acidosis.[57]

Hyperkalaemia, in the presence of hypocalcaemia,

might cause cardiac arrest. It is thought to play a major role in the post-reperfusion syndrome.[72] Potassium changes in liver transplantation should not be seen in isolation, but as part of a complex metabolic system.[57]

# LIVER FUNCTION TESTS

## SERUM ENZYMES.

Serum aspartate- (A.S.T) and alanine (A.L.T) amino transferases as well as lactate dehydrogenase (L.D.H) are enzymes that occur naturally in blood in a normal distribution with established normal values. Iso-enzymes have been measured to differentiate the cellular origin of these enzymes and are used in clinical biochemistry.

A.S.T and A.L.T are widely used as indicators of cellular damage, both in myocardial and hepato-cellular assessment.[52] Lactate dehydrogenase, with A.L.T and A.S.T, is extensively used in ex vivo studies of liver preservation, as a marker of preservation quality.[59] Levels of these enzymes are used as markers of damage and function of transplanted livers in experimental as well as clinical transplantation.[45,46,47,48]

A.S.T. occurs in cytoplasm and mitochondria, whereas A.L.T and L.D.H are found exclusively in cytoplasm. A.S.T release in serum is an indicator of mitochondrial damage, whilst A.L.T and L.D.H indicate cellular membrane disruption.[60] The sensitivity of enzyme release is high but lacks specificity, even when iso-enzyme determinations are done.[52] The use of ratios of A.S.T and A.L.T has been advocated as more sensitive and specific in determining cellular damage, but is still of questionable value.[61]

Lactate dehydrogenase is even more non-specific than A.S.T or A.L.T, but is extensively used as an indicator of cellular damage in ex vivo studies. It is rarely used in assessment of hepato-cellular damage but the iso-enzymes of muscular and cardiac origin are used to assess damage in these two organ systems.

Glutathione S-transferase is a more sensitive indicator of hepatocyte damage in chronic active hepatitis than absolute values or ratios of any other enzymes. It has been suggested that this enzyme is more specific and quantitative of hepatocyte damage than aspartate aminotransferase.[62]

Alkaline phosphatase is also used as an indicator in hepatobiliary disease, but more so as a measure of hepatic excretory function. Levels of this enzyme rise earlier than those of bilirubin (more than 50% of ducts must be obstructed before this is elevated) in obstructive hepatocanalicular disease.[63] The enzyme is actively secreted from hepatocyte canalicular membranes of obstructed bile ducts.[64] It is not used as an indicator of preservation or reperfusion damage in clinical transplantation but is used as an early warning sign in grafted liver rejection.[78]

TOTAL PROTEIN.

The term includes all proteins found in serum such as albumen, alpha and beta globulins as well as a variety of carrier proteins. Fibrinogen is an important protein manufactured in the liver and is included in the concept of total protein.[128]

Total protein in the context of surgical patients is mainly used to assess nutritional status.[130]

ALBUMEN.

Albumen is used as an indicator of liver function especially in end stage liver disease. Intravascular concentration is an important criterion in selection of liver transplant recipients.[141]

Although the liver is the sole source of albumen, plasma concentration or total body albumen pool is not always diminished in advanced liver disease. A distribution change, where the extravascular pool contains a proportionally higher level of albumen relative to the intravascular concentration, might also exist in the presence of ascites.[128]

Albumen plays an important role in the maintenance of oncotic pressure and is also a ready source of calories.[54]

A very important protective mechanism in albumen synthesis and catabolism is the concept of fractional catabolic rate. This means that reduced rate of synthesis is met by a reduced rate of catabolism to protect the available and/or whole body pool of albumen. This mechanism does not exist in the case of fibrinogen and is inverted in the case of carrier proteins such as haptoglobin. The site of albumen breakdown is uncertain and factors controlling synthesis are not fully clarified either.[128]

Transient hypoalbumenaemia in surgical patients is a common but ill understood phenomenon. It usually occurs between 5 and 9 days postoperatively and is thought to be

caused by retention of albumen in an extra, created compartment namely the surgical wound.[161] In rabbits transient sequestration of albumen in the wound site has been shown.[138] In newborn infants with gastroschisis, actual loss of albumen, similar to that seen in burn wound sites, is seen.[139] In renal transplant patients with normal liver function hypoalbumenaemia also occurs, although only after 10 to 14 days, postoperatively. It was postulated that loss of albumen through lymphatic channels was responsible for this reversible phenomenon.[140] An immediate decrease in albumen, upon reperfusion, was reported in recipients of longterm (72 hour) preserved canine liver transplants.[46] In these animals albumen concentration did not show any sign of recovery during ten postoperative days, but fibrinogen concentration, which was also markedly decreased simultaneously on reperfusion, returned to normal levels within two to three days.

In the transplant setting the possible hepatotoxic effect of immunosuppressive drugs might suppress albumen synthesis and worsen existing hypoalbumenaemia.

GLUCOSE

The liver plays a central role in glucose metabolism via the reversible process of glycogenesis. Although glucose is only one of the glycogenic substances that can be used in the liver, it plays a pivotal role in energy metabolism.[65]

Glucose uptake in the liver depends to a large extent on blood glucose level, but is influenced by hormonal action such as insulin, growth hormone, glucocorticoids, epinephrine and glucagon.[54]

Various other tissues such as muscle play a major role in glucose metabolism, but the effect of these in liver transplantation is negligible.[65,67,68]

The liver is the most important site for gluconeogenesis because of the presence of glucose-6-phosphatase. Production of glucose in sites other than the liver can be ignored. Also, metabolism of lactate through the Cori (glucose-lactate) cycle takes place exclusively in the liver.[67,68] This process, as in the case of pyruvate, does not produce net glucose gain. It is, however, extremely important in glucose homeostasis, and even more so during anaerobic glycolysis, to remove excess lactic acid by conversion to $CO_2$ through glucose and nett gain of energy. The liver is the major site for the citric acid cycle in metabolism of pyruvate formed during anaerobic glycolysis in all tissue, although this can occur in all mitochondria.[65]

Hypoglycaemia is a theoretical possibility during the anhepatic phase in liver transplantation, when the only source of glucose-6-phosphate (liver) is absent. It was suggested that glucose containing solutions be infused during the procedure to prevent this condition.[69].

This concept was challenged by a proposition that infused glucose might add to the glucose load given during massive transfusion with development of a hyperglycaemic (hyperosmolar) state in the postreperfusion period.[70]

Hyperglycemia has been shown to occur commonly after liver transplantation, and it has been suggested that glucose was released from the reperfused liver while infused glucose attributed nothing to observed hyperglycaemia. Underutilisation of glucose due to unrecognised hypothermia might also lead to increased blood levels.[71]

The role of the transplanted liver in changes relating to glucose homeostasis, during and after reperfusion, has not been clarified.

LACTIC ACID.

In patients considered for liver transplantation, end stage liver disease is the cause of multiple metabolic derangements, amongst which is a varying degree of lactic acidosis.[75] The formation of serum lactic acid under normal circumstances occurs because of anaerobic glycolysis, of which there is always some degree, resulting in a normal level in humans of ±2 mMol/L. Lactic acid is produced by muscle, skin, gut, brain and erythrocytes. Excess lactic acid is removed by the liver through gluconeogenesis in the Cori cycle. The kidney can also excrete excess lactic acid but functions mainly as a "backup" system.[76]

It has been shown that after low flow states and haemorrhage, up to 90% of lactic acid removal is effected by the liver, and lactic acid blood levels as well as rate of decline thereof, have been used in the prediction of survival after occurrence of low flow states. Liver function therefore is a major modifier in the regulation and evaluation of blood lactic acid levels.[77]

During storage of the donor liver anaerobic metabolism continues and it is likely that lactic acid is formed in the stored liver.[78] In the liver transplant patient, circulating lactic acid is increased as part of the disease process necessitating transplantation, as well as lactic acid flushed out of the liver upon reperfusion.

The concept of "lactate washout" indicates a paradoxical increase in lactic acid concentration after

restoration of flow. The duration of this phenomenon after liver transplantation has not been established. It has been shown in pigs with normal liver function, after controlled cardiac arrest and reflow, that this time period was less than two minutes and that the rate of decrease in lactic acid concentration correlated with survival.[80]

Anaesthesia in patients with hepatic failure may cause a rise in lactic acid by interference with the hyperdynamic state.[75] In this quoted study it has been shown that anaesthesia abolishes the hyperdynamic state, causing hypoperfusion of peripheral tissue with resultant local tissue hypoxia, and an increase in lactic acid levels, both pre- and post-perfusion of the transplanted liver. The resultant metabolic acidosis can further impair hepatic clearance of lactates.[87]

In the experimental model the complexities of preexisting hepatic disease are excluded and anaesthesia is standardised; the effect of storage and transplantation on lactic acid metabolism can thus be evaluated.

CHAPTER 4.

# COAGULATION IN LIVER TRANSPLANTATION

A discussion of the theoretical basis of the clotting mechanism and the effect of liver transplantation upon derangements thereof. Specific reference is made to heparin.

## COAGULATION and HEPARIN.

Haemostasis is the state in the living animal where coagulation and thrombolysis are in equilibrium. Coagulation consists of a series of linked proteolytic enzymatic reactions with the aim of converting fibrinogen into fibrin monomers by the action of thrombin. It is an ongoing process with built in regulation as will be referred to later. The possible mechanism had been postulated by Moravitz in 1905 but definite proof of its existence was only supplied by Seegers in 1940.[105]

The concept of the "cascade" mechanism was established in 1964 by two separate groups.[91,92] Numerous new components, as well as hitherto unknown, but postulated substances in the coagulation process, have been identified. The integral role of platelets and the importance of the platelet release reaction, through various factors released, is well accepted. [93] The basic mechanism is an extremely complex sequence of biochemical events, with inherent positive and negative feedback systems. The role of various co-factors such as $Ca^{++}$, Factor V, Factor VII, prekallikrein and HMW kininogens complicates the understanding of the molecular events, at which levels the basic process of coagulation and its control, takes place. The waterfall concept consists of an extrinsic and intrinsic pathway cascading down onto a common pathway ending with the

formation of fibrin. This mechanism is in dynamic flux at all times.

The intrinsic mechanism is initiated by the interaction of Factor XII with a negatively charged surface to form F XIIa. The hepatocyte is the only source of Factor XII.[94] Activated F XII proteolyses Factor IX in a two step reaction to F IXa. This step is absolutely quantitatively dependent upon $Ca^{++}$, although $Mg^{++}$ may be utilised in the absence of $Ca^{++}$. When $Mg^{++}$ acts as a co-factor, the activation rate is slower. Excess $Mg^{++}$ may competitively inhibit the action of $Ca^{++}$, resulting in a decreased clotting activity.[95]

The extrinsic mechanism is initiated by tissue factor (TF) which has to be released from damaged tissue. Sources are monocytes activated by endotoxin, antigen-antibody complexes and products of complement activation, as well as the platelet release reaction. Tissue factor consists of lipoprotein:phospholipid complex in a ratio of less than 1:450 with an average MW of 43000. This ratio is essential to ensure maximal activation of Factor VII. Factor VII is an obligatory co-factor for TF.[96]

A common pathway exists for the conversion of Factor X to F Xa, which is the prothrombin converting serine protease. Conversion of F X can only take place on the platelet surface. The rate of this reaction is dependent on the presence of Factor VIII. F VIII increases the velocity of F X formation 200,000 times by aligning F X

on the platelet surface for maximum exposure of binding sites. Thrombin will then in complex concert with Factor V act upon fibrinogen to form fibrin.[97]

Haemostasis during and after liver transplantation is of the utmost importance in the clinical setting. Patients undergoing liver transplantation may have grossly deranged haemostatic profiles, which is most often the result of pre-existing liver disease.[98] Surgical bleeding from inadequate haemostasis during dissection, in the portal hypertensive patient, may start a consumption coagulopathy that will lead to uncontrollable bleeding when the replaced liver can not immediately supply essential clotting factors.[99]

In early clinical and experimental liver transplantation heparin was used systemically in the recipient, as well as in preservation fluids, with complete absence of clotting ability on reperfusion: Although heparin is not used routinely during clinical hepatic transplantation currently, even with the use of veno-venous bypass, enough heparin might enter the circulation, through flushing of monitor and access lines, to cause heparin intolerance.[98,100] The role of "endogenous" heparin has not yet been clarified and this or so called "heparin like" substances may play a role in the bleeding tendency of some patients.[101]

The role of heparin in bleeding after liver transplantation has recently been studied and it was concluded that, although not directly responsible, the administration of heparin together with dissection and handling of the liver may precipitate thrombolysis.[153]

Hypercoagulability of blood may also complicate this picture, especially where surgery is performed in the presence of hepatic tumours.[102] This phenomenon may be implicated in the pathology of hepatic artery thrombosis, but should not have an influence in the experimental situation where young healthy animals are used.[103]

Thus it can be postulated that in experimental liver transplantation, if standardised minimal heparin dosages are used in healthy animals, the changes seen in haemostatic parameters, should be due to the effect of the procedure and of storage.

CHAPTER 5.

# TECHNIQUE OF PORCINE LIVER TRANSPLANTATION.

A detailed description of the technique, for harvesting and transplantation, of the pig liver, is presented. The novel technique of autotransplantation developed in this laboratory by Hickman is discussed.

TECHNIQUE IN THE PIG.

The method of harvesting and transplantation of the liver was largely an adaptation of the techniques described by Terblanche, Hickman and Dent [2,3,4]. All animals in all groups were treated in exactly the same fashion to ensure that a uniform approach was applied.

DONOR OPERATION.

Young pigs of either sex weighing between 20 and 30 kg were starved for 24 hours, but had free access to water. Anaesthesia was induced with sodium pentothal (2-3 mg/kg) into an ear vein. An endotracheal tube of adequate size was inserted and $O_2$ and $N_2O$ used by intermittent positive pressure ventilation at 25 cm $H_2O$ to maintain anaesthesia. Halothane was not used at all. A 3.5 to 4 cm longitudinal incision parallel to the posterior border of M.Sternocleidomastoideus was made to approach the external or internal jugular vein for insertion of an intravenous line to administer 500ml 10% dextrose in balanced salt solution (Maintelyte ®)[Table 3] solution during the harvesting procedure.

A long midline incision was made from xiphisternum to ± 4 cm above the symphisis pubis using cutting diathermy. A self retaining retractor (Balfour) was positioned. The small and large bowel were placed in a plastic bag.

The right and left coronary ligaments of the liver were divided by diathermy. The left lobe of the liver was retracted to the right, and the left triangular ligament also divided.

With downward traction on the greater curve of the stomach the gastro-hepatic ligament was displayed. The bile duct was identified and with sharp dissection this was separated from the posteriorly situated portal vein; care was taken not to disrupt the loose areolar tissue around the bile duct.[5] The anterior peritoneal leaf of the gastro-hepatic ligament was picked up to the left of the bile duct and divided cranially and to the left. The left gastric artery was now clearly visible and was ligated and divided. The posterior leaf of the gastro-hepatic ligament was now divided caudally down to the origin of the coeliac axis. The hepatic artery was usually surrounded by lymph nodes in this region but could be demonstrated easily. All small branches to lymph nodes were carefully cauterised, taking care not to damage the hepatic artery proper, and lymphadenectomy was performed. During this dissection the origin of the gastro-duodenal artery from the right was usually easily noticed. This artery was carefully tied about 3 mm from the hepatic artery. The hepatic artery was separated from the portal vein to the origin of the splenic artery.

The portal vein was now approached from the right and dissected free. The pancreatic vein, which was usually easily visible just above the upper border of the pancreas, draining into the anterior aspect of the portal vein was ligated and divided. In autograft procedures this vein was always preserved.

The right lobe of the liver was now gently retracted to the left to display the infrahepatic vena cava. A double layer of peritoneum, extending from proximal to the adrenals glands up towards the diaphragm, and from the inferior vena cava to posterior abdominal wall, was divided by diathermy.

At this stage $50^{u/kg}$ of sodium heparin was given intravenously. The common bile duct was transected as distal as possible. After a minimum time delay of 4 minutes ( circulation time for heparin distribution) an 18F catheter was inserted into the portal vein in experiments where a flush was indicated. Otherwise a vascular clamp was placed at the level of the superior border of the pancreas. In rapid sequence the hepatic artery was clamped as proximal as possible and transected. A curved vascular clamp was placed on the infrahepatic vena cava at the level of the adrenal glands. A large curved (Satinsky) vascular clamp was placed as far cranially as possible on the suprahepatic vena cava including diaphragm and the vena cava was cut on the junction with the diaphragm.

The infrahepatic vena cava was now transected on the clamp and the flushing of the liver immediately started through the portal vein catheter. The liver was removed from the abdominal cavity and flushing completed. In all cases the total volume of flush solution was 1000 ml.

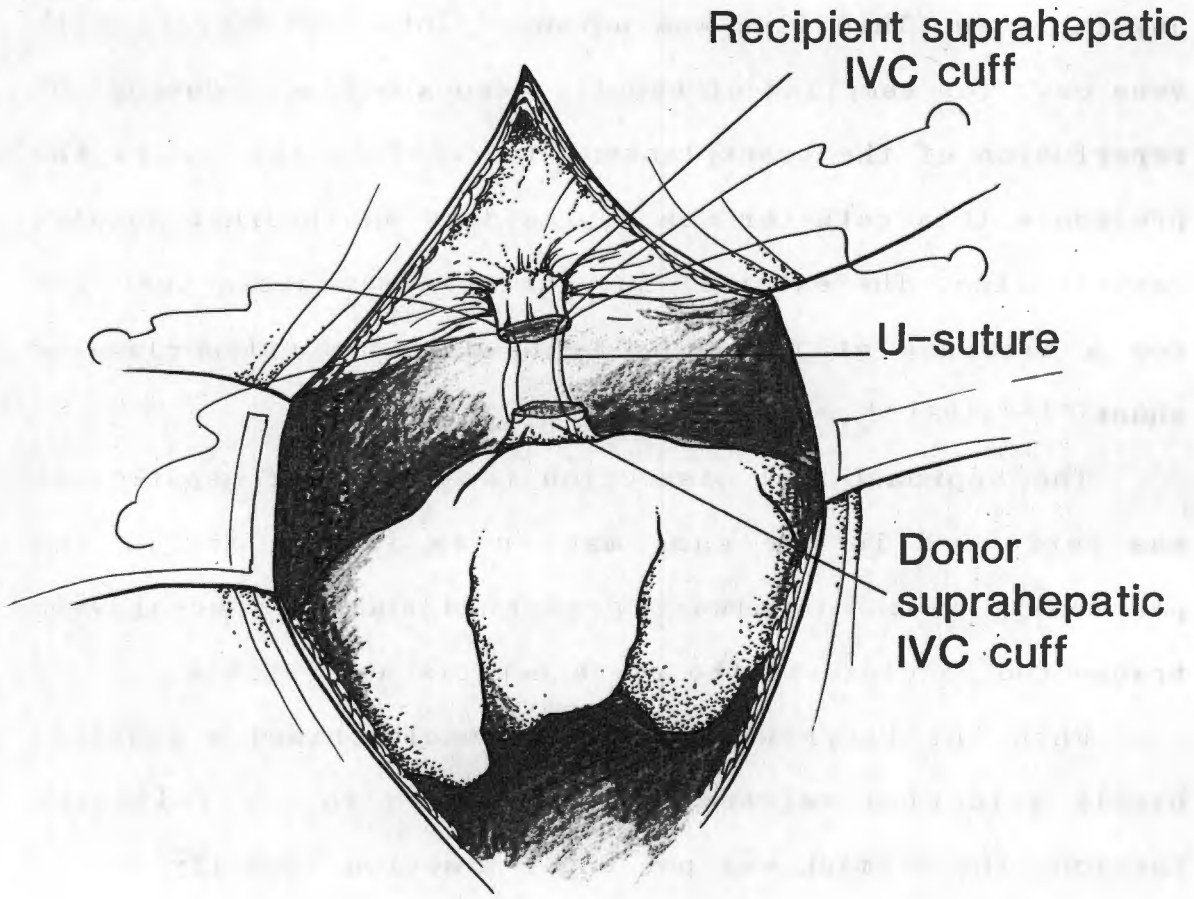
In the autograft groups this technique was modified in the sense that all transections were done at convenient midway points. Special attention was given to preservation of the pancreatic vein draining into the portal vein. Also, the hepatic artery was transected distal to the gastroduodenal artery, rather than more proximal as in the allograft. Atraumatic vascular clamps were always used on all vessels

RECIPIENT OPERATION.

Dissection in the neck was similar as in the donor, except that a catheter was placed in the common carotid artery for monitoring of the systolic blood pressure. The intravenous central line was always put into the internal jugular vein. This line was advanced into the suprahepatic vena cava for sampling of hepatic venous effluent during the reperfusion of the transplanted liver.[167] At the end of the procedure this catheter was replaced as an internal jugular central line. The external jugular vein was dissected free for a distance of 2-3 cm to be used in the spleno-jugular shunt(vide infra).

The approach and dissection for recipient hepatectomy was performed in the same manner as in the donor. The pancreatic vein was always preserved and the portal vein transected as close to the porta hepatis as possible.

When the liver was completely skeletonised a modified highly selective vagotomy was performed in the following fashion. The stomach was put under traction caudally and to the right, by grasping the greater curve on the gastric tube, to display the nerve of Latarjet. The peritoneum was picked up at the level of the incisura and divided along the lesser curve to the level of the gastro-oesophageal junction. Anterior highly selective vagotomy was performed by ligation and sharp division. The lesser sac was now entered through the gastro-colic ligament and a posterior truncal vagotomy performed.




"PARACHUTING" OF DONOR LIVER

Sodium heparin ($25^{U/kg}$) was administered and a passive spleno-jugular shunt inserted as follows. The splenic vein was isolated at the point where it emerges posterior to the pancreas and through a transverse venotomy a 18F silastic catheter placed and secured by 2/0 cotton ligatures. A T-connection to a 1L bag with 0.9% saline and the external jugular vein was set up. Free flow was observed through this venous shunt with an open portal vein. Only then were all vessels clamped seriatim starting with the hepatic artery, followed by portal vein, infrahepatic inferior vena cava and lastly the subdiaphragmatic vena cava; transection of the vessels was performed in the same sequence. All vessels and ducts were cut in a mirror image fashion to those in the donor operation.

After removal of the liver the abdominal cavity was inspected for any bleeding points and if present, these were controlled by ligation or cautery. The replacement liver was now positioned and the suprahepatic vena cava sutures (5/0 polypropylene) placed in a "U" fashion.*** Diagram opposite page.

The liver was then "parachuted" into position. This method of suture placement ensured that no corner leakage occurred in any case as well as endothelium to endothelium anastomosis. The posterior wall was sutured from inside followed by the anterior wall.*** Diagram The portal anastomosis was now performed with exactly the same method

but utilising 6/0 suture material. The spleno-jugular shunt was clamped and the portal clamp released to allow portal venous reperfusion. The liver was allowed to fill with blood and ±30 ml blood allowed to escape from the lower inferior vena cava before it was clamped.

The clamp on the suprahepatic vena cava was released and the effluent of blood controlled over a period of 5-10 seconds to prevent a sudden rush of hyperkalaemic blood into the systemic circulation. The clamp was then completely removed. The infrahepatic vena cava anastomosis was now performed with 5/0 polypropylene thread. The proximal of the two clamps was removed first to vent the small amount of air (less than 1 ml), that was usually present in this venous segment.

The hepatic arterial segments were trimmed to ensure that there were neither excess vessel nor anastomotic tension and the anastomosis performed with continuous 7/0 polypropylene suture. The shunt catheter was removed from the splenic vein and this repaired with 6/0 polypropylene. End to end anastomosis of the bile duct was effected with the same suture material.

A final inspection of the abdominal cavity was done to establish that there was adequate haemostasis. Mass two layer closure of the abdominal wall was effected with 1 chromic and 2/0 nylon sutures respectively.

CHAPTER 6.

# MATERIALS AND METHODS.

## 6.1. Groups

**Autograft without flushing.**

**Autograft with flushing.**

**Coldstorage with Collins solution**

**Coldstorage with U.W. solution.**

**Coldstorage without any solution.**

## 6.2. Management of animals.

## TABLE 1.

**Collins $C_3$ solution.**

| | |
|---|---|
| 1. $KH_2PO_4$ | 2.05 gm/L |
| 2. $K_2HPO_4 . 3H_2O$ | 9.7 gm/L |
| 3. KCL | 1.12 gm/L |
| 4. $NaHCO_3$ | 0.84 gm/L |
| 5. Procaine HCl | 0.10 gm/L |
| 6. Glucose | 25 0 gm/L |
| 7. $MgSO_4 . 7H_2O$ | 7.38 gm/L |

This solution did not contain any heparin.
Components 5, 6 and 7 were added immediately before use.

## TABLE 2.

**University of Wisconsin solution.**

| | |
|---|---|
| Hydroxy-ethyl-starch (Pentafraction) | 50.0 gm/L |
| Lactobionic acid | 35.83 gm/L |
| Potassium phosphate monobasic | 3.4 gm/L |
| Magnesium sulfate heptahydrate | 1.23 gm/L |
| Raffinose pentahydrate | 17.83 gm/L |
| Adenosine | 1.34 gm/L |
| Allopurinol | 0.136 gm/L |
| Glutathione (Reduced) | 0.92 gm/L |
| Potassium hydroxide | q.s.}to adjust |
| Sodium hydroxide | q.s.}pH to 7.4 |
| Water for injection | Volume to 1000 ml. |

Additions prior to use:

| | |
|---|---|
| Dexamethasone | 16 mg/L |
| Insulin | 40 U/L |
| Co-trimoxasole | 5 ml/L |

No heparin is used in this solution.

## TABLE 3.

**Maintelyte.(SABAX)**

| | |
|---|---|
| Sodium | 35mmol/L |
| Potassium | 25mmol/L |
| Magnesium | 2.5mmol/L |
| Chloride | 65mmol/L |
| pH | 4 |
| Osmolarity | 683mosmol/L. |

## 6.1 GROUPS.

5 Groups of animals were used as follows.

Autograft without flushing.

(Group 1)

In six animals the livers were completely dissected free, all the relevant vessels as well as the bile duct were transected and the liver removed out of the abdominal cavity and immediately re implanted.

Autograft with flushing.

(Group 2)

In seven animals the liver was dissected free, the portal vein cannulated, all relevant vessels and bile duct transected, the liver removed and flushed ex situ with one litre of lactated Ringers solution at $4^0$C. The liver was immediately re-implanted.

Allograft coldstorage with Collins solution.

(Group 3)

In seven animals transplantation was performed with a liver flushed through the portal vein (cannulated in situ), immediately after removal with one litre of modified Collins $C_3$ solution (no heparin) (Table 1) at $4^0$C and stored in a plastic bag, containing one litre of the same, in an outer plastic bag containing one litre of lactated Ringers solution, on crushed ice for six hours.

Allograft after coldstorage with U.W. solution.

(Group 4)

In seven animals transplantation was performed with a liver flushed through the portal vein (cannulated in situ), immediately after removal with one litre of University of Wisconsin solution Table 2 at 4°C and stored in a plastic bag, containing one litre of the same, in an outer plastic bag containing one litre of lactated Ringers solution, on crushed ice for six hours.

Allograft after coldstorage without flushing.

(Group 5)

In five animals transplantation was performed with a liver harvested and stored on crushed ice for six hours. The liver was dissected free, removed and put into a double plastic bag. The inner bag contained only the liver whilst the outer bag contained one litre of lactated Ringers solution to prevent direct contact of the liver with the crushed ice.

No liver, in any of the groups, was flushed out with any type of solution prior to reperfusion, except the controlled escape of ±30ml blood as described.Chapter 3: Technique in recipient.

## 6.2 MANAGEMENT OF RECIPIENTS.

(INTRA AND POSTOPERATIVE)

An infusion of Maintelyte ® was started as soon as the intravenous line had been inserted. This was run at 1ml/min during the entire procedure. A 4.5% solution of $NaCHO_3^-$ was set up to administer 25 mEq of $NaCHO_3^-$ during the anhepatic phase. The spleno-jugular shunt was very slowly infused with 0.9% NaCl solution at 3-5 drops (10 drops per ml) per minute. The total volume of crystalloid fluid amounted to ±500 ml during the first six hours. Another 1500 ml Maintelyte ® was given in the first 24 hours to a total of 2000 ml.

Sampling for the following determinations was done.

1. Blood for pH, $pCO_2$, $pO_2$, $HCO_3^-$, Base excess, $Na^+$ and $K^+$.
2. Activated clotting time on whole blood.
3. Serum aspartate and alanine transaminases.
3. Serum lactates.
4. Serum bilirubin.
5. Full clotting profile.
6. Total and ionised calcium.
7. Total magnesium.
8. A biopsy of about 2 gm of liver tissue for tissue calcium determination.

The sampling schedule was as follows.

1. Pre-hepatectomy. At ± 10 minutes after initiation of intermittent positive pressure ventilation.

2. Five minutes after restitution of portal flow. At this point the liver had been perfused with mesenteric venous blood for ± 5 minutes.

3. Five minutes after restitution of vena cava flow. The pooled blood from the lower systemic circulation (legs and renal) was back into circulation.

4. Five minutes after hepatic arterial anastomosis (full hepatic revascularisation ).

5. Thirty minutes after full revascularisation.

6. Six hours after full revascularisation.

7. A total of 26 ml of blood was taken daily for biochemical analysis for 7 days as set out. Vide supra

At time points 1 through 5, samples were obtained from the hepatic vein catheter for determinations 1, 6 and 7. See: Chapter 6: Sampling schedule. In group 1 this was not done at all and in groups 2 and 3 in only three animals in each group.

It should be noted that the actual time between sampling points was more than Five minutes; Timepoint (TP) 1 at 10 minutes into the operation, TP 2 at ±40 minutes, TP 3 at ±50 and TP 4 at ± 90 minutes after starting anaesthesia.

No blood or plasma was given at any point during the procedure. Co-trimoxasole (5ml) was given in the first litre of Maintelyte ® and daily thereafter for three days. No liver was flushed prior to being revascularised. During the second 24 hours after transplantation, 1 litre of Maintelyte ® was given. No parenteral fluid was given after the second day.

No immunosuppression was given at any stage.

CHAPTER 7.

# RESULTS

## 7.1 SURVIVAL OF ANIMALS.

## 7.2 BLOOD AND SERUM MEASUREMENTS

## 7.1 SURVIVAL OF ANIMALS.

Six animals in each group survived for seven or more days. The simple cold storage allograft and Ringer lactate flushed autograft groups had six consecutive survivors. In all recipient animals total operating time was less than $1^1/_2$ hours. Operating time in autograft procedures was comparable to that in the allografted animals. Recipient hepatectomy on average took 15 minutes whilst highly selective vagotomy and placement of the spleno-jugular shunt required 10 to 12 minutes. Anhepatic time (until restoration of portal flow) was always less than 15 minutes. Arterial anastomoses and splenic vein repair were always performed with magnification. The same sequence of anastomosis was used in all transplants.

No technical failure occurred. There were no incidences of gastric ulceration. Hepatic artery thrombosis did not occur. Pigs that died before seven days ( three ) did not have a discernible cause but metabolic parameters indicated non function of the grafted liver. All animals that died were in severe irreversible metabolic acidosis, with low bicarbonate and high lactic acid levels. Haemorrhage was not a cause of death in these pigs, nor did any coagulopathy occur.

## 7.2 BLOOD AND SERUM MEASUREMENTS.

In description of the changes occurring in laboratory data extensive use will be made of reference sampling points designated as TP (timepoints). For convenience sake an abbreviated key is presented as a footnote on each page that contains descriptions relating to changes in laboratory values at timepoints. The full description is as follows. Characters in parentheses are those used in graphs to describe timepoints on the X-axis.

TP 1 (PRE.) This is also referred to as baseline value. Pre-hepatectomy. At ± 10 minutes after initiation of intermittent positive pressure ventilation.

TP 2 (5'P.V.) Five minutes after restitution of portal flow. At this point the liver had been perfused with mesenteric venous blood for five minutes.

TP 3 (5'I.V.C.) Five minutes after restitution of infrahepatic inferior vena cava flow. The pooled blood from the lower systemic circulation (legs and renal) was back into circulation.

TP 4 (5'H.A.) Five minutes after opening hepatic arterial anastomosis (hepatic revascularisation).

TP 5 (30'Revas) Thirty minutes after full revascularisation.

TP 6 (6 HR) 6 hours after full revascularisation.

Day 1-6. Refers to postoperative day. Day 1 denotes 24 hours after transplant.

Following description of changes in each measured parameter, a graphic representation of changes in autograft and allograft groups will be presented. The X-axis represents time. It needs to be noted that the reference points on the X-axis are not to scale, but are presented in this manner to highlight the acute changes that do take place early in the procedure, during and following revascularisation.

All results were subjected to statistical evaluation by means of Student's t-test, both in groups with reference to baseline values and between groups at comparable timepoints. All statistically significant differences (by t-test) were also subjected to non-parametric evaluation before being reported as significantly different. Statistical calculations were done by hand, by the author utilising a Hewlett-Packard C11 scientific calculator. Differences between mean values with a p-value of $< 0.05$ were regarded as statistically significant.

CHAPTER 8.

# CHANGES IN BIOCHEMICAL VALUES.

8.1. Calcium.

8.2. Magnesium.

8.3. Acid base metabolism.

pH.

Bicarbonate.

Sodium.

Potassium.

8.4. Liver function.

Enzymes.

Aspartate aminotransferase.

Alanine aminotransferase.

Proteins.

Total protein.

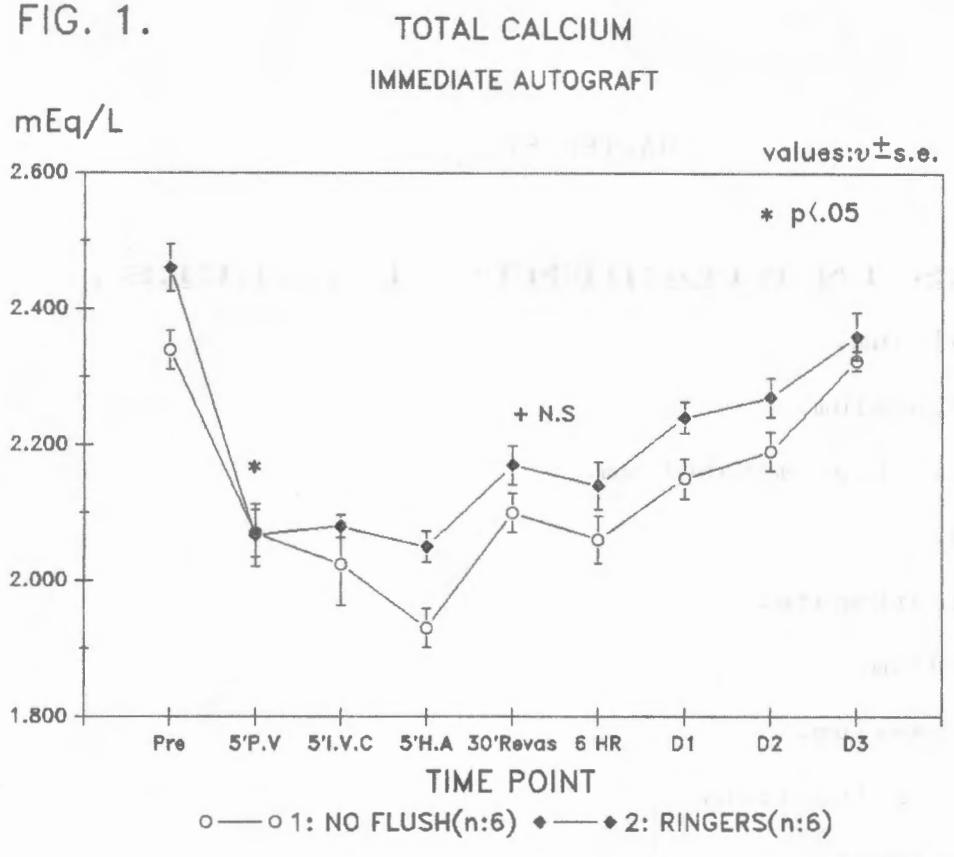
Albumen.

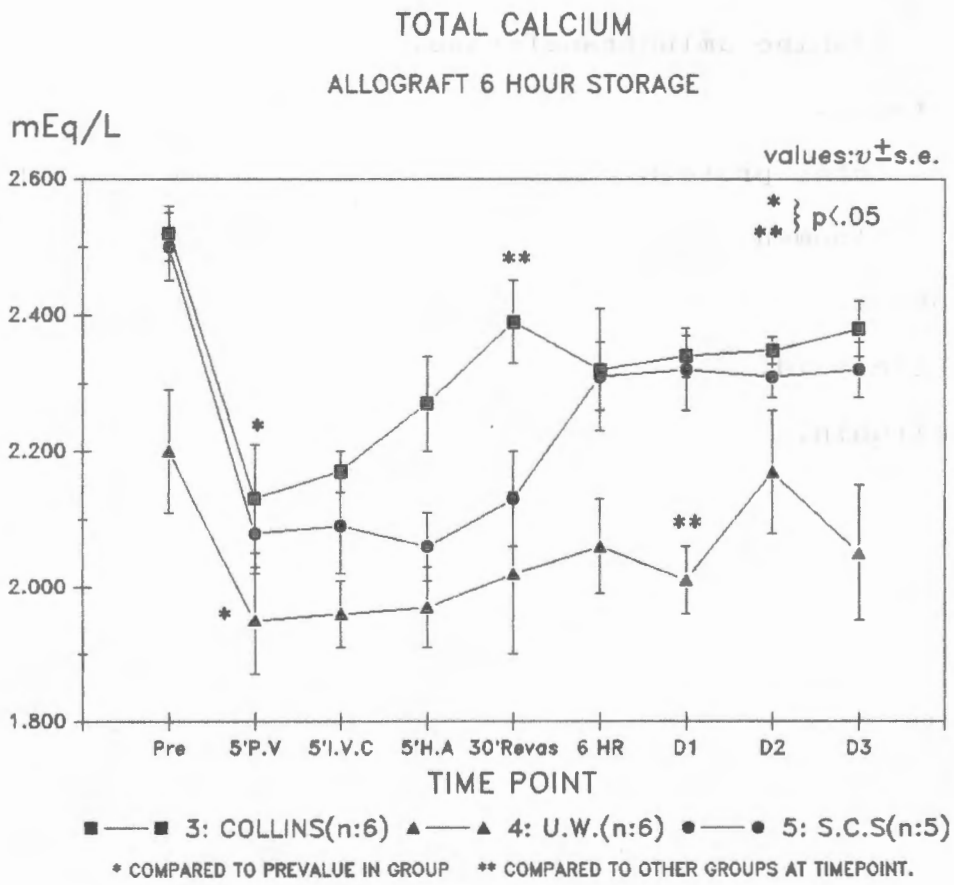
Glucose.

Lactic acid.

Bilirubin.

FIG. 1.


TOTAL CALCIUM
IMMEDIATE AUTOGRAFT
mEq/L
values:υ±s.e.
* p<.05
+ N.S
2.600
2.400
2.200
2.000
1.800
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
6 HR
D1
D2
D3
TIME POINT
1: NO FLUSH(n:6)
2: RINGERS(n:6)


TOTAL CALCIUM
ALLOGRAFT 6 HOUR STORAGE
mEq/L
values:υ±s.e.
* ** } p<.05
2.600
2.400
2.200
2.000
1.800
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
6 HR
D1
D2
D3
TIME POINT
3: COLLINS(n:6)
4: U.W.(n:6)
5: S.C.S(n:5)
* COMPARED TO PREVALUE IN GROUP
** COMPARED TO OTHER GROUPS AT TIMEPOINT.

8.1 TOTAL CALCIUM.

Arterial: Fig.1.

Mean baseline values in 4 groups (1,2,3 and 5) lay between 2.34±0.12 and 2.52±0.12 mmol/L. Only in group 4 (storage in UW solution) was the mean baseline value lower at 2.20±0.23. This difference was not statistically significant and could not be attributed to anything specific. There was a decline in calcium in all groups at TP 2,3 and 4 which was significantly ($p<0.05$) lower than baseline. This represented a fall of 11.3 - 16.8% in various groups.

In group 3 (Collins) by TP 3 and group 5 (S.C.S) by TP 4, total calcium values had returned to almost normal and remained so for the rest of the study.

In groups 1, 2 and 4 there was a slower return to normal, although from TP 5 and 6, the values were not significantly reduced.

TOTAL CALCIUM.

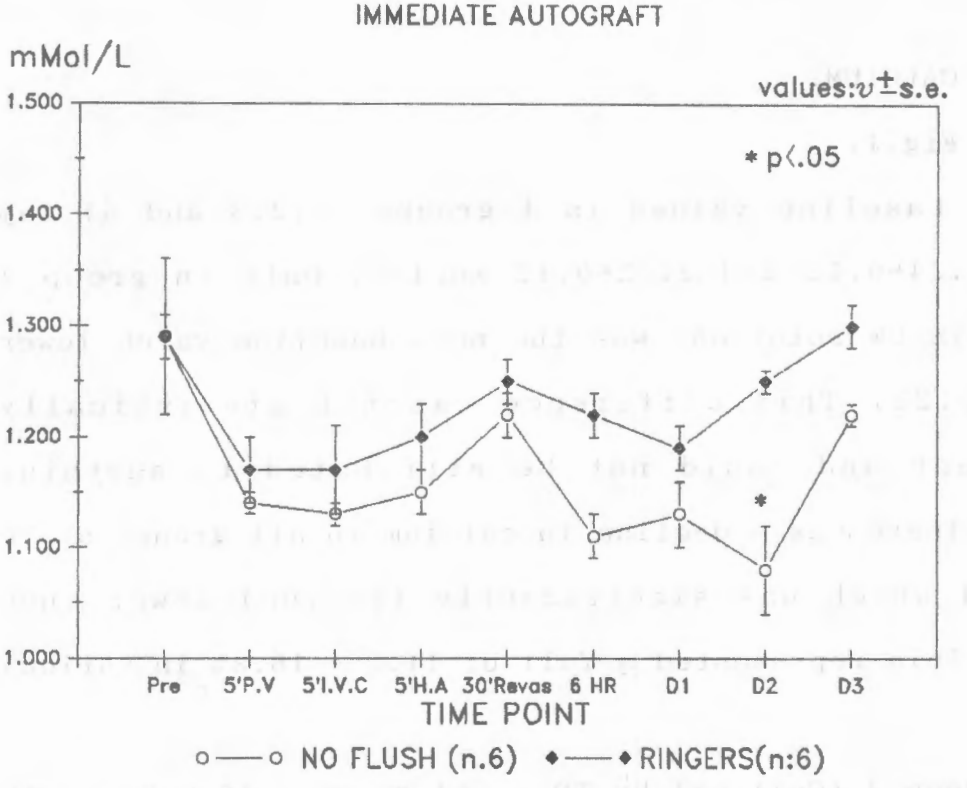
Venous Not shown

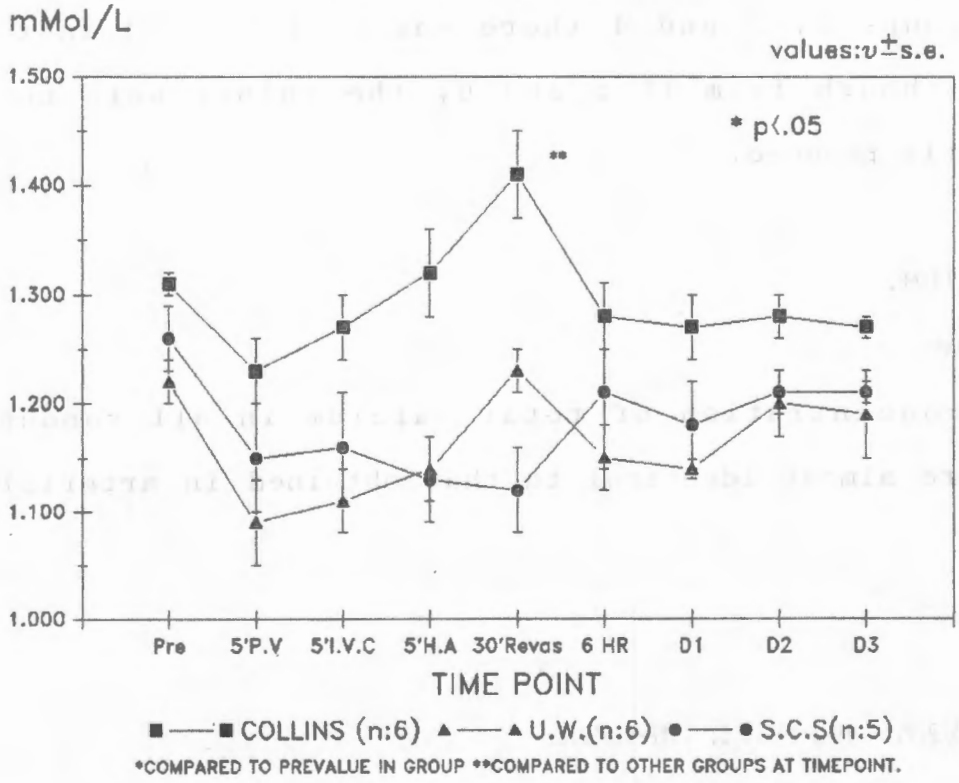
The concentration of total calcium in all venous samples were almost identical to that obtained in arterial blood.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 2.




*COMPARED TO PREVALUE IN GROUP **COMPARED TO OTHER GROUPS AT TIMEPOINT.

IONISED CALCIUM.

Arterial: Fig.2. .

Mean baseline arterial values ranged between 1.22±0.17 and 1.31±0.02 mmol/L in all groups except 4, where values were again lower as with total calcium. However, the ratio of total to ionised calcium was similar in all groups.

The pattern of decline of ionised calcium after revascularisation occurred in all groups. The return towards normal was similar in groups 2, 4 and 5. In group 1, there was a continued decline, which was highly significant on day 2. In group 3, mean values exceeded baseline value at TP 5, but then returned to baseline levels.

IONISED CALCIUM.

Venous Not shown.

Baseline values were almost identical to those from arterial samples. Values at TP 2 showed the same percentage point fall as in arterial samples. Values at time points 3 to 5 were showing an upward trend to have higher than baseline values in groups 2, 3 and 4 at TP 5, although not statistically significant.

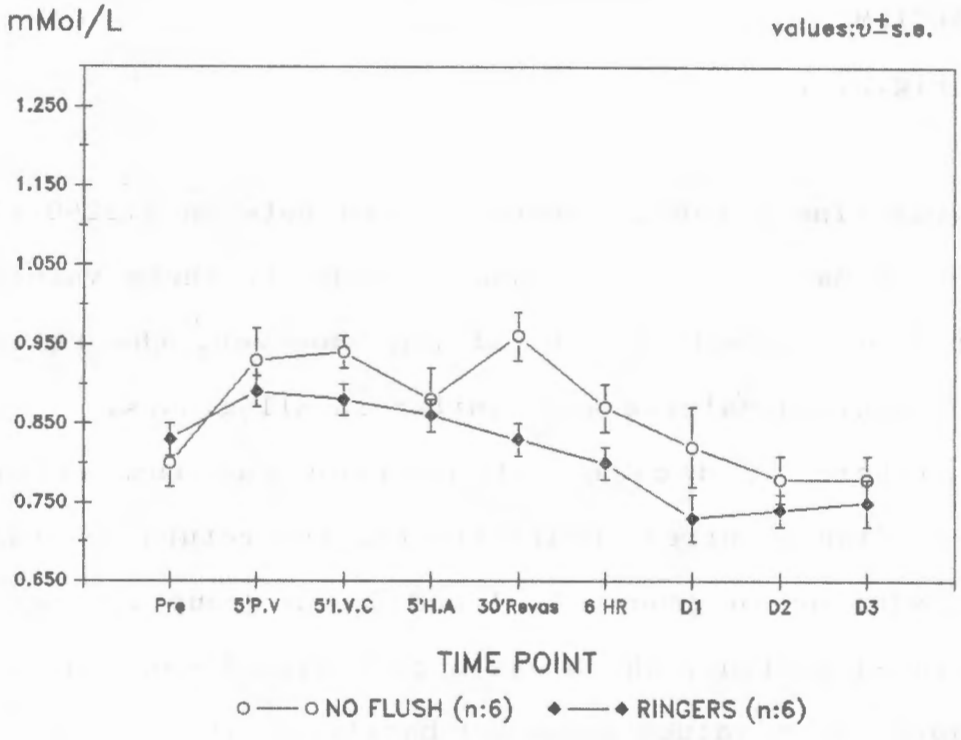
TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 3.

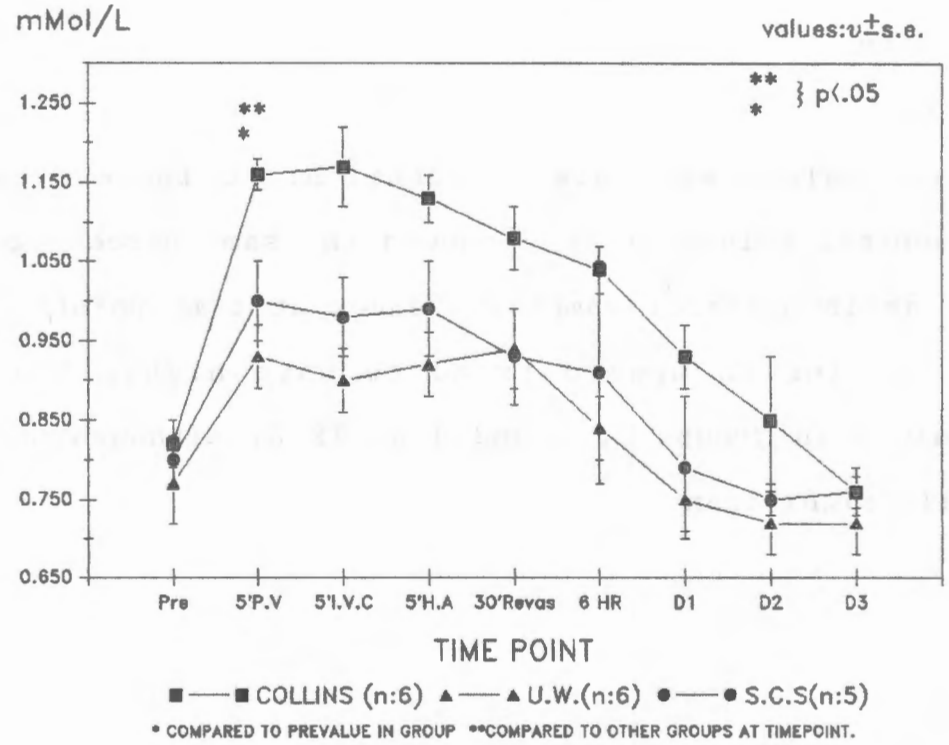
TOTAL MAGNESIUM

IMMEDIATE AUTOGRAFT




TOTAL MAGNESIUM

ALLOGRAFT 6 HOUR STORAGE




* COMPARED TO PREVALUE IN GROUP **COMPARED TO OTHER GROUPS AT TIMEPOINT.

8.2 TOTAL MAGNESIUM.

Arterial:Fig.3.

Mean baseline values were within the range 0.77±0.13 to 0.83±0.07 mmol/L. There were limited increases at TP 2, 3 and 4 in groups 1, 2, 4 and 5. Thereafter values declined to or slightly below baseline.

In group 3, there was a highly significant increase in total magnesium at TP 2, 3, 4 5 and 6, which only returned towards baseline on day 2.

TOTAL MAGNESIUM.

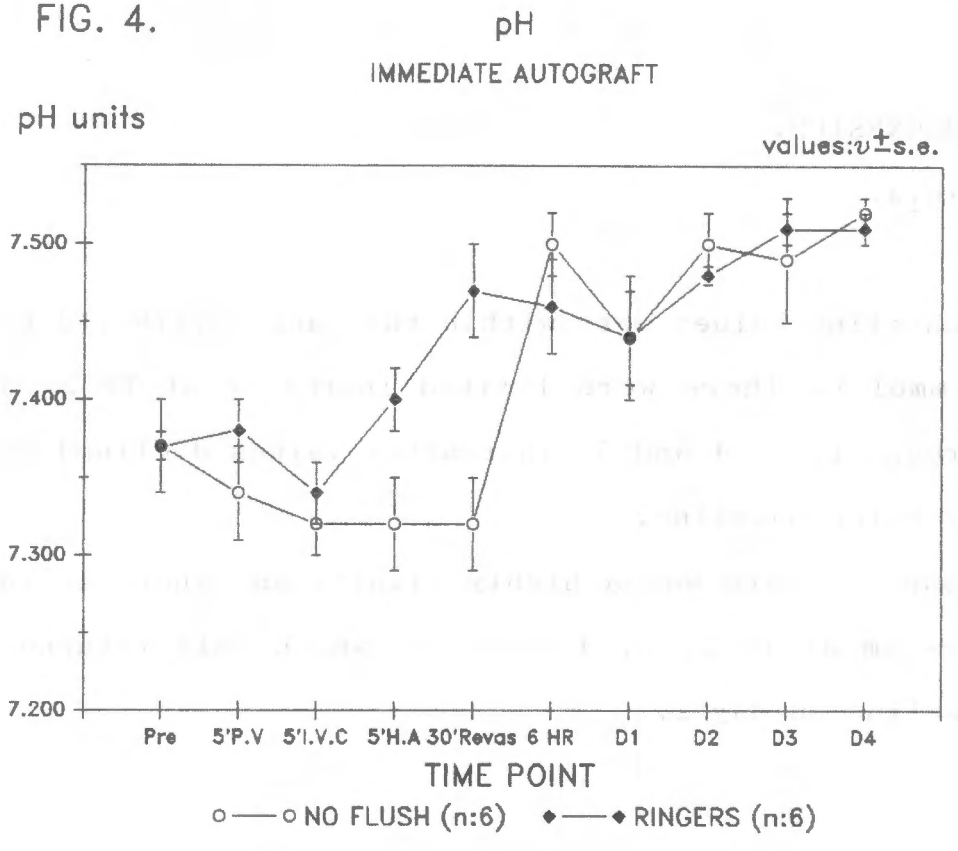
Venous Not shown

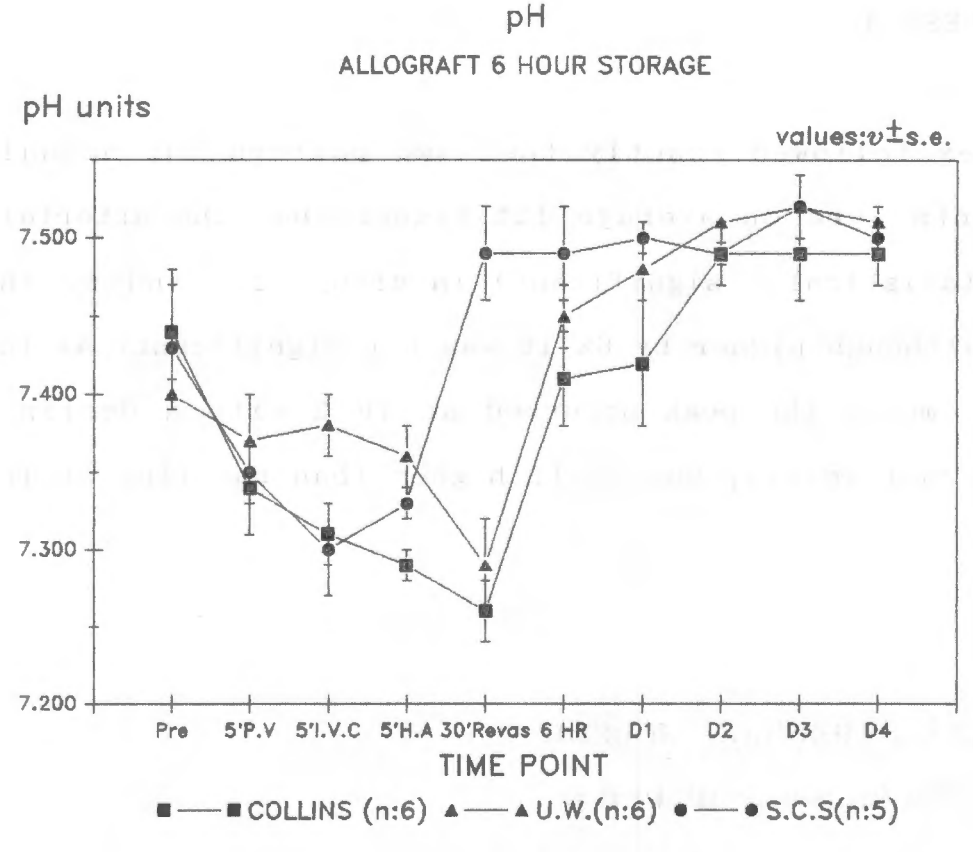
Values followed exactly the same pattern but actual measurements were on average 12% higher than the arterial values (statistically significant) in groups 2,4 and 5. In group 3, although higher by 6% it was not significant. As in arterial samples the peak occurred at TP 2 with a decline towards normal levels, but still higher than baseline at TP 5.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 4.


pH
IMMEDIATE AUTOGRAFT
pH units
values:v±s.e.
7.500
7.400
7.300
7.200
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
6 HR
D1
D2
D3
D4
TIME POINT
NO FLUSH (n:6)
RINGERS (n:6)


pH
ALLOGRAFT 6 HOUR STORAGE
pH units
values:v±s.e.
7.500
7.400
7.300
7.200
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
6 HR
D1
D2
D3
D4
TIME POINT
COLLINS (n:6)
U.W.(n:6)
S.C.S(n:5)

## 8.3 ACID BASE METABOLISM.

### pH CHANGES.

Arterial: Fig.4.

The baseline pH values ranged between 7.37 and 7.44. There was a decline of varying degrees between TP 2 and 4 in all groups. In groups 1, 3 and 4 this decline continued throughout revascularisation, but in groups 2 and 5 the levels increased above baseline from TP 4.

By TP 6 all values had increased to or above baseline and remained so for the rest of the study.

### pH CHANGES.

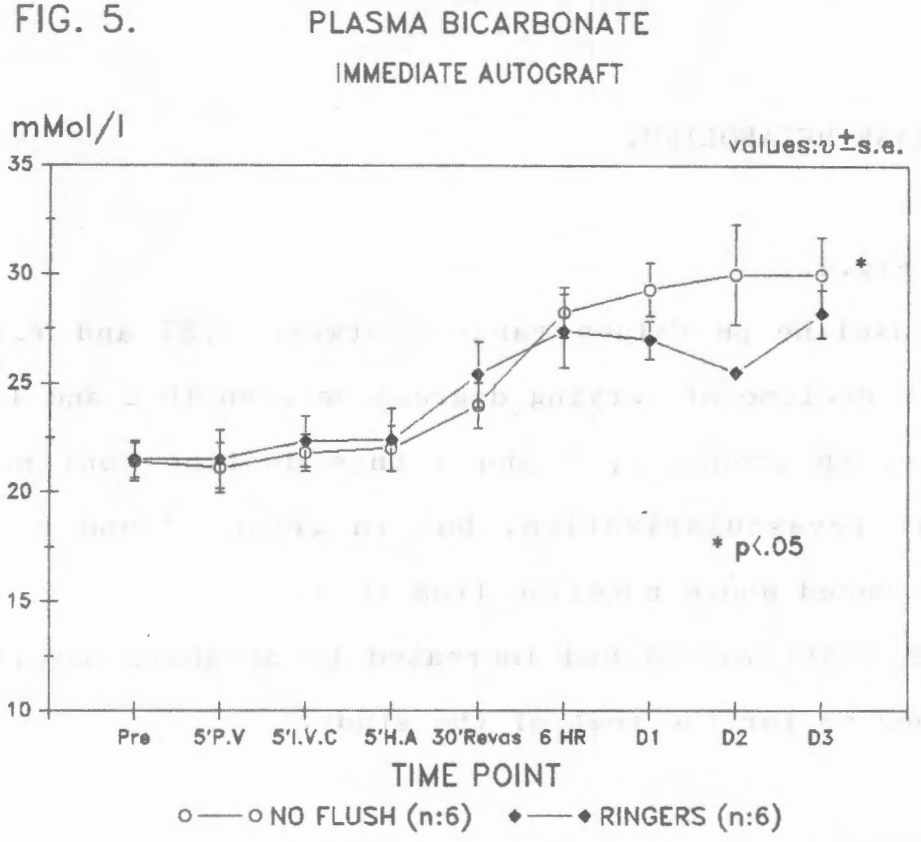
Venous Not shown.

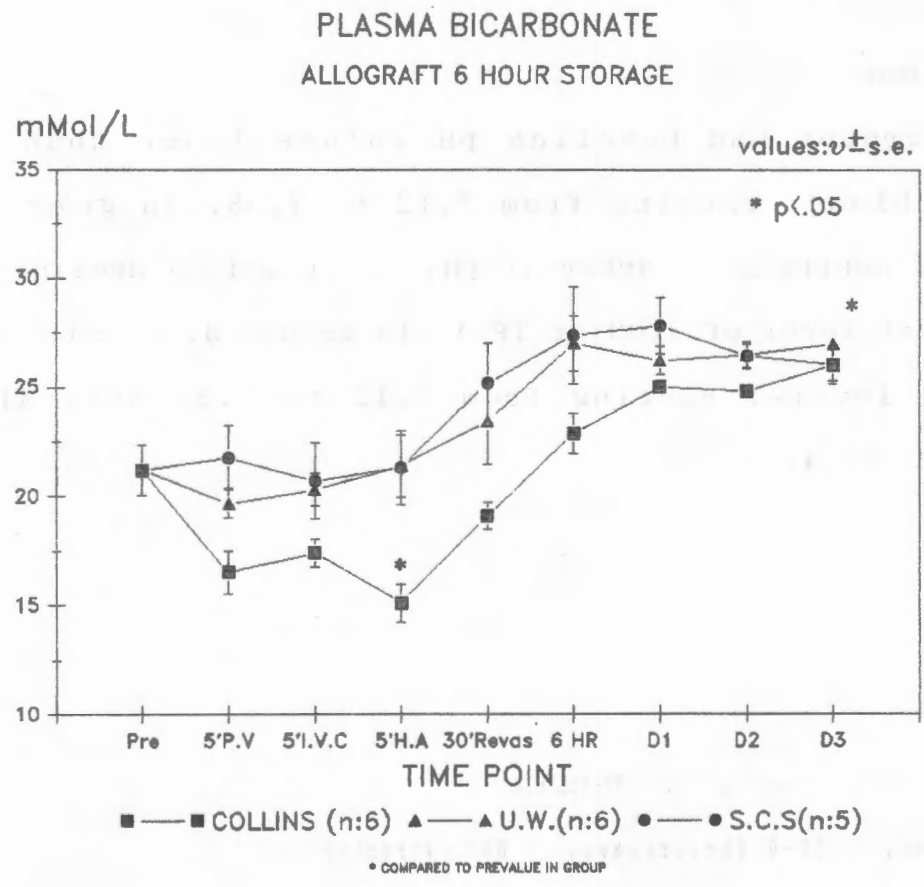
All groups had baseline pH values lower than in arterial blood, ranging from 7.12 to 7.38. In group 2, venous pH, contrary to arterial pH, continued to decline to reach lowest level of 7.09 at TP 4. In groups 3, 4 and 5 the lowest pH levels, ranging from 7.12 to 7.23 were also recorded at TP 4.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 5.


PLASMA BICARBONATE
IMMEDIATE AUTOGRAFT
mMol/l
values:υ±s.e.
35
30
25
20
15
10
*
* p<.05
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
6 HR
D1
D2
D3
TIME POINT
o—o NO FLUSH (n:6)
◆—◆ RINGERS (n:6)


PLASMA BICARBONATE
ALLOGRAFT 6 HOUR STORAGE
mMol/L
values:υ±s.e.
* p<.05
35
30
25
20
15
10
*
*
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
6 HR
D1
D2
D3
TIME POINT
■—■ COLLINS (n:6)
▲—▲ U.W.(n:6)
●—● S.C.S(n:5)
* COMPARED TO PREVALUE IN GROUP

PLASMA BICARBONATE.

Arterial: Fig.5.

Bicarbonate values were calculated on a Siggaard-Anderson nomogram using pH and $pCO_2$ values. All determinations were done within minutes on anaerobically obtained arterial blood samples.

Baseline values at TP 1 varied from 20.2 mMol/L in group 2 to 25.9 mMol/L in group 5. There was no statistically significant difference between groups at this TP.

From TP 3 to TP 5 there was no change in plasma bicarbonate of groups 1, 2, 4 and 5; in group 3 there was a significant decline to TP 4. Thereafter, in all groups there was an increase which persisted to remain above baseline for the rest of the study.

There was a strong association between a rise in serum bicarbonate, decline in lactic acid concentration and clinical impression of early recovery from anaesthesia as well as eventual survival of animals. In the animals that did not survive bicarbonate levels remained at low (< 16 mmol/L) levels after revascularisation until demise after 6 to 8 hours.

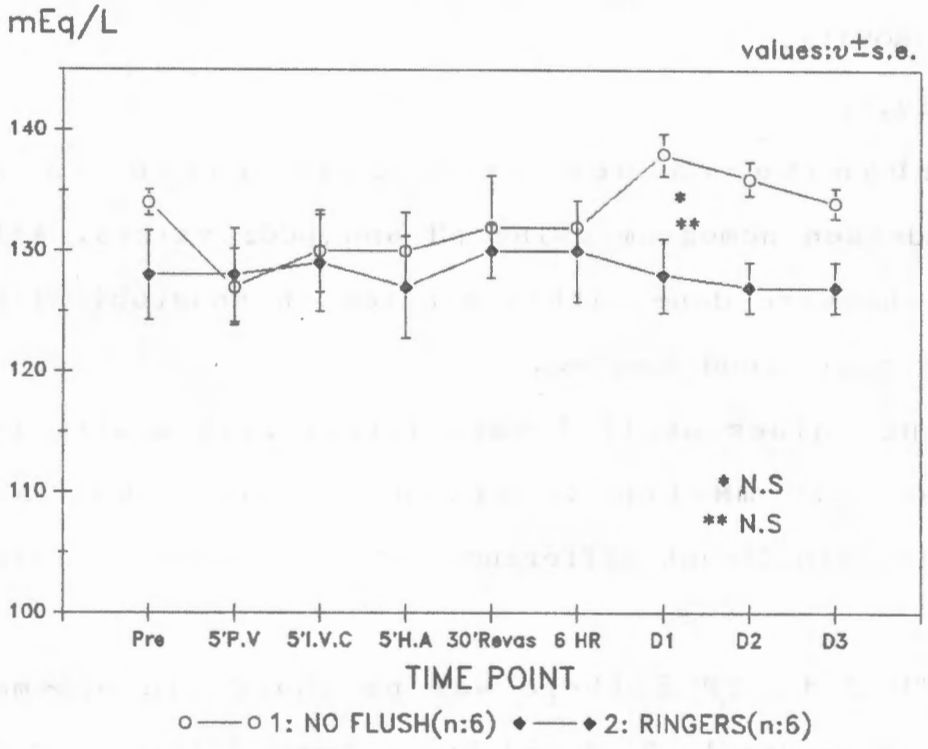
TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 6.

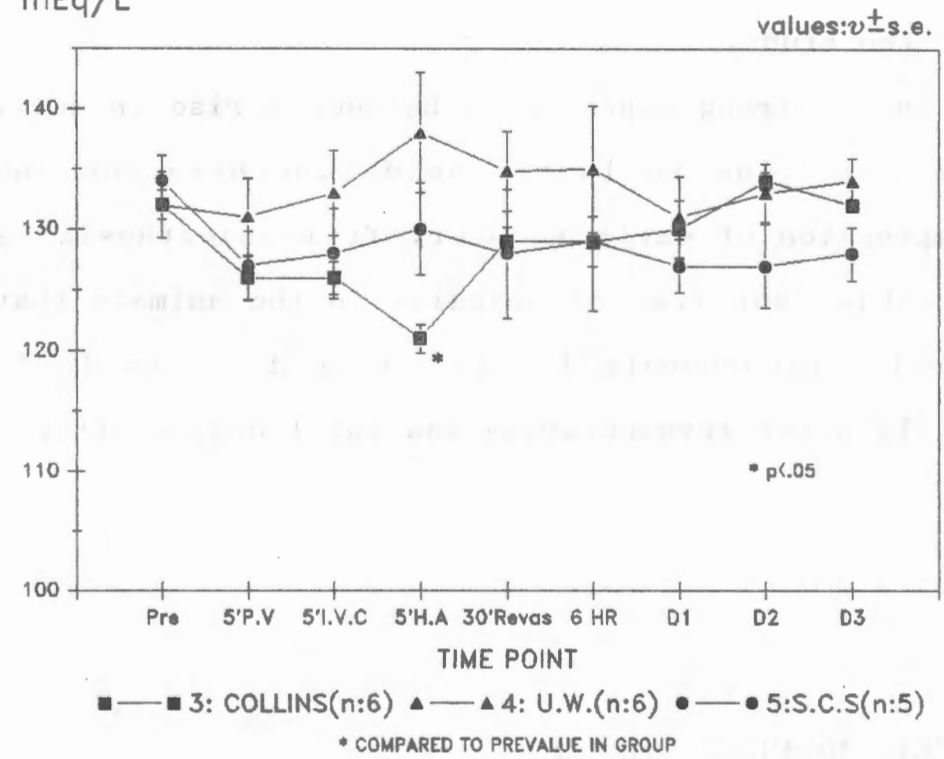
SODIUM

IMMEDIATE AUTOGRAFT




SODIUM

ALLOGRAFT 6 HOUR STORAGE




* COMPARED TO PREVALUE IN GROUP

SODIUM.

Arterial: Fig.6.

Baseline mean sodium values ranged from 122±1 to 134±6 mmol/L. Thereafter there was no significant change except in group 3, where a significant reduction ($p<0.05$) occurred from TP 1 to TP 4. However, from TP 5 these values were in the normal range.

SODIUM.

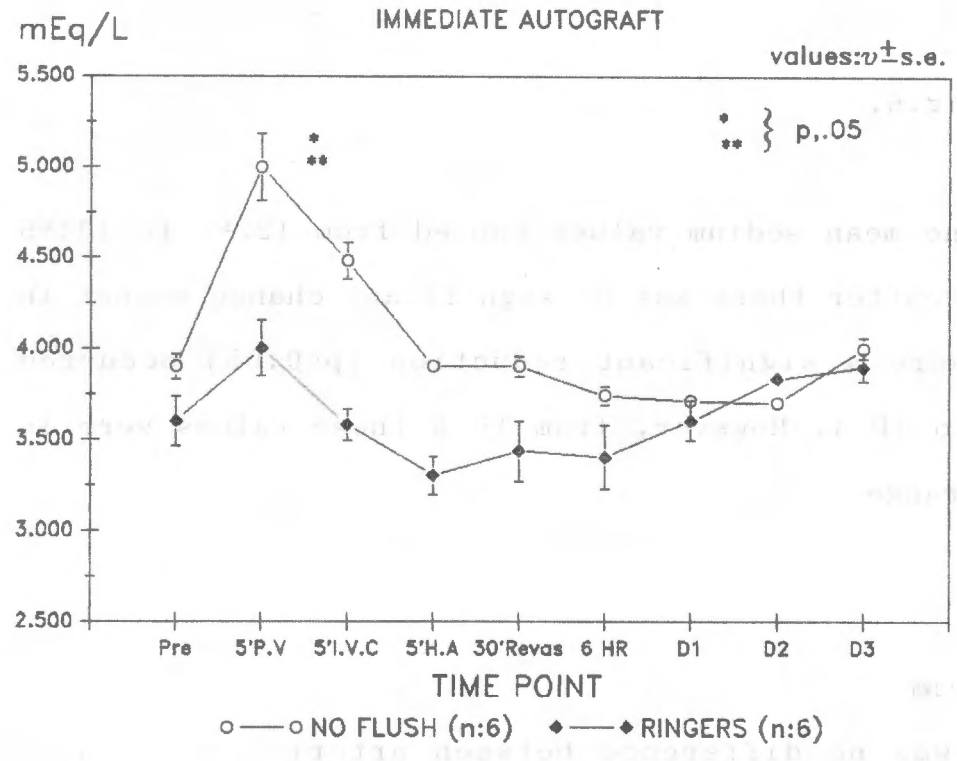
Venous: Not shown

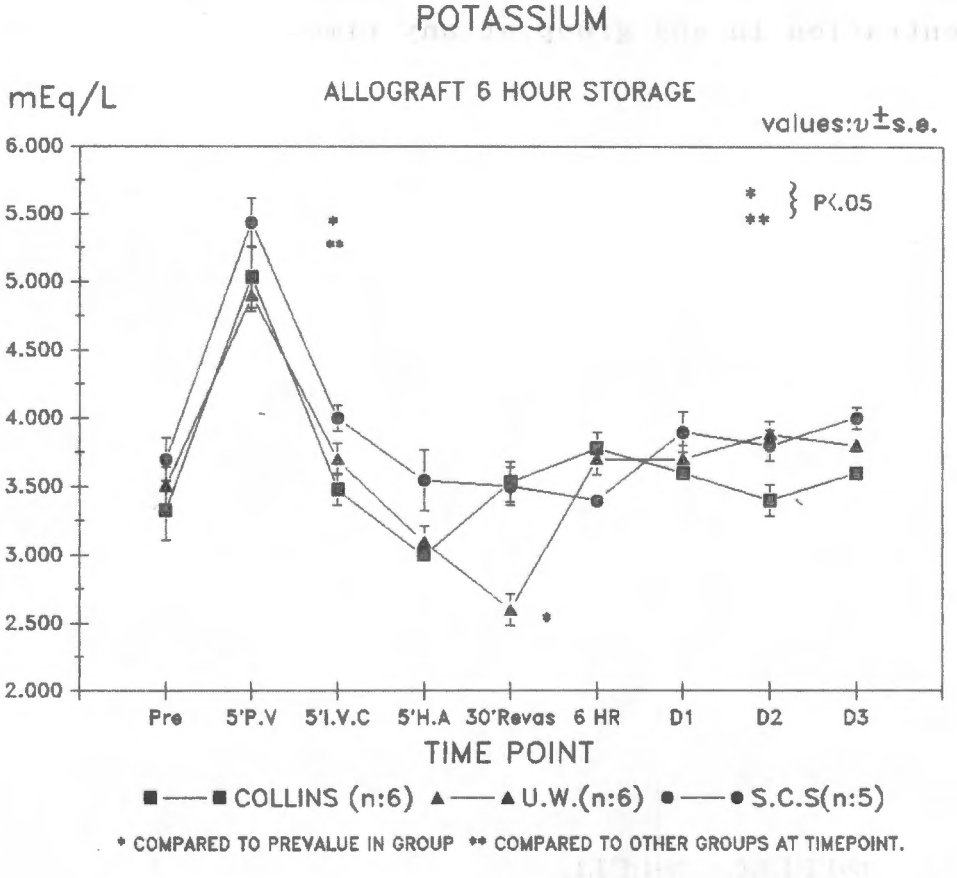
There was no difference between arterial and venous sodium concentration in any group at any time.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 7.


POTASSIUM
mEq/L
IMMEDIATE AUTOGRAFT
values:v±s.e.
5.500
5.000
4.500
4.000
3.500
3.000
2.500
* p,.05
**
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
6 HR
D1
D2
D3
TIME POINT
o—o NO FLUSH (n:6)
◆—◆ RINGERS (n:6)


POTASSIUM
mEq/L
ALLOGRAFT 6 HOUR STORAGE
values:v±s.e.
6.000
5.500
5.000
4.500
4.000
3.500
3.000
2.500
2.000
* P<.05
**
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
6 HR
D1
D2
D3
TIME POINT
■—■ COLLINS (n:6)
▲—▲ U.W.(n:6)
●—● S.C.S(n:5)
* COMPARED TO PREVALUE IN GROUP ** COMPARED TO OTHER GROUPS AT TIMEPOINT.

POTASSIUM.

Arterial: Fig.7.

Potassium values at TP 1 ranged from 3.32±0.38 to 3.90±0.12 mEq/L. These values were in the normal range accepted in our laboratory.

A statistically significant mean increase ($p<0.05$) of between 28 and 51% occurred in groups 1, 3, 4 and 5 at TP 2. In group 1 concentration increased by 28%, in group 3 by 51% and groups 4 and 5 by 38 and 37% respectively. There was no difference between groups.

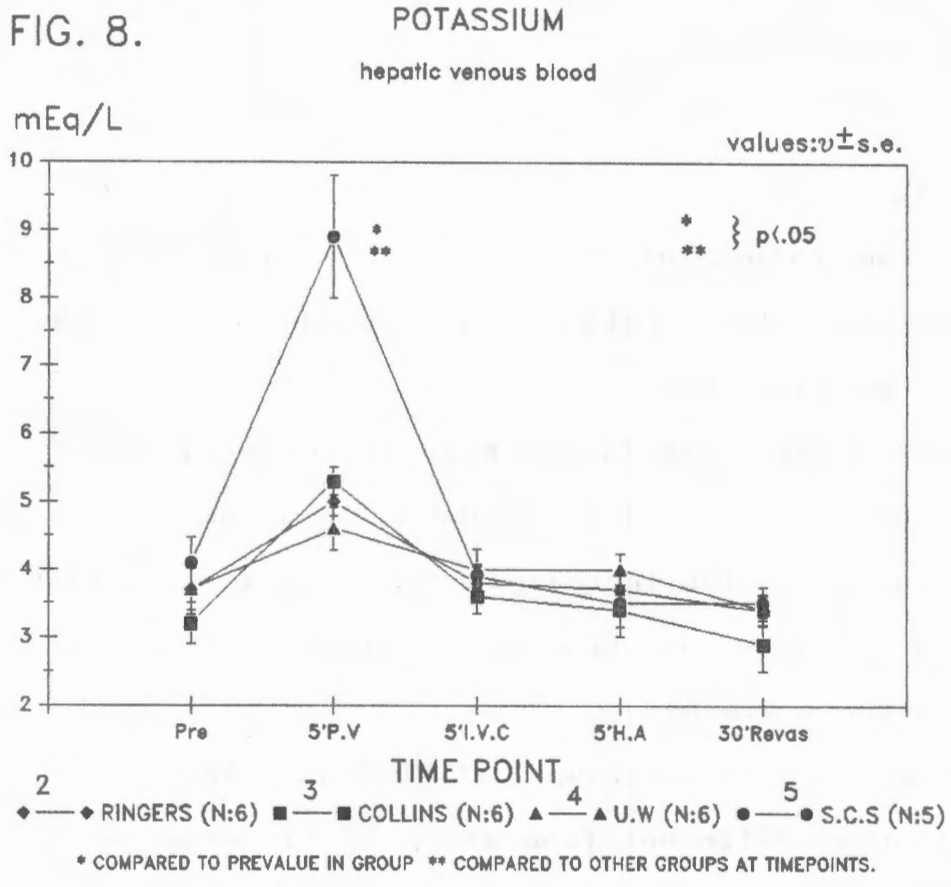
The mean increase in group 2 at TP 2 was 11%; which was not significantly different from group 1. The mean increase was, however, significantly lower than that seen in the other groups, at this TP.

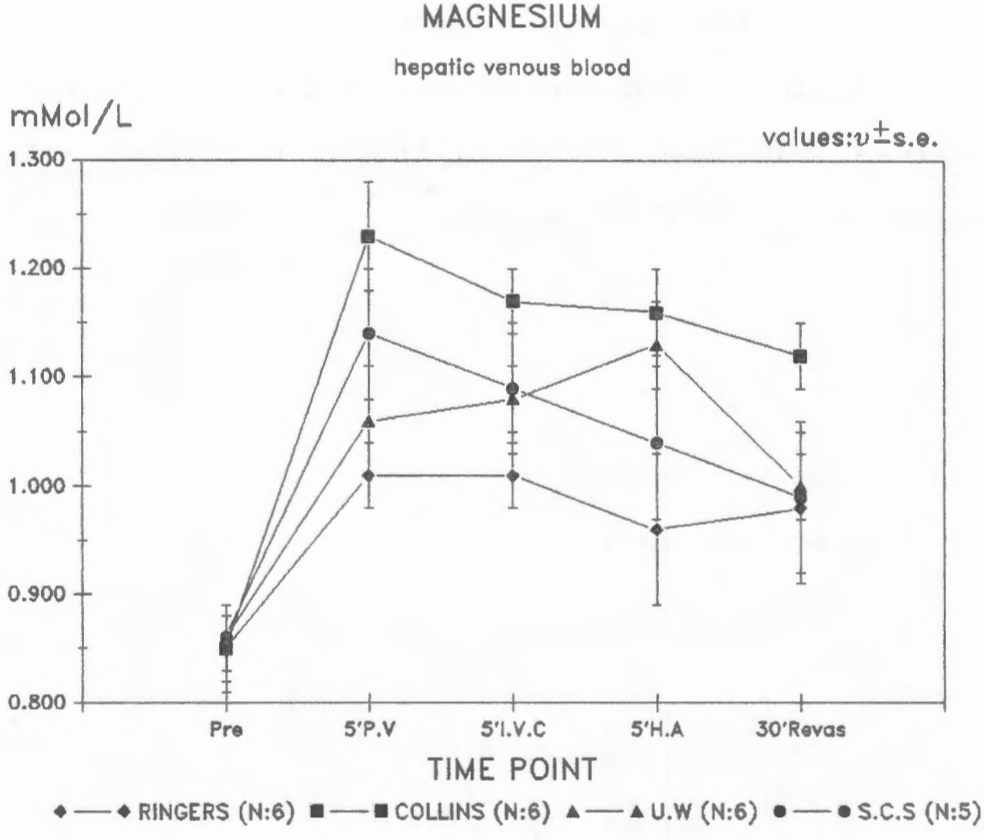
From TP 3 all values in all groups were within normal range, albeit higher than baseline, . In all groups potassium values were completely in the normal range and remained so for the rest of the study.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 8.


POTASSIUM
hepatic venous blood
mEq/L
values:υ±s.e.
10
9
8
7
6
5
4
3
2
*
**
*
**
} p<.05
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
TIME POINT
2
3
4
5
◆—◆ RINGERS (N:6) ■—■ COLLINS (N:6) ▲—▲ U.W (N:6) ●—● S.C.S (N:5)
* COMPARED TO PREVALUE IN GROUP ** COMPARED TO OTHER GROUPS AT TIMEPOINTS.


MAGNESIUM
hepatic venous blood
mMol/L
values:υ±s.e.
1.300
1.200
1.100
1.000
0.900
0.800
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
TIME POINT
◆—◆ RINGERS (N:6) ■—■ COLLINS (N:6) ▲—▲ U.W (N:6) ●—● S.C.S (N:5)

POTASSIUM.

Venous: Fig.8.

Baseline potassium levels were higher than corresponding arterial levels in all groups except group 4, where the mean value was 0.18mEq/L (4.3%) lower. None of the differences were statistically significant.

At TP 2 venous potassium concentrations in all groups were higher than corresponding arterial levels.

In groups 2 and 4 the increase in venous $K^+$ was double that seen in the arterial $K^+$. Due to small numbers in these groups (3 and 2 respectively), statistical evaluation was not performed.

In groups 3 and 5 $K^+$ levels at TP 2 were statistically significantly ($p<0.01$) higher than baseline venous values. Although venous $K^+$ values were higher than the corresponding arterial concentrations, no statistical significance was reached.

Potassium levels at TP 3, 4 and 5 were higher than baseline venous and corresponding arterial levels, but statistical significance was not reached in either instance.

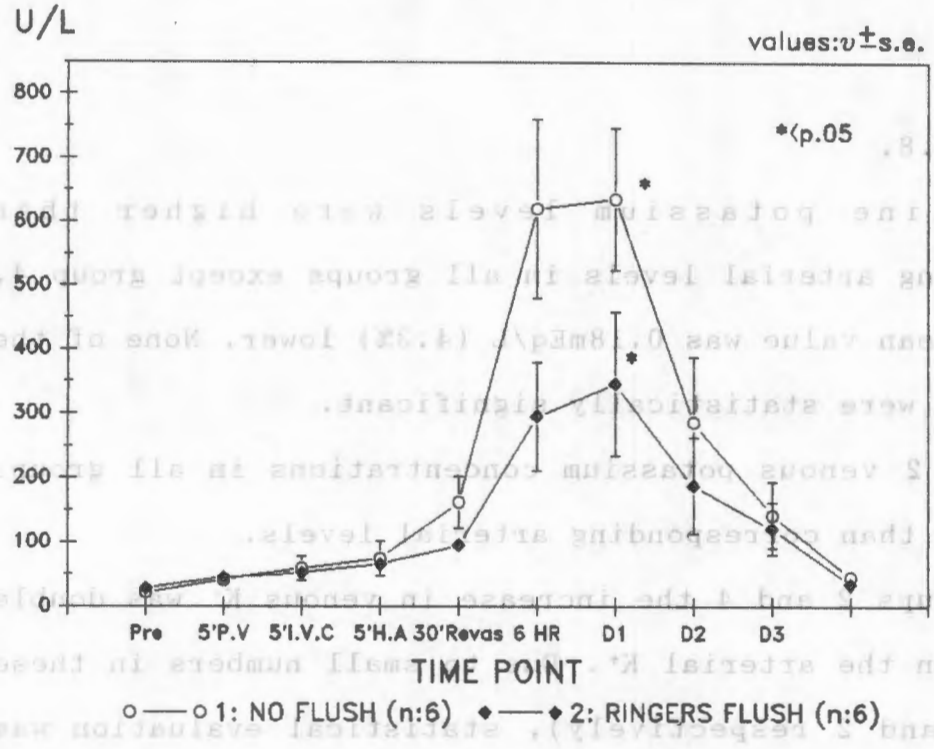
TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 9.

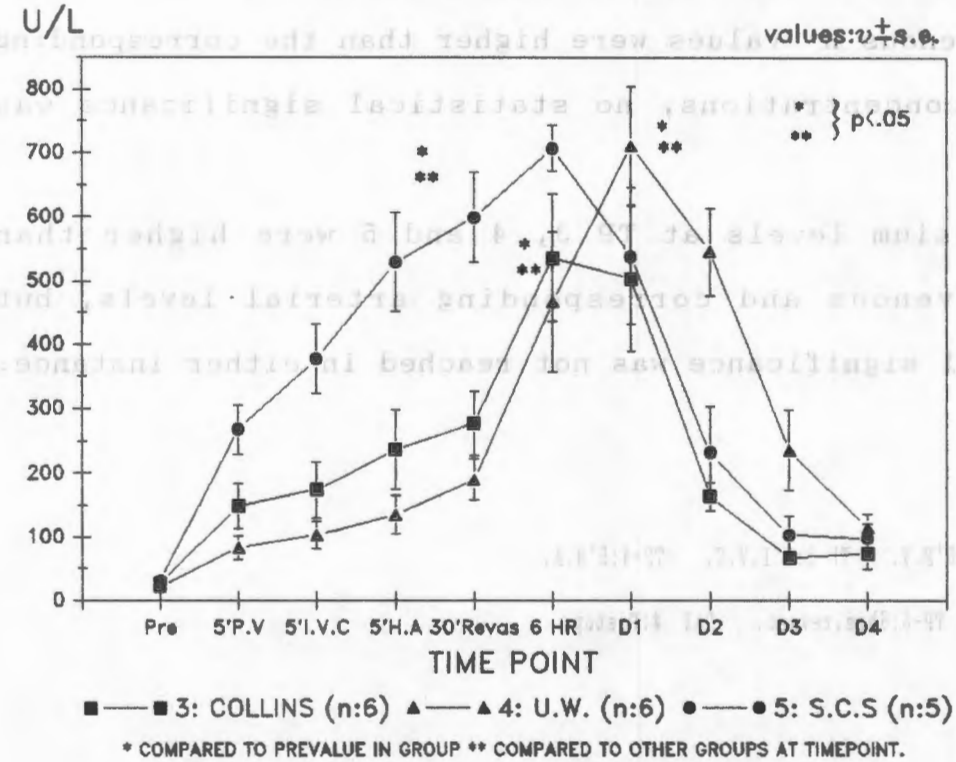
SERUM A.S.T

IMMEDIATE AUTOGRAFT




SERUM A.S.T

ALLOGRAFT 6 HOUR STORAGE




* COMPARED TO PREVALUE IN GROUP ** COMPARED TO OTHER GROUPS AT TIMEPOINT.

8.4 LIVER FUNCTION TESTS.

ASPARTATE AMINOTRANSFERASE (A.S.T).

Fig 9.

The mean preoperative minimum and maximum values ranged from 21.3(±4.5) to 30.8(±12.5) units/L. There was no significant difference in or between group baseline values.

In groups 3, 4 and 5 there was a progressive statistically significant increase at all timepoints in mean plasma AST which reached a peak of 500-700 units/L between 6 hours and day 1, and declined thereafter. In groups 1 and 2 the increase was only significant at 6 hours; values had returned to the normal range by two days postoperatively.

Aspartate amino transferase concentrations at all timepoints from 2 to 6, in group 5, were statistically significantly ($p<0.05$) higher than all other groups.

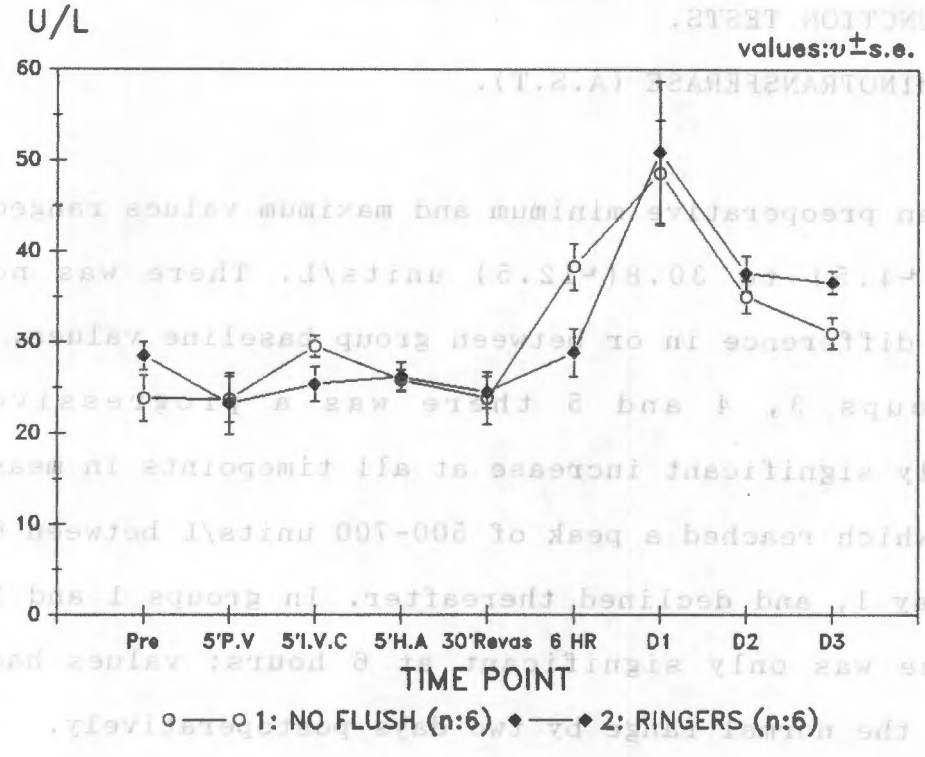
TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 10.

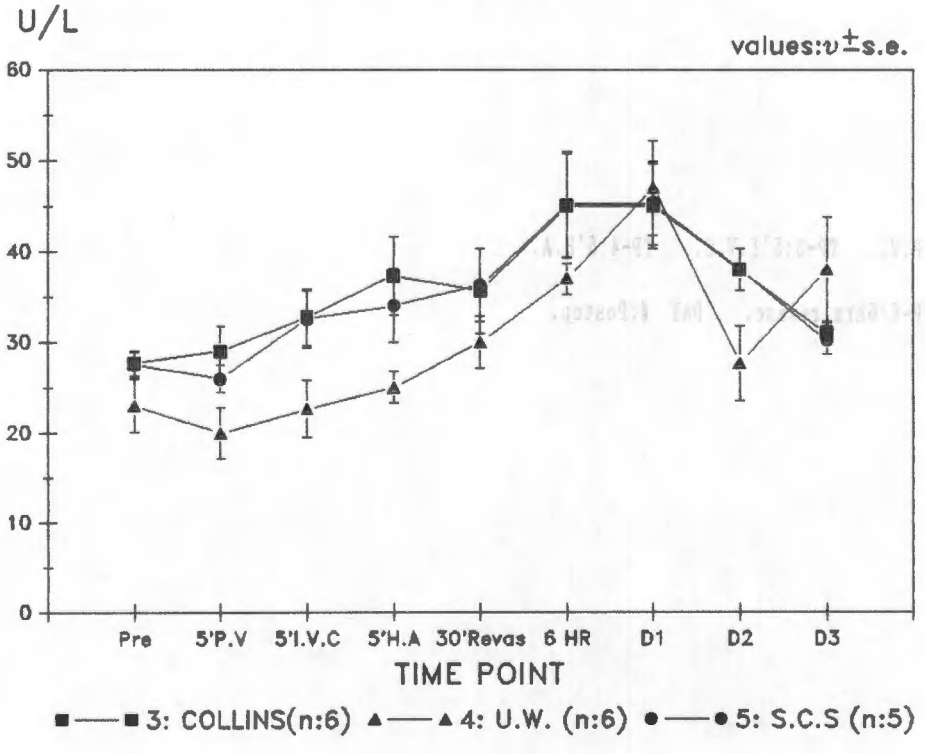
SERUM A.L.T

IMMEDIATE AUTOGRAFT




SERUM A.L.T

ALLOGRAFT 6 HOUR STORAGE



ALANINE AMINOTRANSFERASE.

Fig.10.

Mean baseline values varied between 20(±10) and 40(±10) units/L minimum and maximum respectively.

There was a mean increase to a maximum of 60 units/L in all groups between TP 4 and day 2. However, due to the wide variation in values, there was no significant difference from baseline or between groups at any time.

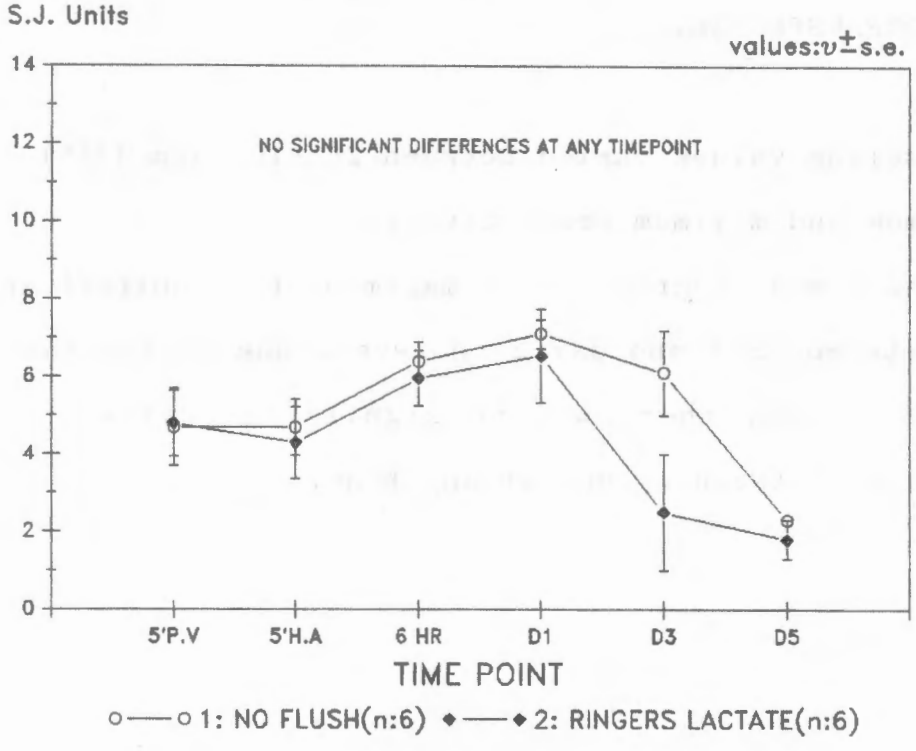
TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 11.

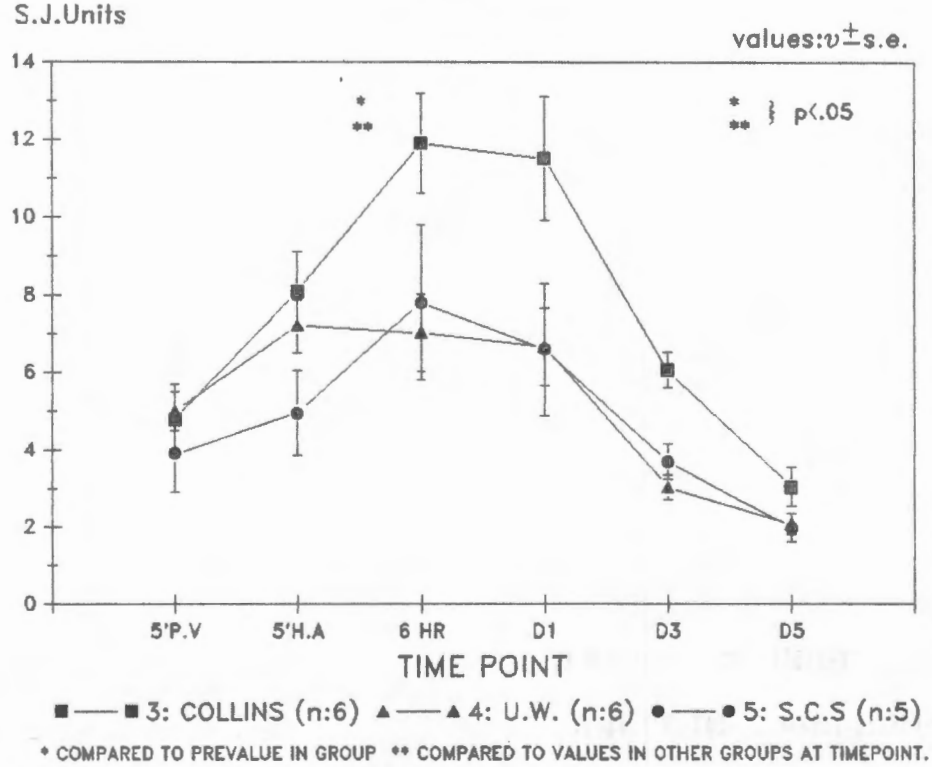
ALKALINE PHOSPHATASE

IMMEDIATE AUTOGRAFT




ALKALINE PHOSPHATASE

ALLOGRAFT 6 HOUR STORAGE




* COMPARED TO PREVALUE IN GROUP ** COMPARED TO VALUES IN OTHER GROUPS AT TIMEPOINT.

ALKALINE PHOSPHATASE.

Fig.11.

Determinations were done at TP 1, 2, 4, 6 and days 1, 3 and 5 and reported as Shinowara-Jones units (S.J).

There was no difference between mean baseline values of any of the groups.

Only in group 3 was there a significant ($p<0.05$) increase - at TP 5 and on day 1, to baseline and other groups peak and/or day 1 values. The maximum values in this group were 11.9 ($\pm$1.3) and 11.5 ($\pm$1.6) S. J units/L at TP 5 and day 1 respectively.

There was a marked decline in mean values in all groups after day 1, which reached significance only in groups 2 and 4 when compared to peak values. Only in group 4 were levels at day 5 significantly lower than baseline values.

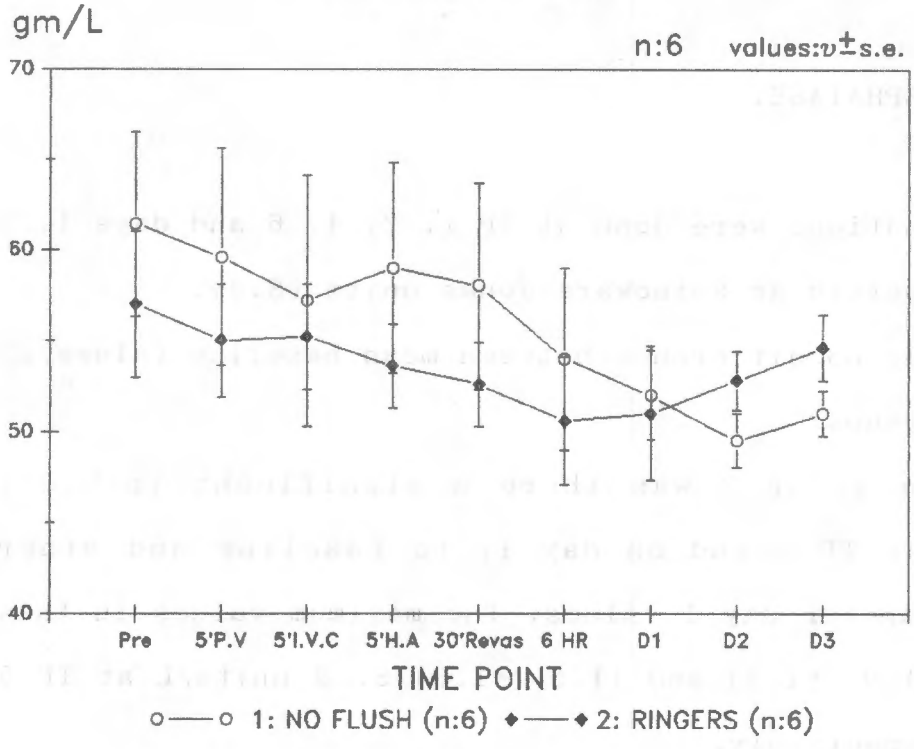
TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 12.

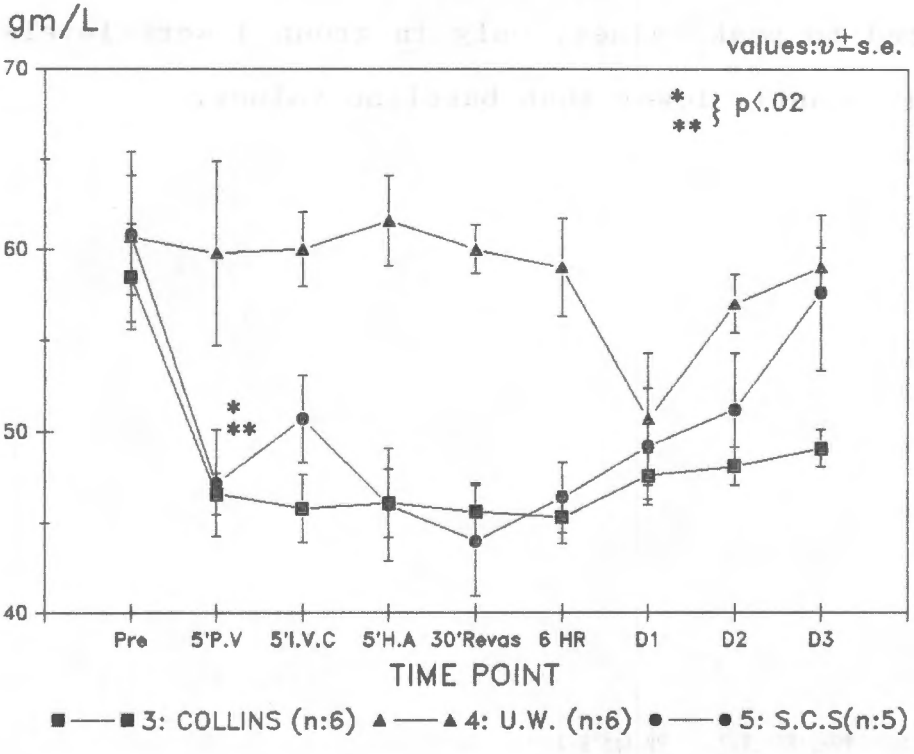
TOTAL PROTEIN

IMMEDIATE AUTOGRAFT




TOTAL PROTEIN

ALLOGRAFT 6 HOUR STORAGE



TOTAL PROTEIN.

Fig.12.

Total protein values for all groups at TP 1 ranged from 55.6(±5) to 61.4(±5.1) gm/L. These differences were not significant. There was a slow decline during the operation in groups 1 and 2 where the lowest level (20% decrease) was seen in group 2 at day 2. In these groups however, values returned to baseline by day 3.

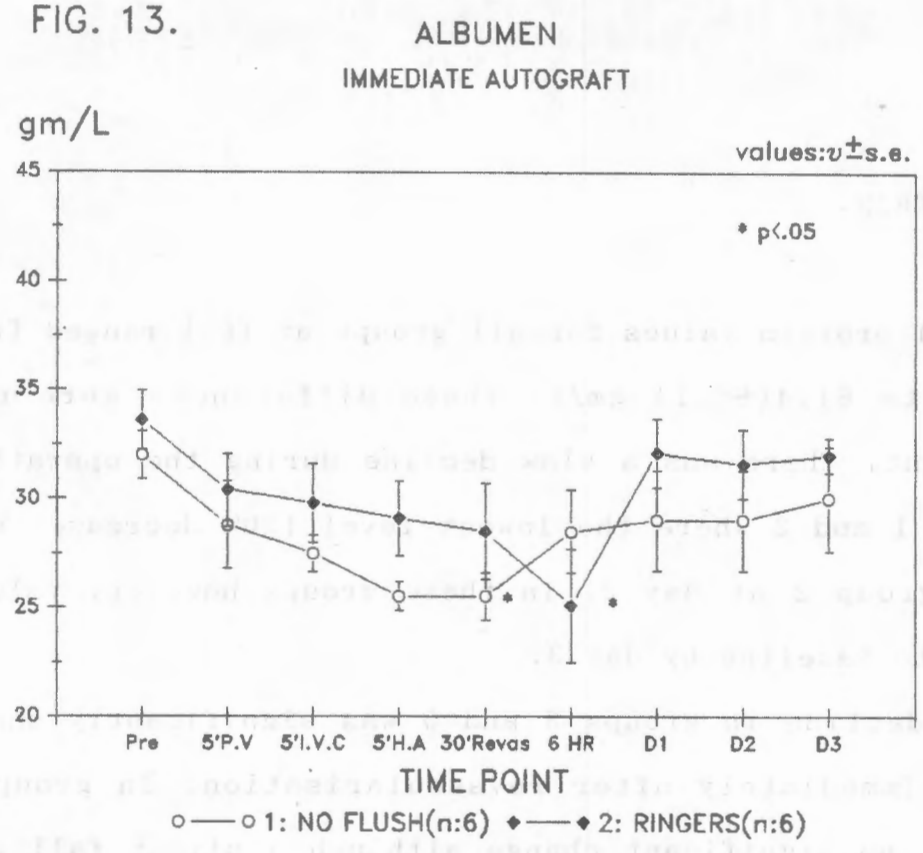
The decline in groups 3 and 5 was significantly sharp ($p<0.02$) immediately after revascularisation. In group 4 there was no significant change although a slight fall was noted on day 1.

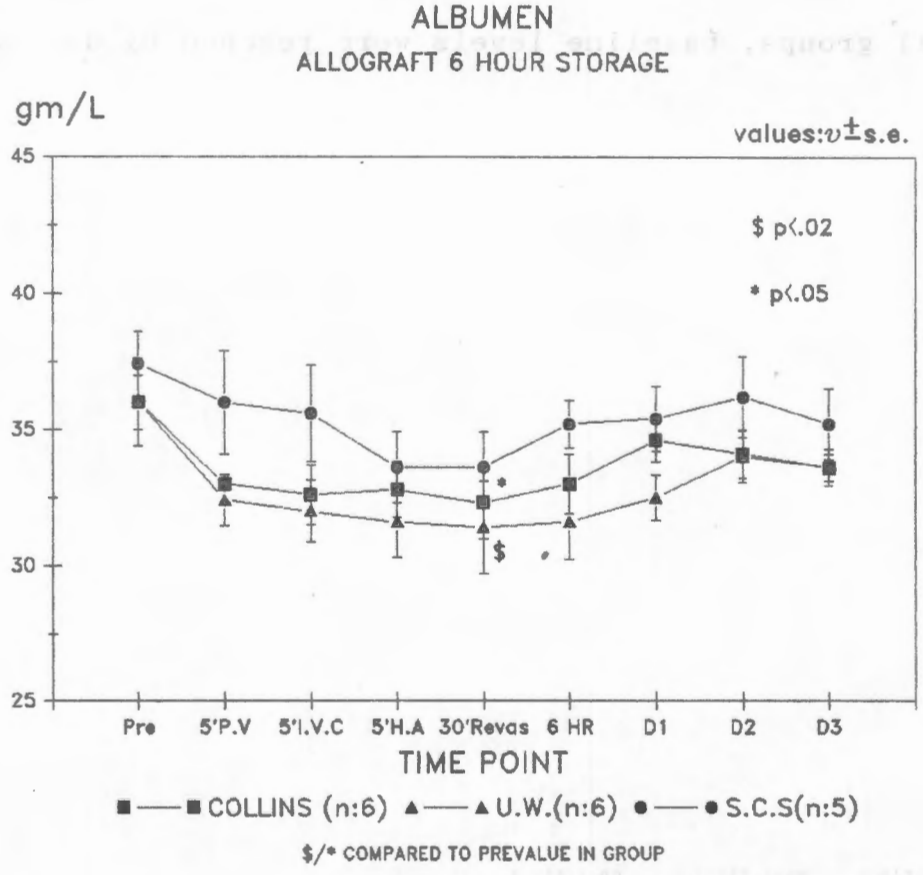
In all groups, baseline levels were reached by day 3.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 13.


ALBUMEN
IMMEDIATE AUTOGRAFT
gm/L
values:v±s.e.
45
40
35
30
25
20
* p<.05
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
6 HR
D1
D2
D3
TIME POINT
o—o 1: NO FLUSH(n:6) ◆—◆ 2: RINGERS(n:6)


ALBUMEN
ALLOGRAFT 6 HOUR STORAGE
gm/L
values:v±s.e.
45
40
35
30
25
$ p<.02
* p<.05
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
6 HR
D1
D2
D3
TIME POINT
■—■ COLLINS (n:6) ▲—▲ U.W.(n:6) ●—● S.C.S(n:5)
$/* COMPARED TO PREVALUE IN GROUP

ALBUMEN.

Fig.13.

Mean baseline albumen concentration ranged from 32(±2.7) to 37.4(±2.7) gm/L.

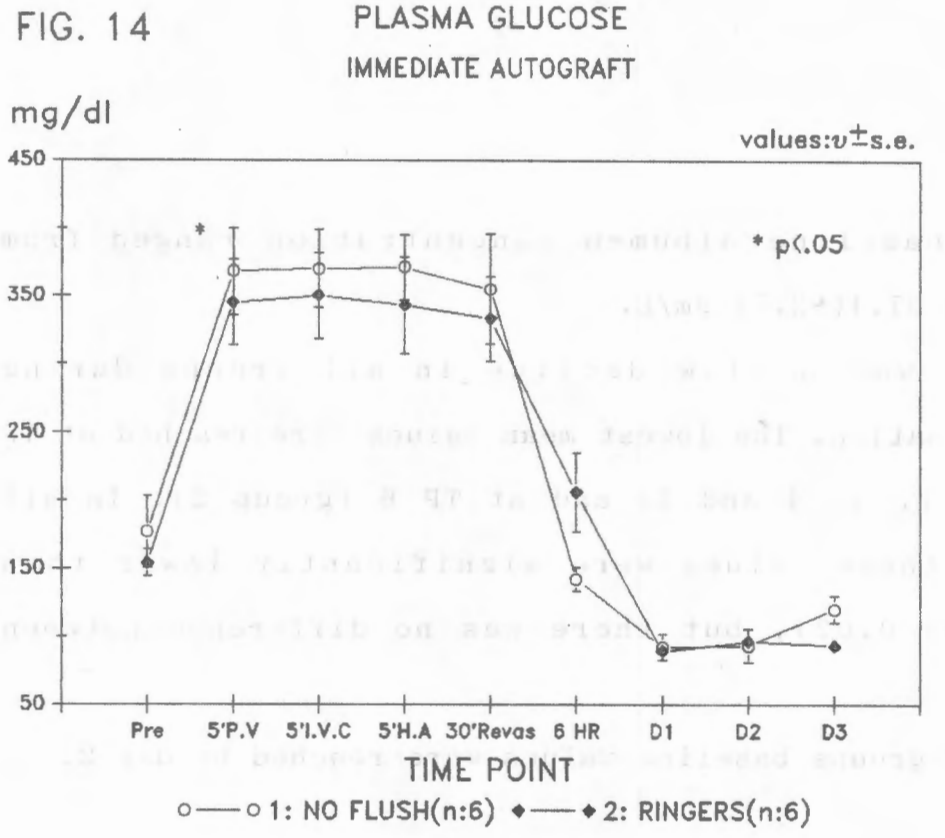
There was a slow decline in all groups during revascularisation. The lowest mean values were reached at TP 5 (groups 1, 3, 4 and 5) and at TP 6 (group 2). In all instances these values were significantly lower than baseline ($p<0.02$), but there was no difference between groups.

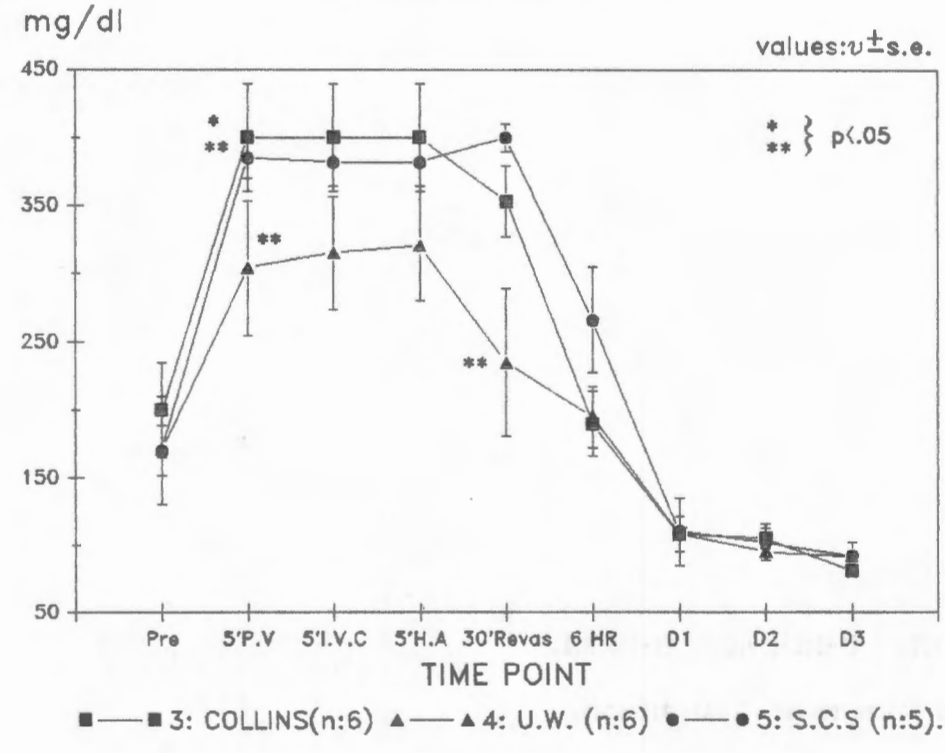
In all groups baseline values were reached by day 2.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 14


PLASMA GLUCOSE
IMMEDIATE AUTOGRAFT
mg/dl
values:υ±s.e.
450
350
250
150
50
* p<.05
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
6 HR
D1
D2
D3
TIME POINT
○—○ 1: NO FLUSH(n:6) ◆—◆ 2: RINGERS(n:6)


PLASMA GLUCOSE
ALLOGRAFT 6 HOUR STORAGE
mg/dl
values:υ±s.e.
450
350
250
150
50
* ** p<.05
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
6 HR
D1
D2
D3
TIME POINT
■—■ 3: COLLINS(n:6) ▲—▲ 4: U.W. (n:6) ●—● 5: S.C.S (n:5).

GLUCOSE.

Fig.14.

There was a wide range of blood glucose levels at TP 1 from 150(±24.3) to 250(±82.1) mg/dL. This wide range was probably due to the infusion of a 10% dextrose solution (Maintelyte®). There was no statistical difference between values in the groups.

In all groups, high levels were sustained until TP 5. In group 4, however, levels were significantly ($p<0.05$) lower than those in groups 3 and 5.

Although glucose containing infusion continued there was a significant decline in mean values from TP 5 to day 1. Thereafter values remained within the normal range regardless of the discontinuation of glucose containing infusion.

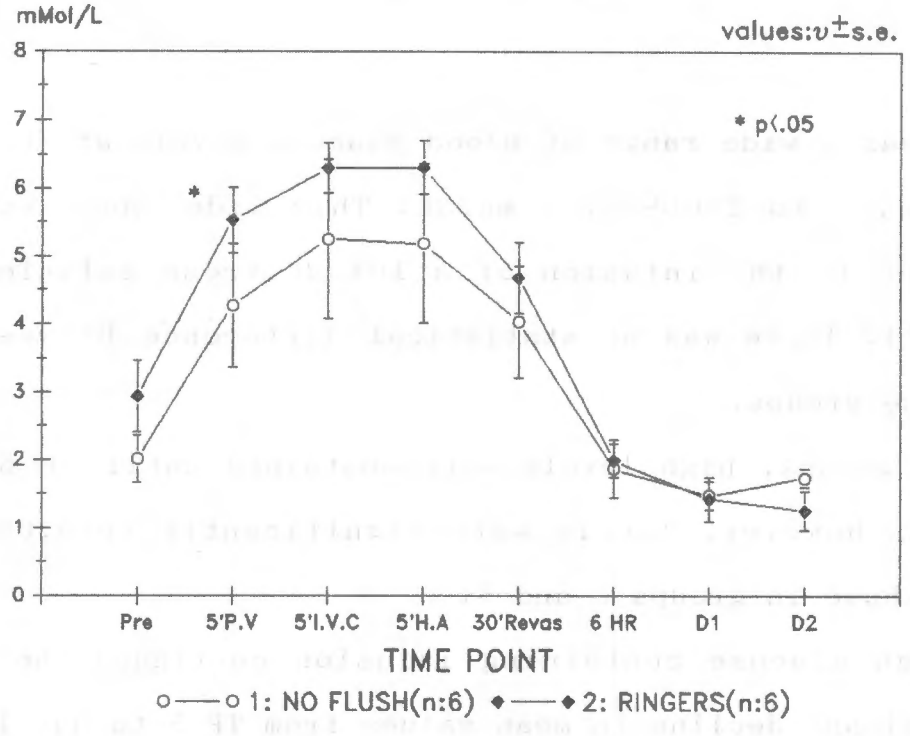
TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 15.

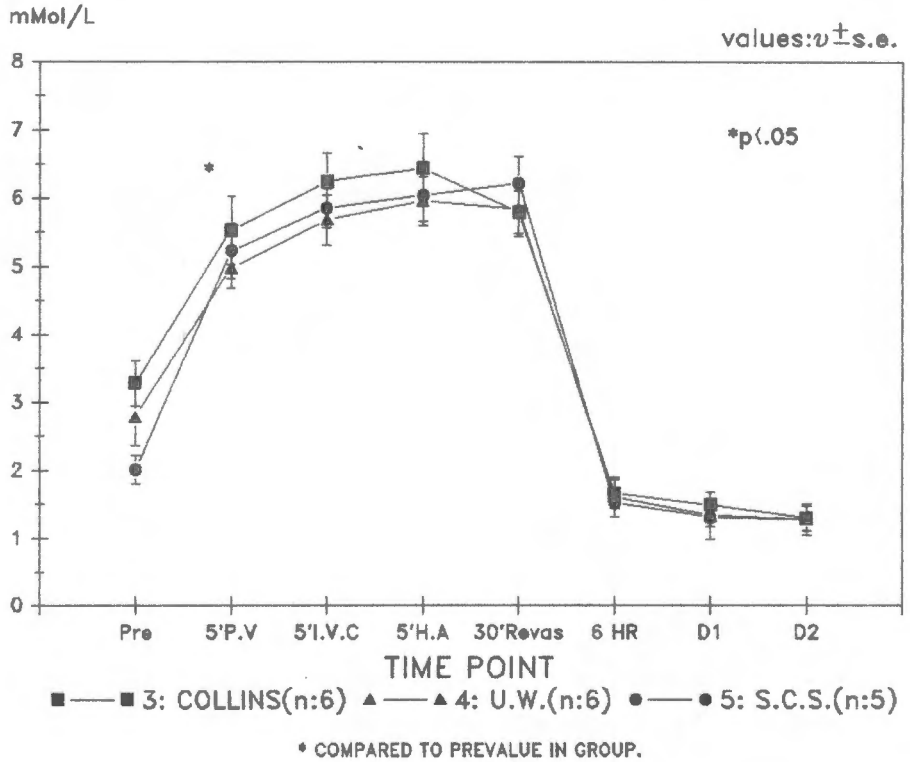
LACTIC ACID

IMMEDIATE AUTOGRAFT




LACTIC ACID

ALLOGRAFT 6 HOUR STORAGE




* COMPARED TO PREVALUE IN GROUP.

LACTIC ACID.

Fig.15.

Mean baseline plasma concentration of lactic acid varied between 2.01(±.46)and 3.2(±.82) mMol/L for all groups.

There was a significant twofold increase upon initial reperfusion and a further increase upon opening of the I.V.C in all groups: thirty minutes after complete revascularisation the levels of lactic acid in allografts were still significantly elevated and were different from those in autografts ($p<0.02$).

By day 1 however, all values were within baseline range. There was a strong association between a decline in lactic acid concentration, rise in serum bicarbonate and clinical impression of early recovery from anaesthesia as well as eventual survival of animals. In the animals that did not survive, lactic acid levels remained at high (>5 mmol/L) levels after revascularisation until demise after 6 to 8 hours.

Lactic acid in plasma remained between 1 and 2.5 mMol/L in all groups from day 1 to 7.

In summary, a similar pattern was observed in all animals, except that in the allograft groups levels remained higher for thirty minutes following full revascularisation.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

BILIRUBIN.

Data not shown.

Bilirubin concentration did not change from values at TP 1 in any of the groups during the first six hours, or for the first three days. In some animals a rise in bilirubin was evident at day 3. This was not confined to a specific group and occurred in about 30% of all animals.

In half these animals bilirubin rose to a peak at day 5 or 6, but with marked reductions in concentration over subsequent 24 hour periods, to baseline values.

If a spontaneous decrease did not occur, a continuing increase in bilirubin concentration coincided with a clinical post-mortem diagnosis of rejection. Choledochal anastomoses did not show stricture at post-mortem in any of the animals.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

CHAPTER 9.

# CHANGES IN COAGULATION PARAMETERS.

Prothrombin time.

Partial thromboplastin time.

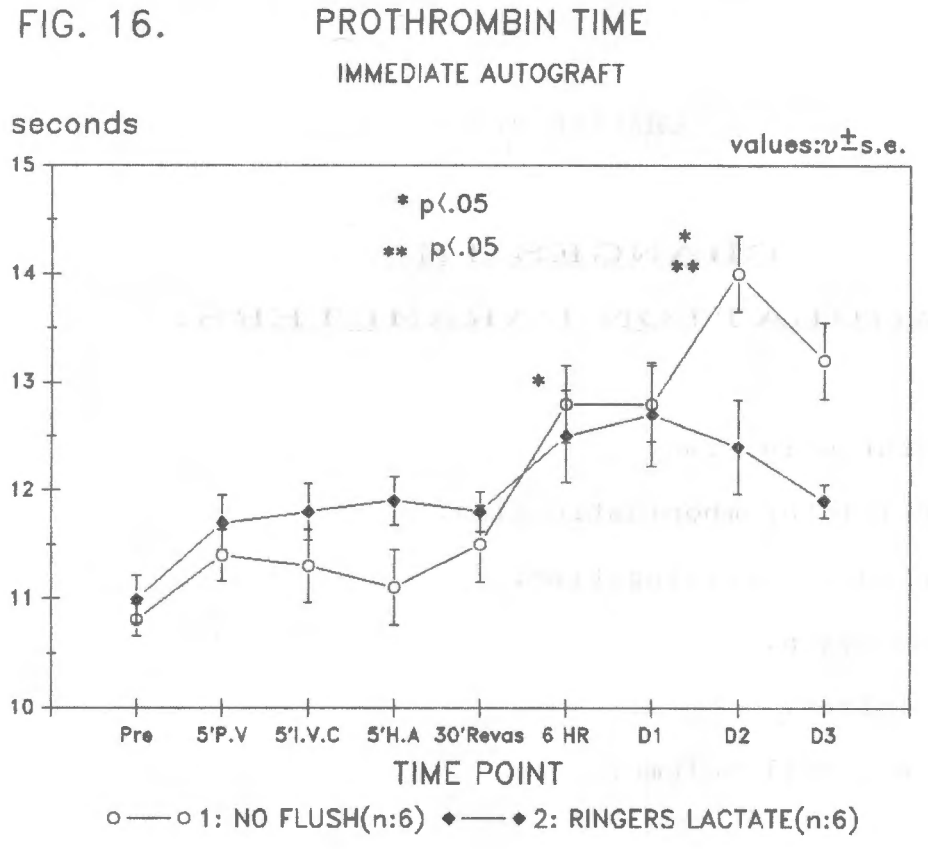
Activated clotting time.

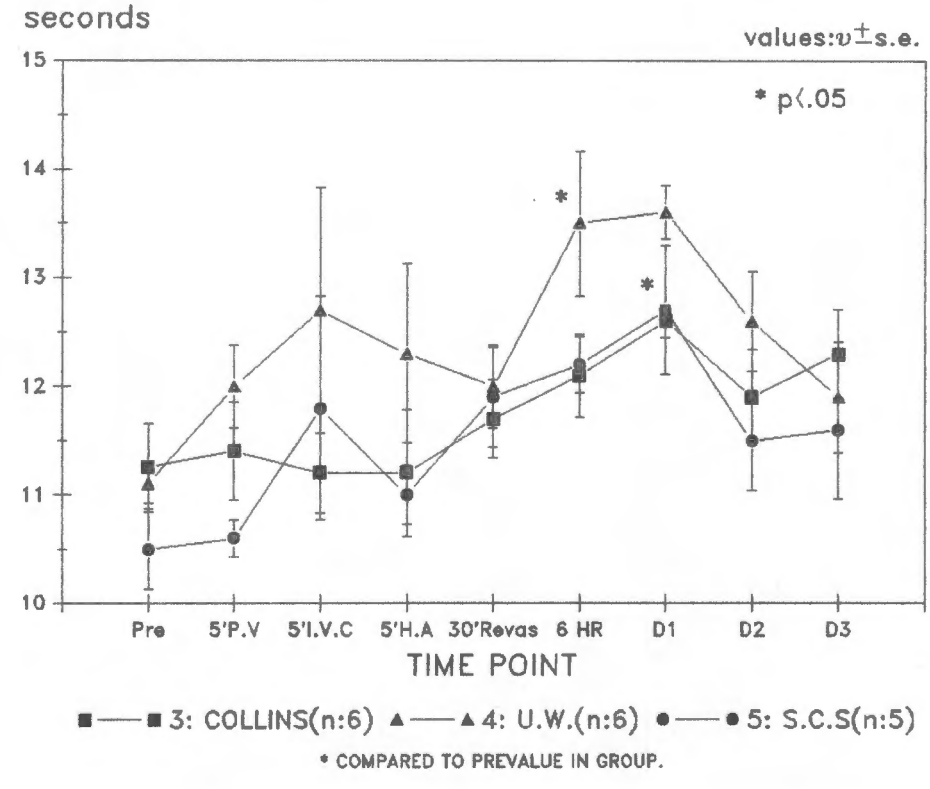
Fibrinogen.

Platelets.

Packed cell volume.

FIG. 16.




* COMPARED TO PREVALUE IN GROUP.

PROTHROMBIN TIME.

Fig.16.

Baseline values at TP 1 varied from 10.5 to 11.5 seconds in all groups. There was a significant increase in all groups at TP 6 and day 1 ($p<0.05$). This increase persisted in group 1 until day 3, but in all other groups values at day 3 were not significantly raised. In all groups values were back to baseline values by day 4.

PARTIAL THROMBOPLASTIN TIME.

Data not shown.

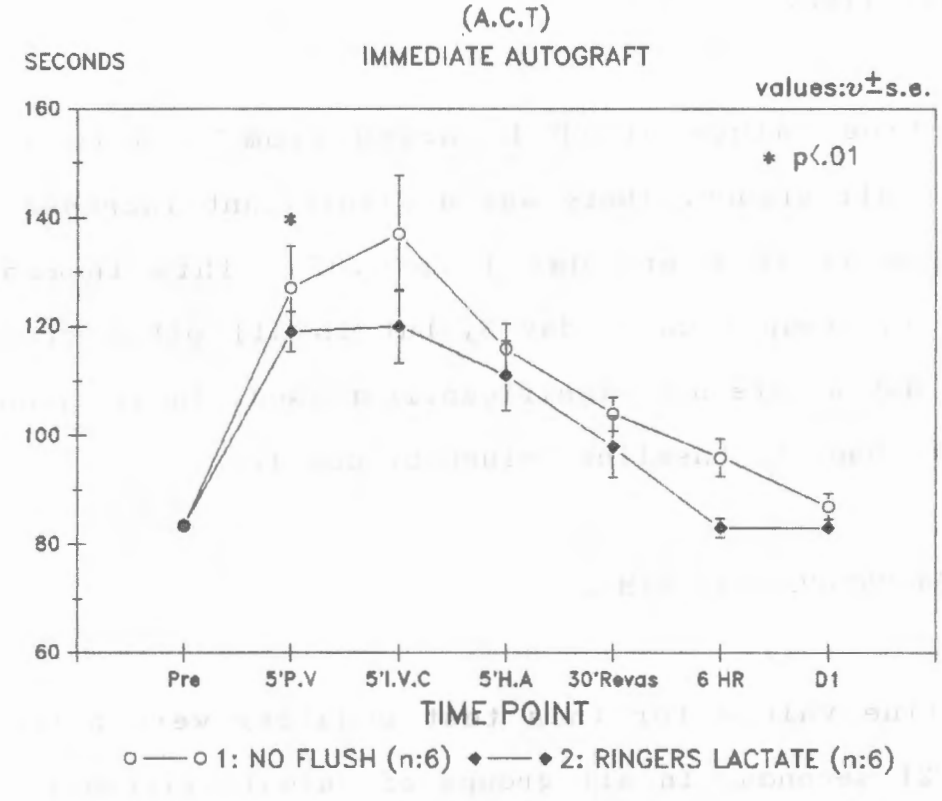
Baseline values for this test modality were between 20.5 and 21 seconds. In all groups of animals virtually no changes occurred during the procedure or in the first 24 hours.

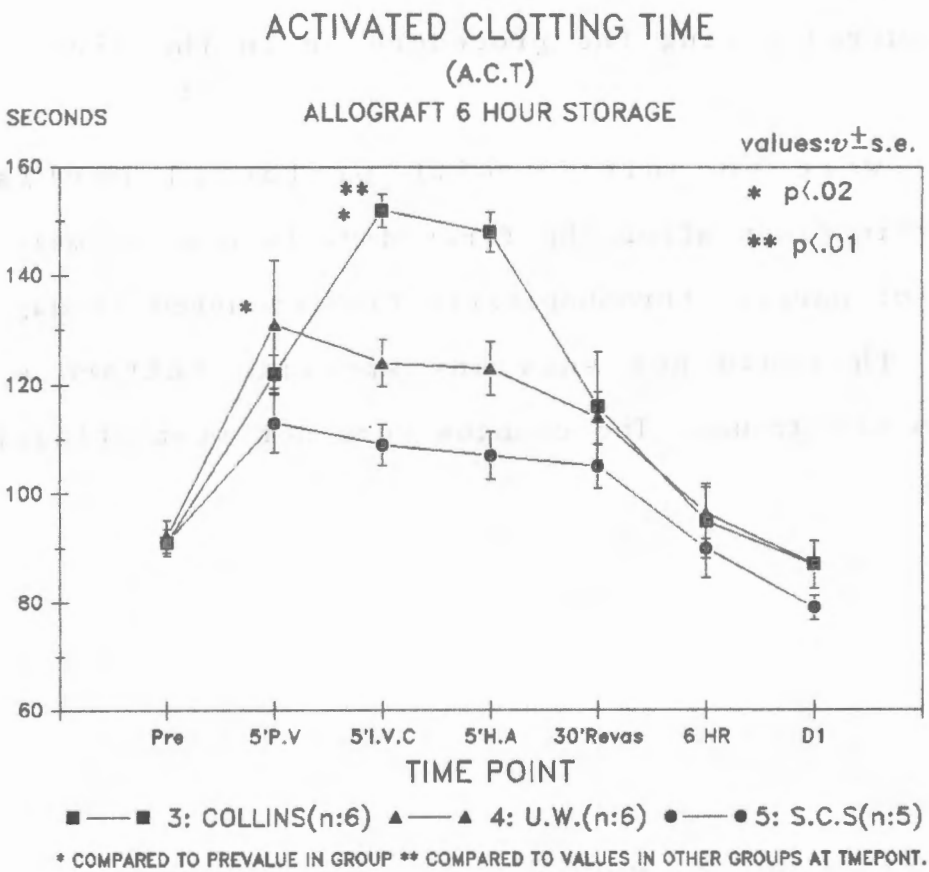
There were no animals with prolonged partial thromboplastin times after the first day. In some animals a shortening of partial thromboplastin time occurred at day 4 or later. This did not show any specific pattern and occurred in all groups. The changes were not statistically significant.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 17. ACTIVATED CLOTTING TIME




* COMPARED TO PREVALUE IN GROUP ** COMPARED TO VALUES IN OTHER GROUPS AT TMEPONT.

ACTIVATED CLOTTING TIME.

Fig.17.

Baseline activated clotting times (A.C.T.), taken before administration of heparin, were between 83 and 92 seconds.

The mean times increased in all groups at TP 1 ($p<0.01$-$p<0.02$). The peak times were reached between TP 1 and TP 3. Thereafter all time values declined towards normal except in group 3 where values remained prolonged to TP 4. Activated clotting times in group 3 were significantly prolonged ($p<0.02$) at TP 3 and 4 both to baseline and all other group values. In all groups values were back to baseline levels by 6 hours (TP 5), and remained so for the duration of the study.

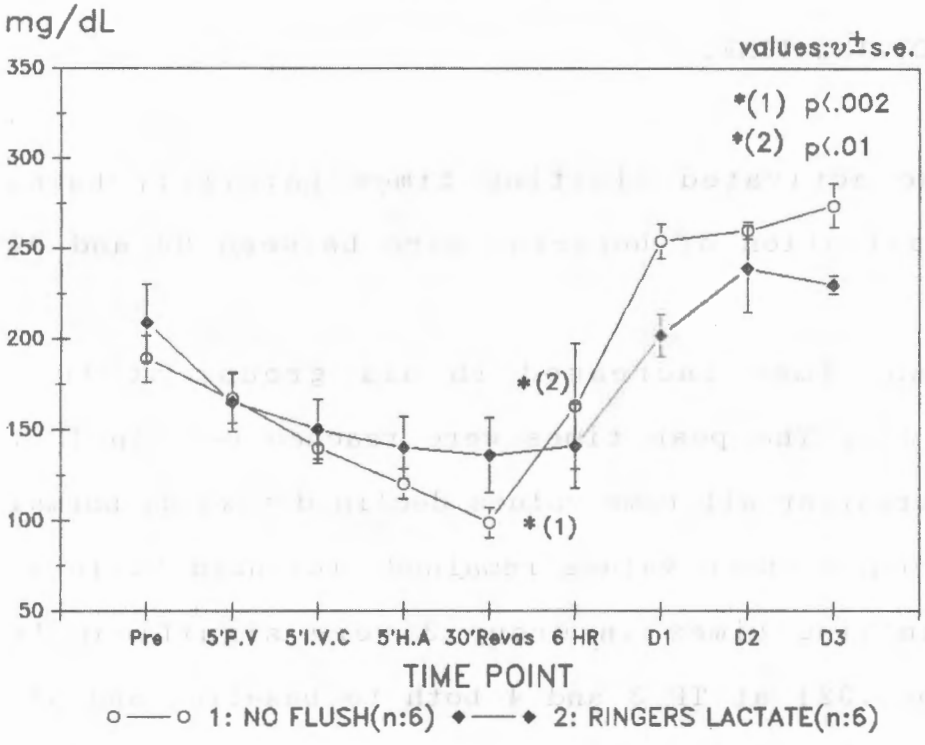
TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 18

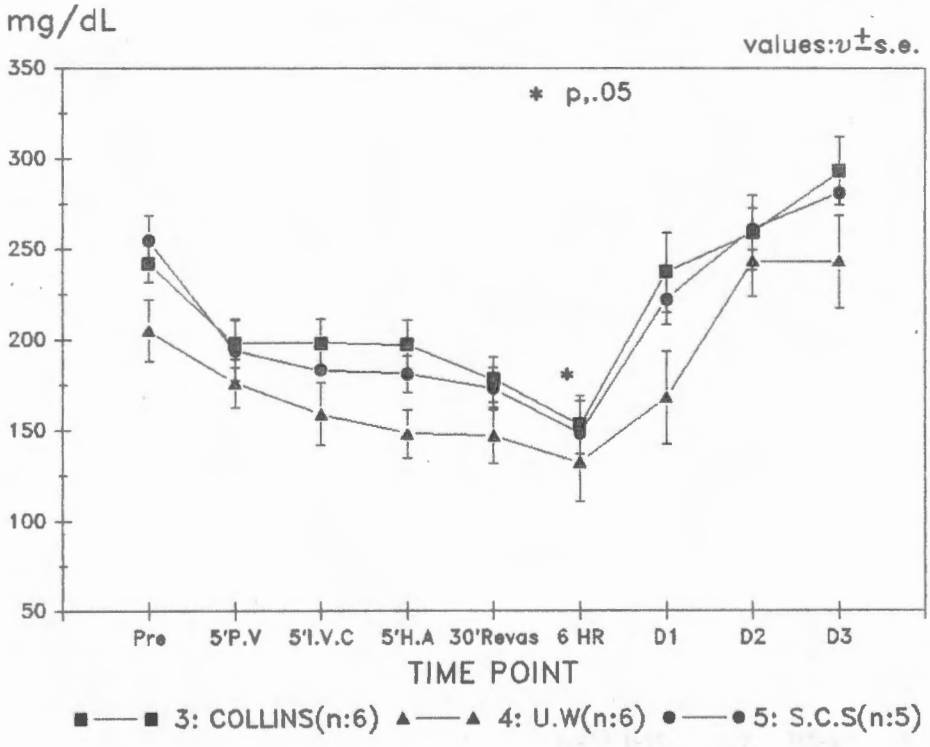
FIBRINOGEN

IMMEDIATE AUTOGRAFT




FIBRINOGEN

ALLOGRAFT 6 HOUR STORAGE



FIBRINOGEN.

Fig.18.

Mean baseline values ranged from 189 (±31.6) to 255 (±30.1) mg/dL.

In all groups there was a slow decrease in fibrinogen concentration during revascularisation (similar to that noted for albumen). At TP 5 this decrease was significant for group 1 ($p<0.002$) and group 2 ($p<0.05$), and at TP 6 for groups 3, 4 and 5 ($p<0.05$).

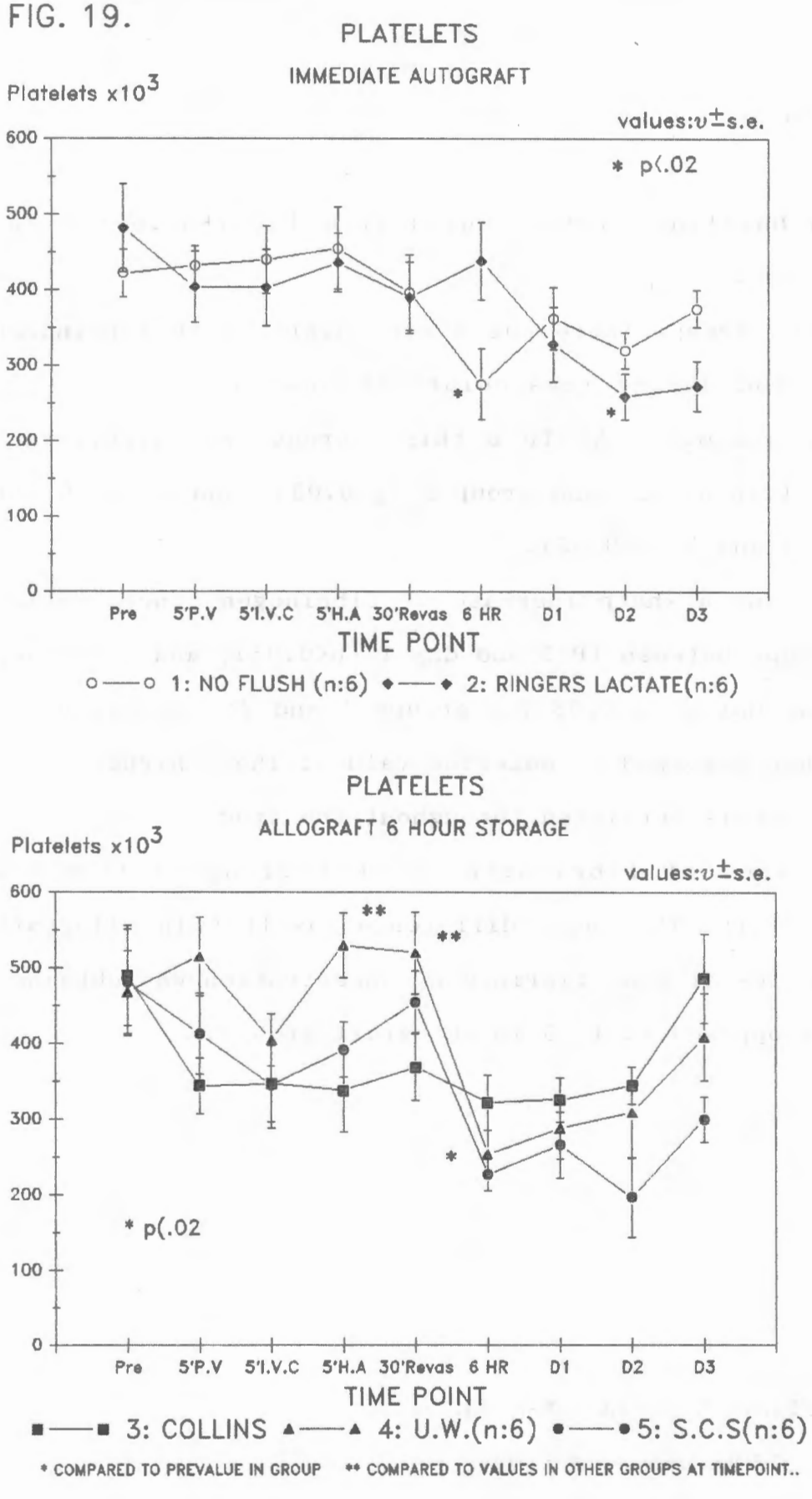
There was a sharp increase of fibrinogen concentration in all groups between TP 5 and day 1 ($p<0.05$), and a further increase at day 2 ($p<0.02$ for groups 1 and 2; N.S for other groups) when compared to baseline values. These higher (than baseline) levels persisted throughout the study.

Decreases of fibrinogen in both groups followed a similar pattern. The major difference was that in allograft groups the lowest mean fibrinogen concentration was obtained at TP 6 as opposed to TP 5 in autograft groups.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 19.


PLATELETS
IMMEDIATE AUTOGRAFT
Platelets x10^3
values:υ±s.e.
* p(.02
600
500
400
300
200
100
0
Pre
5'P.V
5'I.V.C
5'H.A
30'Revas
6 HR
D1
D2
D3
TIME POINT
1: NO FLUSH (n:6)
2: RINGERS LACTATE(n:6)
PLATELETS
ALLOGRAFT 6 HOUR STORAGE
Platelets x10^3
values:υ±s.e.
**
**
*
* p(.02
3: COLLINS
4: U.W.(n:6)
5: S.C.S(n:6)
* COMPARED TO PREVALUE IN GROUP
** COMPARED TO VALUES IN OTHER GROUPS AT TIMEPOINT..

PLATELETS.

Fig.19.

Mean platelet counts in all groups varied from 422(±76) to 482(±142) x $10^3$/ml. There were no significant differences between any of the groups.

There was a decline in platelet count to a significant lower level in autografted animals (group 1, TP 6 $p<0.02$; group 2, day 2 $p<0.05$). In group 3 and 5 an immediate significant decrease ($p<0.05$) was seen at TP 2 which persisted to TP 6. No immediate change was observed in group 4, but the decline in this group occurred at day 1.

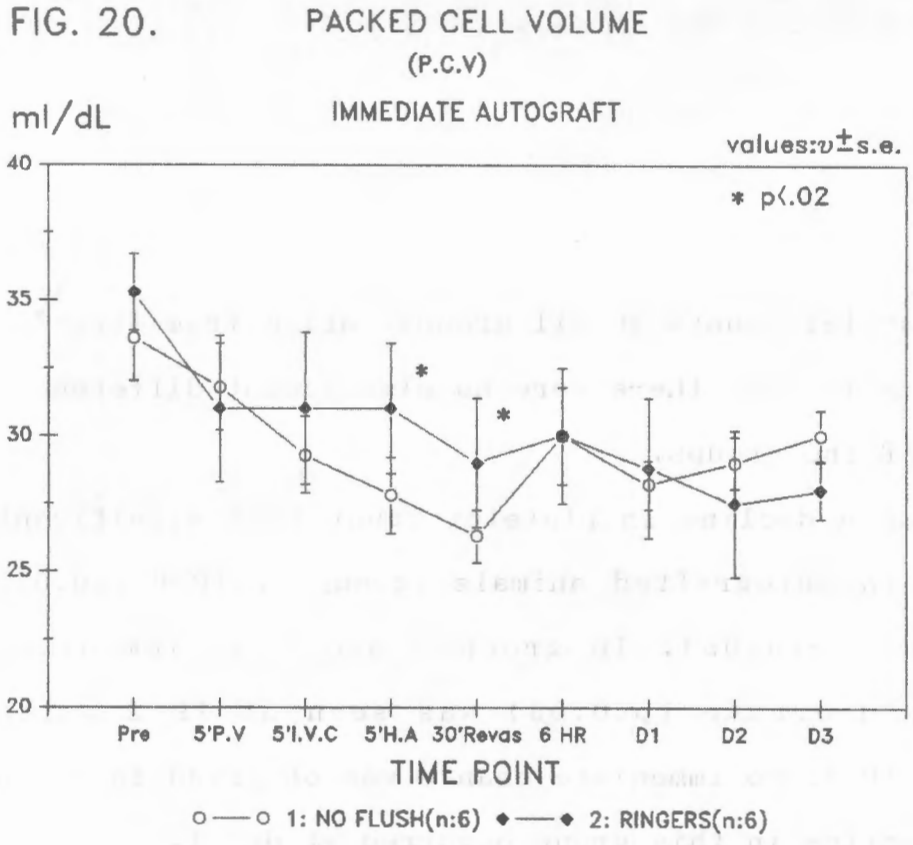
There was no difference between groups at given timepoints.

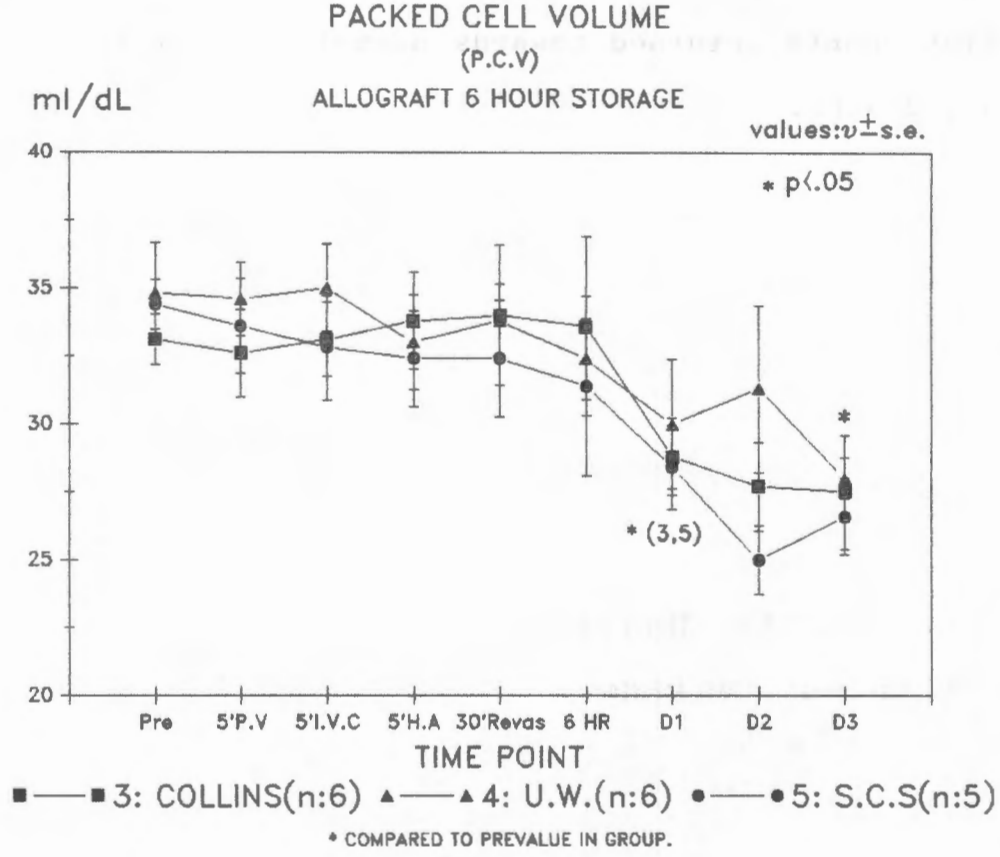
Platelet counts returned towards normal between days 2 and 3 in all groups.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

FIG. 20.




* COMPARED TO PREVALUE IN GROUP.

PACKED CELL VOLUME.

Fig.20.

Mean baseline packed cell volume in all groups lay within a narrow range of 33.1(±2.3) to 35.3(±2.3) ml/dL with no difference between groups.

There was a slow decline in groups 1 and 2 to a mean nadir at TP 4 of 18-22% ($p<0.02$) and this remained so to the end of the study.

In allografts (groups 3, 4 and 5) there were no changes until after 6 hours at day 1. In groups 3 and 5 mean values on day 1 were significantly lower than baseline ($p<0.05$). In group 4, only the value on day 3 was significantly lower. From day 3 to 7, the values in groups 3, 4 and 5 were significantly ($p<0.05$) lower than baseline.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc. DAY #:Postop.

# PROLOGUE TO DISCUSSION.

Analysis of data is divided into three chapters. Firstly, because of the large amount of data, the important correlation between technical aspects of transplantation and post-operative function, as assessed biochemically, is dicussed in general and then secondly considering the individual procedures with special emphasis on the method that is currently regarded as the best available (U.W. solution)[171,172] and the simple cold storage technique, which is novel.

CHAPTER 10.

# DISCUSSION: PART I

## THE EFFECT OF TECHNICAL ASPECTS IN LIVER TRANSPLANTATION.

Ischaemia.

Operative technique.

Immunosuppression.

ISCHAEMIA.

This method of donor hepatectomy allowed harvesting in less than 20 minutes. All dissection was done in the donor animal whilst the liver was normally perfused. The possible harmful effect of dissection on a cold, ischaemic organ was avoided.[6] This technique also allowed for exactly the same procedure in allo- and autografts. In cases where portal vein cannulation was performed, arterial perfusion of the liver was not interrupted until removal of the liver. The warm ischaemic time of the liver prior to flushing, utilising this method, was usually less than 30 seconds. Some authors pay extraordinary attention to minimising this period of warm ischaemia. Results obtained in this series of experiments questions the relevance of brief warm ischaemia time[164], as do previous studies by Kahn et al who demonstrated that minimal changes occurred in liver enzymes after 30 minutes' total vascular occlusion under normothermic conditions.[123]

OPERATIVE TECHNIQUE.

All implantations were done in exactly the same manner and anastomotic sequence. Operative times were similar in all groups regardless of the procedure being allo-or autograft. Total intra-operative times were less than one and a half hours in all cases. This differed markedly from previously described methods and techniques. Also, heparin administration was much lower than used earlier in our

laboratory as well as described in the literature, especially by the earliest workers such as Cordier.[1,2,4,6] The postoperative fluid and drug management was standardised and kept within narrow limits.

IMMUNOSUPPRESSION.

No immunosuppression was given, as was standard procedure in our laboratory, following the observations made by Terblanche [2], Calne [7] and others. The teaching, that the allogeneic transplanted pig liver does not reject easily, however, has recently been questioned. (Sacks D.; Personal communication.) However, acute or hyper-acute rejection of the liver was not evident in this series of experiments.

In more than 50% of transplanted animals in this series, a rise in serum A.S.T, coinciding with cholestatic jaundice occurred between days 4 and 6. In 50% of these animals this was a transient occurrence, but the rest succumbed to what seemed a rejection episode, after day 7.

The use of immunosuppressive drugs, however, is not without side effects. Immunosuppressive drugs are non-specific and cause decreases in all immune responses.[159] In this study rejection did not seem to play a role early in the postoperative period; liver function and changes thereof could be assessed, without the possible harmful effects of immunosuppression on liver function, immediately and in the first week following liver transplantation.

Survival of 91% was obtained, in transplanted animals, for the duration of the study (30 out of 33).

CHAPTER 11.

# DISCUSSION: PART II

## THE EFFECT OF LIVER TRANSPLANTATION ON BIOCHEMICAL VALUES.

**Calcium.**

**Magnesium.**

**Acid base.**

**Liver function tests.**

**Protein.**

**Glucose.**

**Lactic acid.**

**Bilirubin.**

## CALCIUM.

Calcium is the 5th most abundant element in the human body and the most common of the mineral ions comprising approximately 2% of body weight. The ECF concentration of the ion is kept within narrow limits to permit its action in a multitude of metabolic processes, one of the most important of which is coagulation of blood. It also plays an important role in the control of enzyme functions and as a messenger in the transmission of hormone actions.[106]

The vital role that calcium plays in myocardial contraction was proved more than a 100 years ago by Ringer.[120] The observation that calcium in plasma existed in more than one fraction was made in 1911, but these were only defined in 1934.[108,109] Definition of the three plasma fractions of calcium universally accepts ionised (50%), complexed but diffusible or ligand bound (10%) and protein bound (40%) forms.[110]

The complexed form of calcium in plasma is bound to citrate, bicarbonate and phosphate. This is the smallest fraction of calcium and no physiological activity or role has yet been ascribed to it. Determination of this fraction is difficult and depends

on factors such as pH, concentration and affinity of the various ligands to ionised calcium.[111]

The ligand bound form of calcium might be important in the acute control of calcium concentration in plasma. It has been shown that in lactic acidosis ionised calcium levels will decrease by formation of calcium lactate.[125]

Protein-bound calcium accounts for a major portion of calcium in plasma. Calcium binds to globulins (all four fractions) and albumen with the following affinity.

Albumen>gamma->alpha 1->alpha 2->beta globulin[111]

It is thus clear that any change in the relative quantities of the different protein fractions will exert major changes on the total to ionised calcium ratio. This has been shown to be true in patients with liver cirrhosis, where measured plasma ionised calcium concentration were higher than calculated values, in order to maintain calcium homeostasis.[112]

It has previously been noted in clinical liver transplantation that there was a marked fall in the concentration of total calcium postoperatively.[113,168,169] This was ascribed to the use of citrated blood during the procedure, where up to 30 or 40 units of blood may be used. These authors suggested that 2.5 mMol of calcium chloride

should be given for each unit of blood administered. They observed that most of the patients in their study had raised ionised serum calcium levels postoperatively (after receiving intravenous calcium chloride as suggested) but this was corrected spontaneously.[113] Other authors have shown that even after massive transfusion, ionised calcium levels returned to normal as soon as citrate was metabolised and that empirically administered calcium might be deleterious.[126]

The importance of maintaining normal calcium levels rests in its contribution to cardio-vascular function and stability. Some investigators have attributed the inability to maintain the hyperkinetic cardiac state seen in some liver transplant recipients to calcium deficiency.[103] It has also been suggested that immediate correction of changes in ionised calcium should be made by administration of calcium chloride to prevent citrate toxicity.[124] A second reason for calcium supplementation would be prevention of hypocalcaemic tetany.[86]

The liver has the lowest intracellular concentration of calcium in the body at 1.6 mMol Ca++/kg wet weight (muscle 8.9, kidney 6.4).

This is significant because of the action of calcium as a messenger in the cytosol, control of membrane permeability to itself as well as other ions and also because of its direct action upon processes such as phosphorylation and DNA synthesis. If a breakdown in the integrity of the ion channels in the cell membrane occurs, there will be a flux of calcium and other ions into cells, based on concentration only.[106]. It has been noted that there is an extremely steep gradient between intra- and extracellular ionised calcium levels with an important functional purpose.see Chapter 3: Calcium.

During organ storage for transplantation cellular and membrane damage occurs.[114,115] The role of calcium as initiator as well as mediator in cell death has been described[87], and upon reperfusion of the organ, the action of oxygen free radicals would cause further cell wall damage.[116,117] It is clear that if excess calcium is circulating, further damage to cells already damaged by hypothermic storage and reperfusion injury may be caused by an increase in intracellular calcium and inability of energy dependent mechanisms to extrude this.

In critically ill patients calcium homeostasis is grossly deranged; such patients tend to have low total calcium levels, but it has been found that even in them ionised calcium remains within a narrow, albeit low

normal, range. Myocardial and other cellular functions are adapted to these lower levels of calcium.[118]

The level of ionised calcium may be chronically low in relation to established "normal" values. In the patient population for liver transplantation, organ systems other than the liver, such as renal (hepato-renal syndrome) and respiratory (intra pulmonary shunting) are often compromised. These patients are often hypoalbuminemic and hypokalaemic, and might appear on laboratory data alone to be hypocalcaemic (total as well as ionised). Functionally, however, these patients are in calcium homeostasis. In the absence of acute symptoms, prophylactic treatment of presumed or projected hypocalcaemia may be deleterious eg. disturbances of cardiac rhythm.[107]

Ionised calcium is the functionally active form of this essential mineral. It has been shown that ionised calcium concentration changes inversely to changes in pH associated with respiratory alkalosis or acidosis at a rate of 0.4mMol/L per pH unit.[121] It has also been established in a model of metabolic acidosis that a decrease in pH will raise ionised calcium, but that very little effect is exerted on total or protein bound calcium .[122]

In the groups of transplanted animals in this study reference values of calcium correlated well with those reported by other investigators using pigs as

experimental models.[122]

In both auto- and allograft graft groups there was an immediate decrease of total calcium upon reperfusion. There was no transfusion of blood or blood products, at any time, to account for this decrease. No drugs that could affect calcium values were given to any animal.

Variables in the composition of the solution (pH, calcium) used for flushing of the liver may contribute to changes in measured values after reperfusion. It is noted that Eurocollins and U.W solutions do not contain calcium, whereas lactated Ringers solution does. However, there was no difference in calcium values of auto- and allograft groups regardless of flushing or not.

In group 2 (lactated Ringers solution) a 0.1 mEq/L higher value was seen at TP 3 through to day 3. This was only statistically significant after reperfusion of the hepatic artery. Lactated Ringers solution contains 2 mMol/L of calcium ion. It could be argued that this would account for the higher values in this group of animals, but this is an unknown quantity as no actual measurement of the volume remaining in the liver was made. Some of this was flushed out by blood after anastomosis and restitution of flow through portal vein.See Chapter 5: Technique.

The hepatic venous concentration of ionised calcium showed no change up to thirty minutes after full revascularisation. In the same period marked fluctuations

in total calcium values occurred.

The pH in the Ringers flushed group rose earlier than in the no-flush group which might account for the slightly higher levels of calcium up to thirty minutes post full revascularisation. It does not explain the statistically significantly lower ionised calcium at TP 6 and days I and 2 in group 1 animals.

Levels of plasma bicarbonate, lactic acid and albumen were similar in both groups. Theoretically competitive binding sites for ionised calcium would have been similar and would thus not account for discrepancies in values between these two groups either.

In Collins solution stored livers total and ionised calcium concentration in arterial and venous blood showed an almost identical picture. Ionised calcium was higher than reference values in the first 24 hours, but normalised at day 1. It is of note that Collins solution does not contain calcium, but does contain magnesium.Table 1.

Changes in pH would account for changes in ionised calcium in group 3 (Collins), but not for that in total calcium.[122] Plasma bicarbonate in this group remained statistically significantly lower than in the other allograft groups yet ionised calcium levels were higher at comparative timepoints.

Lactic acid concentrations in the allograft groups

were identical and thus calcium complexing to lactic acid cannot account for variation in values.[125]

Mechanisms for the acute control of calcium homeostasis have not been clarified.[120,122] It seems that in the transplant setting changes in calcium homeostasis occur independent of transfusion of citrated blood, pH changes or protein metabolism. Variation in calcium values does not follow theoretical, predicted paradigms.

In considering plasma levels of aspartate amino transferase as an index of hepatocyte damage, no correlation could be found with total or ionised calcium levels; e.g. plasma aspartate amino transferase was highest in recipients of simple cold stored livers yet changes in total and ionised calcium were moderate.

This consideration of recognised factors in calcium homeostasis has not revealed consistent correlations and thus, in this setting, changes in calcium levels seem to occur independantly of changes in pH, albumen or hepatocyte damage. There does not appear to be any consistent correlation between levels of plasma calcium and electrolyte composition of flushing solutions.[Vide supra.]

It is apparent that further studies will be necessary to elucidate causes, but from this data it can be concluded that changes in plasma calcium occur as part of the liver transplant procedure per sē.

## MAGNESIUM.

The importance of magnesium is recognised in a wide range of biological processes but homeostasis is not as well understood as in the case of calcium, although it is recognised as an essential mineral.[54]

There is no reference to changes in magnesium in transplant literature, or the possible effect thereof upon metabolism in the recipient.

Hypocalcaemia leads to increased absorption of magnesium in the loop of Henle which leads to mild hypermagnesaemia.[127] As it has been shown that hypocalcaemia is a common finding in chronic liver disease[128], there may also be an increase in magnesium concentration.

Hypercalcaemia leads to hypomagnesaemia by a reversal of the renal mechanism alluded to above.[127] The clinical effects of magnesium deficiency manifest as altered mental state as well as hyperexcitability in neural, cardiac- and somatic muscular tissue. Very subtle changes in plasma magnesium concentration may cause symptoms in neural and cardio-vascular systems, but tissue concentrations (especially muscle) will show depletion much earlier. Hypomagnesaemia is often seen in intravenously fed patients and potentiates the effects of hypokalaemia and digitalis on cardiac function; it is essential that magnesium should be added to intravenous hyperalimentation regimens.[130]

No adverse effects of hypermagnesaemia have been described, except that on a theoretical basis reduced absorption of calcium in the distal tubule might occur.[129]

In the liver transplant setting the importance of magnesium lies in the possibility of renal mediated hypomagnesaemia caused by excessive administration of calcium. Intravenous alimentation, without additional magnesium, may increase this defect.

In all transplanted animals an increase in total magnesium occurred during the first 24 hours. In autograft groups this increase was significantly less than in allografted animals and levels had returned to normal by 6 hours post-reperfusion.

The significantly higher magnesium levels in group 4 up to day 2 may have been due to magnesium contained in Collins solution.Table 1. Influx of water into liver cells drags potassium as well as magnesium into the cell.[131] It has been shown that cell swelling with this type of preservation solution (Collins) is much more than with U.W. solution, which has been shown to decrease cell volume.[60] However, U.W solution also contains magnesium in the same concentration as Collins solution and no similar increase was seen in recipients in that group. Increased plasma levels of magnesium might be an indicator of cellular damage in the liver with release upon reperfusion. This is also indicated

by increased plasma magnesium levels in hepatic venous blood at TP 2, whereas at TP 3 (return of pooled blood from the lower limbs and kidneys) plasma levels were lower than at TP 2 in all but group 4. Thus, it is possible that after reperfusion, swollen cells would lose magnesium along with potassium, as part of the reperfusion phenomenon. The magnitude of increase in magnesium concentration, in this series of experiments is in keeping with this postulate. The increase in serum magnesium should be correlated in the experimental and clinical situation with known parameters of functional assessment.

Maintelyte® intravenous solution contains 5 mEq of magnesium per litre but as it was given to all animals in the same quantities, the effect of administered magnesium should be ignored.

As in the case of calcium, it would be difficult to deduce the concentration of ionised magnesium, by methods other than direct measurement. It is likely that calculated values for ionised magnesium would be inaccurate as with ionised calcium. It is accepted that with magnesium, as with calcium, the ionised fraction is the metabolically active form. This would explain symptoms of hypomagnesaemia in the presence of normal or near normal plasma magnesium.

These results indicate that magnesium metabolism

after liver transplantation, should be investigated further as an early marker of preservation damage or return of function.

# ACID BASE METABOLISM.

pH

As is well described in literature, pH declined upon reperfusion of the transplanted liver. The return of pH to normal did not differ between allo- and autograft groups, except for group 2 (lactated Ringers flush) and 5 (no flush), where the significantly earlier return to normal started immediately after portal reperfusion. Metabolism of residual lactate ion present in vascular spaces from flushing with lactated Ringers solution via the Cori cycle is a theoretical explanation for this phenomenon. Serum lactic acid in group 2 was higher than in non-flushed livers but not statistically significantly so, as in the case of pH.

The reason for the earlier return of pH to baseline levels in group 5, which was statistically significant, can only be speculated upon. Anaerobic metabolism in the blood left in vascular spaces could have been the source of extra lactic acid compared to the other groups, but on measurement of plasma lactic acid there was no difference. Furthermore, in group 5 at TP 6 lactic acid, bicarbonate ion as well as $pCO_2$ were at the same levels as in groups 3 and 4, but the pH was 0.2 units higher. This would suggest the existence of another organic acidic metabolite not removed by the liver

TP-5:30'Revasc. TP-6:6hrs.revasc.

in groups 3 and 4. This remains to be identified.

Arterial pH levels from day 1 through 7 were higher than baseline levels. The metabolic alkalosis that is seen after human and canine liver transplantation, has been ascribed to metabolism of citrate administered with blood.[83] It is clear from the present account, where no blood or citrate was administered, that the cause for this phenomenon does not rest with citrate and the cause for this alteration in pH has also not been clarified yet. It is known that primary graft failure is associated with persistently low pH values. The identification of substances associated with persistently low pH might be an early marker of primary non functional grafts.

BICARBONATE.

Metabolic alkalosis often occurs in surgical patients due to loss of acid or addition of base. The causative process can usually be identified from the patient's condition or therapeutic management. In complex procedures, like liver transplantation, however reasons for development of this condition might not be clear.[74] The frequent development of metabolic alkalosis post liver transplant has been noted and ascribed to the quantity of blood products used and metabolism of citrate contained therein.[83,132]

Variables in the clinical setting that affect bicarbonate ion levels include various drugs eg. diuretics and corticosteroids, loss of acid via nasogastric drainage, ventilator management via $pCO_2$ and blood product infusion (citrate metabolism). Apart from nasogastric drainage during the operation only, none of these was present to influence bicarbonate production or loss in this series of liver transplants.

In autograft groups bicarbonate did not decrease after reperfusion, but from 6 hours onward a metabolic alkalosis was evident. Bicarbonate ion concentration was not affected by lactic acid concentration . Also, the drop in pH did not affect bicarbonate concentration or vice versa.

The significant decrease of bicarbonate upon reperfusion in allografted animals could only have been

caused by reperfusion of the stored liver (period of tissue hypoperfusion in both groups was similar).

As was noted in autografted animals, lactic acid concentration did not have an effect on bicarbonate concentration. In group 5, although pH increased at least 6 hours before that in group 4, bicarbonate levels were almost similar. In group 3 conversely, although pH at day 1 was still lower than other groups, bicarbonate concentration was at a comparable level.

In all animals a metabolic alkalosis was present until day 7, with increased plasma bicarbonate concentrations. The development of a metabolic alkalosis in the first 6 hours post liver transplant is associated with survival of animals, whereas persistence of low levels of bicarbonate is seen as a poor prognostic sign.[134] In a model of poor liver preservation, an irreversible metabolic acidosis with non-reversible low bicarbonate levels always resulted in demise of transplanted animals. Even administration of exogenous sodium bicarbonate and mechanical hyperventilation did not raise measured serum bicarbonate.[134] In the three animals that died in this series, with no demonstrable cause, bicarbonate levels continued to decline. These animals were not ventilated nor given bicarbonate.

The prognostic indicative value of post transplant bicarbonate levels is a novel preliminary observation

documented from our laboratory. The results obtained in this series of experiments suggest that post transplant bicarbonate levels should be assessed in the clinical situation as an indicator of function.

SODIUM

Sodium changes in the entire series of experiments were unremarkable throughout. Although statistically significant changes were found, when compared to baseline values, all of these were well within normal range and clinically not of note. Even flushing the liver with lactated Ringers solution, with a high sodium content compared to other solutions, had no measurable effect on sodium metabolism.

It seems that the suggested important role of the liver in volume regulation[82], as a function of sodium concentration, does not come into effect in liver transplantation. The total denervation of the liver is presumed to be the cause for this phenomenon in the transplanted liver. Hormonal effects mediated via the adrenal gland is not thought to exert influence in the immediate post transplant period.

POTASSIUM.

Arterial

An immediate increase in serum potassium levels was seen in all groups upon reperfusion. Since no exogenous potassium was given in group 1, the source in all groups was most probably due to the reperfused liver. The only sources for this which could be identified would be haemolysis of red blood cells upon reperfusion or release from hepatocytes.

Both Collins and U.W. solutions contain intracellular concentrations of potassium, yet the increase in potassium concentration was not different from that seen in the non-flushed groups. This would confirm that the increases in potassium were due to release from reperfused hepatocytes. Accepted transplant dogma requires livers to be flushed with large volumes of low potassium solutions prior to reperfusion. Although all livers in these experiments were flushed with autologous blood this did not change the transient hyperkalaemia. The observation of hyperkalaemia, after reperfusion, even in non-flushed livers questions the advisability or need to flush livers prior to reperfusion with large volumes of lactated Ringers solution.[135]

Similar conclusions explaining the increase in potassium level during liver transplantation have been drawn by other authors.[83,86]

A significantly lower potassium was seen in group 4 at TP 5. This phenomenon was also seen in canine liver transplants and was ascribed to uptake of potassium by functioning hepatocytes.[45,46,78] This was corrected spontaneously by TP 6.

Reperfusion of the distal systemic circulation (release of infrahepatic inferior vena clamp) did not appear to cause any rise in potassium concentration in any group.

Potassium homeostasis in all groups was normal at 6 hours postransplant.

No animal showed severe cardiac reactions relating to higher levels of potassium circulating at time of declamping of the suprahepatic vena cava, although ECG tracings were not taken.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc.

POTASSIUM.

Venous

Concentrations of $K^+$ at all timepoints were almost identical in all groups except 5. The significant difference of 3.5mEq/L between arterial and venous samples, at TP 2 only, with virtually no difference in other groups cannot be explained. The lung, as far as could be ascertained, has never been described as a potassium trap. At TP 3 potassium levels were comparable to those in other groups as well as to those in arterial blood.

The importance of this discrepancy lies in the fact that potassium rich venous blood enters the cardiac circulation first and might cause serious rhythm abnormalities. In these experimental animals no untoward reactions were seen despite high serum potassium concentrations.

The clinical implication of this would be to sample central venous blood for potassium determination, especially on portal venous reperfusion, as arterial blood might not reflect true potassium concentration fluctuation.

TP-1:PRE. TP-2:5'P.V. TP-3:5'I.V.C. TP-4:5'H.A.

TP-5:30'Revasc. TP-6:6hrs.revasc.

# LIVER FUNCTIONS.

## ASPARTATE AMINOTRANSFERASE (A.S.T)

Levels of this enzyme are often used to indicate damage during storage in vitro in storage medium.[60] The serum level post transplantation is used as a parameter for return of function as well as repair of storage induced injury to hepatocytes.[45,46,47] Isoenzymes are present and forms originating from the liver can readily be identified. The important other sources of this enzyme are cardiac muscle and red blood cells.[59] In these experiments with healthy young pigs, cardiac muscle was unlikely to be abnormal and thus the possible cardiac origin of the enzyme was excluded.

In autograft groups virtually no increase of A.S.T was seen during reperfusion. The increase in both groups up to day 1 indicated ongoing cellular disruption and mitochondrial breakdown. Because liver grafts were returned to the same animals there was no possibility of immune mediated cytotoxic reaction in these groups. A likely cause was oxygen free radical damage, which occurs upon reperfusion.[136] The reason for lower levels, although not statistically significant, in lactated Ringers flushed autografts is not clear. This is not an intracellular solution, but contains an extra cellular like concentration of sodium and is slightly hypo-osmolar. This solution causes

marked hepatocyte swelling in perfused livers.[78] Also, if A.S.T was from red blood cells left behind in the non-flushed groups the rise in plasma concentration would have been expected earlier.

In allografted groups the haemolytic theory would explain early high values in group 5. From groups 3 and 4 it appears that an ongoing process is causing a continuous rise in A.S.T levels.

University of Wisconsin solution has been reported to give superior results in clinical, rat and canine liver preservation studies when compared to Euro Collins solution; one of the main parameters of preservation quality in these studies is postoperative serum levels of A.S.T and the rate of return to normal levels in the postoperative period.[45,46,47,78] The reason for peak levels of A.S.T in U.W solution preserved livers, twenty four hours later, and higher serum levels for two days longer than in other groups, is obscure. The inability to preserve viability in porcine livers for extended periods, up to 24 hours, might be linked to this observation.[134], Sheil R.;personal communication.

## ALANINE AMINOTRANSFERASE (A.L.T)

Alanine aminotransferase is used in conjunction with aspartate aminotransferase to assess preservation damage.[45,46,47] It as also used in conjunction with A.S.T to indicate rejection episodes.[137, Belzer F.:Personal communication.]

Although values in some instances doubled at day 1, there were no obvious differences between groups. In some pigs fulminant rejection, shortly after day 7, could not be predicted from serum value changes in this enzyme. Biochemical changes which were compatible with transient rejection episodes likewise were not associated with any rises in serum concentration of this enzyme.

Neither A.S.T nor A.L.T or ratios of the two enzymes could be used to indicate the damage caused by different length of storage in these experiments. Serious doubts must exist about the use of these enzymes as an indicator of cellular damage in the transplant situation.

The use of glutathione S-transferase assays might be a more sensitive assessment of preservation and reperfusion damage and should possibly be investigated in the transplant setting.

ALKALINE PHOSPHATASE (A.P)

In autograft groups slightly increased values were seen which peaked at day 1. In the absence of rejection phenomena this would indicate some degree of biliary canalicular obstruction, resulting from cell swelling. As would be expected bilirubin levels were normal in these animals.[63]

The increase in plasma alkaline phosphatase in group 3 (Collins solution) may also be explained by cellular swelling. It was shown that Collins solution caused markedly more swelling of cells during hypothermic storage than U.W solution.[114] As with autografted animals bilirubin levels were within baseline ranges in all allografted groups. Levels of alkaline phosphatase in these animals at day 7 were still within the baseline range, despite increases in aspartate aminotransferases and a clinical picture of graft rejection.

The specificity, as well as sensitivity, of plasma alkaline phosphatase levels as a marker of pathology in the post liver transplant setting seems to be less than that of other, already questionable, measurements.

TOTAL PROTEIN

Baseline values of total protein varied widely and the same wide variation existed in the postoperative period in all groups. Actual intravascular fluid volume is important in protein concentration determination.[140] The gradual decline seen in autograft groups up to day 2 postoperatively could be explained by the dilutional effect of intravenous fluid therapy, since concentration increased from day 3, when intravenous infusion was stopped.

In allograft groups the significantly higher total protein in group 4 (U.W. solution) up to 6 hours postoperatively might also have been due to fluid shifts. It has been shown that U.W. solution actually reduces hepatocyte volume and total tissue water.[60] Movement of intravascular water into the transplanted liver during the first 6 hours could have resulted in a relative higher intravascular total protein level.

When the values for packed cell volume are assessed in conjunction with total protein values it appears that changes are not interrelated. The marked reduction in total protein in Collins and no-flush autografts has no effect on packed cell volume or vice versa. In the U.W group both packed cell volume and total protein remained very close to baseline reference values.

ALBUMEN

Contrary to reported literature albumen levels in all groups remained within reference values for the first week. The only decrease was seen in the immediate post-reperfusion period and that was transient and insignificant.

There is no readily available explanation for the discrepancy in albumen:total protein ratio observed in group 4 (U.W. solution). In this group albumen was lower relative to total protein. On speculative basis, fluid drag of albumen into hepatocytes might be implicated in causing relative loss of intravascular albumen, into other fluid compartments. In order to prove this hypothesis the actual size of relevant compartments could be established by $I^{125}$-labelled tracer albumen. This discrepancy was rectified spontaneously and did not prove to be significant in the postoperative course.

It was shown that, although protein synthesis and breakdown increase up to 50% in the perioperative period[142], the most likely cause for acute changes in albumen concentration during this time is alteration in fluid composition. These authors furthermore suggested that albumen concentration per se was not an indication of rate of synthesis or catabolism unless volume of albumen containing pools was taken into consideration.

The rate and magnitude of changes in albumen

concentration were similar in all groups regardless of storage or perfusion solution variation. Synthetic function of the liver does not appear to have been compromised in this series of experiments. This was contrary to the observation made in ultra long term liver preservation in dogs where albumen levels at 6 hours after grafting were very low and never returned to normal.[46]

These authors suggested that the albumen synthetic facilities in the liver were irreversibly damaged by long term preservation damage.

GLUCOSE

Glucose levels in all pigs during the dissection phase were higher than post-absorptive normal levels (50-70 mg/dL). Elevated levels were most probably due to infusion of 10% Maintelyte® initiated ±15 minutes prior to sampling.

No sampling was done during the anhepatic phase which was shorter than 20 minutes in all cases. Anhepatic time was recorded from disconnection of the liver till restoration of portal flow.

The increase in glucose levels on reperfusion was in keeping with experimental and clinical reports.[85,144,146] Experimental work done on dogs showed a similar increase in Phase I (pre-anhepatic) to what was seen in all pigs, except that no dextrose was administered during the entire procedure in dogs.[144] Also, in clinical transplants, it was shown by Atchison that the amount of dextrose administered in blood products, did not bear relation to the rise in glucose on reperfusion of the donor liver.[71] In groups 3 (Collins) and 4 (U.W.) extra glucose was present in the storage fluid. However, blood glucose levels on reperfusion showed a similar increase in all animals.

The overall lower glucose levels in group 4 (U.W.) immediately after reperfusion, although not statistically significant, indicated either less leakage or earlier uptake of glucose. It is more likely that leakage of glucose out of cells was responsible for high blood levels.[146]

The statistically significantly lower level at thirty minutes after reperfusion indicates that livers were actively metabolising glucose. Also, it is noted that in group 3 there was a downward trend in glucose levels at thirty minutes postreperfusion. These two groups had additional glucose (added to the preservation solution) compared to the others. It could be suggested that less glycogenolysis and glucose loss from hepatocytes due to glucose loading, would result in earlier availability of hepatic substrate and thus earlier return of energy production by the liver.

In three non-surviving pigs (data not shown) blood glucose levels at 6 hours postreperfusion were above 400 mg/dL. This concurred with the view that persistent hyperglycemia in the postoperative period is a poor prognostic indicator.[143] As shown by Hickman, hepatectomised pigs with persistent hyperglycaemia have an extremely short survival period compared to those where the blood glucose is kept at normal levels.[160]

Khoury and his group theorised that the decrease of glucose on reperfusion, was due to decrease in the glucose load administered in blood, because transfusion requirements diminished during the same period.[145] These results show that the theory is unfounded; in all animals in this series, despite the continuous infusion of a 10% dextrose solution, glucose levels were maintained comparable to what would be

expected in the absorptive (postprandial) state (90-100mg/dL). Thus, glucose levels in the postreperfusion period, decrease due to active metabolism by hepatocytes. On discontinuation of dextrose infusion, early morning blood glucose levels were similar to those seen in the pre-prandial state (50-70 mg/dL).

LACTIC ACID

Lactic acid levels in all groups were compatible with normal levels reported in the literature and accepted in J.S. Marais laboratory.[80]

Increase in lactic acid levels upon reperfusion was expected for theoretical reasons. The seemingly higher values in group 2 (lactated Ringers) was not of statistical significance and percentage increases were exactly similar to those seen in other groups. This indicates that an insignificant volume of remaining lactated Ringers solution was put into circulation on reperfusion.

Opening of the inferior vena cava, in both groups, resulted in an increase in lactic acid levels. This indicates introduction of lactic acid from the temporarily occluded distal systemic circulation. The magnitude of this increase was statistically, and in real terms, insignificant. In autograft groups the isometric maintenance of this level could indicate earlier return of hepatocyte function, because the influx of lactic acid from distal systemic circulation in both groups must have been equipollent.

The increase in plasma levels of lactic acid on reperfusion did not differ between the prolonged storage and autograft groups, and was unexpected. A possible explanation for this might be a rate limiting effect of anaerobic metabolism by decreasing pH intracellularly.[136]

The significantly lower levels at thirty minutes postreperfusion, in the autograft groups, indicate earlier return of metabolic capacity. It remains a question whether the reason for this is qualitative at a cellular level or a function of hepatic volume as a quantitative factor. It seems logical to accept that quality of function is more likely.

When this part of carbohydrate metabolism (lactic acid) is compared to that of glucose it is noted that in the first thirty minutes after complete revascularisation, in autograft groups, lactic acid concentration was lowered before that of glucose. This is in keeping with the concept of no nett gain in glucose by aerobic conversion of lactic acid to glucose. Conversely, in group 4 (U.W. solution) glucose levels declined whilst lactic acid concentration remained stable. There is no readily available explanation for this phenomenon.

The alkalosis that developed in all animals from 6 hours and remained till day 7 happened despite normal lactic acid levels. This finding indicates that metabolism of excess circulating lactate cannot be the cause of ongoing metabolic alkalosis seen after liver transplantation as suggested.[145]

In three pigs that did not survive, lactic acid concentration was increased at 6 hours postoperatively. This phenomenon was also seen in pigs transplanted with extended preservation time (24 hours) livers,[Unpublished data] and all of these animals died in metabolic acidosis.

BILIRUBIN

Bilirubin changes, as mentioned, were not indicative of storage related functional abnormalities. The changes seen in 50% of the animals were indicators of rejection and had no relation to the storage time or perfusion fluid.

The fact that no choledochal strictures were seen at 7 days could be artefactual due to the short time span. However, this type of anastomosis is used clinically and experimentally with success.[46,47,48] Furthermore, the pig is not susceptible to biliary complications after liver transplant, probably due the excellent blood supply to the common hepatic duct as shown by Northover.[43]

It seems that bilirubin levels are not an indicator of preservation damage in the experimental porcine liver transplant setting.

CHAPTER 12.

# DISCUSSION: PART III

## THE EFFECT OF LIVER TRANSPLANTATION ON COAGULATION.

COAGULATION CHANGES IN LIVER TRANSPLANTATION.

Coagulation profiles in the clinical and laboratory settings of liver transplantation vary.

Clinically, the indication for transplantation often is extensive liver pathology and this is frequently the direct cause of pre-and intraoperative haemostatic abnormalities.[147,148]

In the spectrum of liver disease there is a qualitative as well as quantitative difference, in clotting abnormalities presenting, directly relating to underlying pathology.[149]

The basic pathology may vary from a defect in the manufacture of essential clotting components such as factors V, VII, VII and others to plasminogen activators.[149] The existence of a plasminogen activator in bile has been known since 1983, although the role of this substance in liver transplantation has not been investigated.[150] Tissue plasminogen activation as well as other coagulation parameters are extensively investigated in liver transplantation with a view to identifying prognostic indications, as well as the origin of coagulation defects.[98]

Clinical liver transplantation is the surgical procedure associated with the use of highest volume transfusion of blood and or blood products and all possible should be done to decrease requirements.[151]

The situation in experimental transplantation is quite different from the clinical as far as haemostasis is concerned. Normal haemostatic mechanisms should be expected in donor as well as recipient. Any changes in haemostatic parameters will be affected by the procedure or preservation flushing solutions and iatrogenically administered drugs such as heparin.

In experimental transplantation defects in haemostasis have been shown to cause mortality.[152] In early experiments high doses of heparin were used both in donors and recipients. The role of "heparin-like" or endogenous heparin has not been clarified. It is possible that administered heparin might bind to endothelial surfaces and be released on reperfusion of the grafted organ to cause clotting defects.[153]

In experiments under discussion the only significant differences in clotting parameters used, were prolongation of activated clotting time (A.C.T), which is specifically designed to detect heparin action. Although the autograft groups only received a total of 500 Units of heparin, less than the 1500 Units in the donor and recipient together in the allograft groups, there was no difference between these two groups, except in livers preserved in Collins solution. In all animals A.C.T levels were back to normal by 6 hours postoperatively. Judging from this data it seems that the heparin given to the donor did not cause prolongation of

A.C.T. in transplanted animals except in the livers preserved with Collins solution, where it was transient.

The "haemorrhagic diathesis" reported in the literature by Perkins, as well as Plevak and Porte, occurred in livers preserved with crystalloid solutions such as Collins or lactated Ringers.[152,154,155] This grave complication did not play any role upon reperfusion of the donor liver in any of the experiments reported in this study.

Similarly, a decrease in fibrinogen concentration is a common finding after major surgery or resection.[155] The insignificant differences of values between autograft and allograft groups indicates that the decrease in fibrinogen was related to the magnitude of the surgery, and had nothing to do with storage or dilutional effects of preservation solutions.

Platelet counts, although differing widely, did not change significantly in any of the groups. The decline seen after 6 hours in all groups and the return to normal levels, is in keeping with the trend reported by other investigators.[155]

Decreases in platelet counts associated with liver transplantation have been ascribed to sequestration in the donor liver spaces of Disse.[156] It was suggested that platelets aggregated as a faculty of reticulo-endothelial stimulation, with a resultant decrease in absolute

numbers, and were then released as non-functioning units with a low normal absolute platelet count as result, but functionally deficient. It was also thought that platelets were actively ingested by the donor liver Kupffer cells.[157]

In patients with liver disease, necessitating hepatic transplantation, thrombocytopenia as well as abnormal platelet function is known to co-exist. Platelet transfusion is an essential element of haemostatic management intra- and postoperatively.[149]

In these experiments platelet counts showed some decrease in absolute numbers. In no case did an absolute thrombocytopenia or haemostatic defect attributable to platelet dysfunction develop. The lower values in allograft groups could have been due to platelet aggregation on damaged endothelial surface due to storage or as part of a immune response by the reticulo-endothelial system. These experiments indicate that the decrease in platelet numbers does not cause a bleeding diathesis and also that the donor liver is not a cause for qualitative or quantitative host platelet defects.

Packed cell volume is an indicator of blood composition and as such specifically of the red cell mass. The massive transfusion requirement of liver transplantation is well known.[158] Notwithstanding total absence of blood transfusion, packed cell volume decreased to clinically

acceptable levels in this series and remained at that level. It seems that neither transplanted liver nor host immune system played a major role in the lowered packed cell volume.

Data collected in this series of experiments indicate that the transplanted liver per se, does not have any effect on packed cell volume. Changes which do occur are due to blood loss or compensatory fluid shifts that are seen in all major surgery.

CHAPTER 13.

# CONCLUSIONS.

## Conclusions.

In formulating conclusions from the vast amount of data collected in this series of experiments, it is important to briefly paraphrase the hypothesis or questions asked in this study, namely if the handling and storing moiety of the liver transplant procedure plays any role in changes in standard parameters seen after transplantation. It should also be kept in mind that this study was not used to compare different methods of storage, but that variations seen were used in an attempt to elucidate the mechanism responsible for the observed changes.

The comprehensive conclusion regarding the five groups of transplanted animals definitively affirms that the process of transplantation of the liver, per sẽ, does cause major changes in biochemical parameters of liver function. Contrary to earlier beliefs, transplantation had minimal effect upon coagulation, and it is clear that changes in coagulation parameters reported by earlier studies, were due either to iatrogenic pharmacological manipulation by heparin administration or preservation damage. It can be postulated that the effect of the donor state and/or preservation damage will cause exponential decline of function, which will be cumulative to those changes elicited by the transplant process. The excellent function of non-flushed livers challenges current preservation dogma that flushing of livers is essential.

The decrease in total, as well as ionised calcium,

that occurred seems to be an integral part of complex biochemical changes that occur in the transplanted liver. Changes in calcium levels occurred independently of recognised intrinsic influencing factors such as pH, albumen and hepatocyte damage or the extrinsic effect of blood transfusion. It was also shown that the only correct quantification of ionised calcium is by direct measurement. The significance and mechanism of changes in magnesium levels, that mirrored those of ionised calcium, could not be explained. Further studies are essential to elucidate the causes of plasma level changes, in these two minerals, in association with liver transplantation.

Liver transplantation profoundly affected acid-base metabolism. Metabolic alkalosis occurring after liver transplantation is not due to the metabolism of citrate or lactic acid, but does indicate functional hepatocytes with good prognosis for survival. This phenomenon should be investigated at cellular level.

Measurement of "liver enzymes" as an indicator of storage damage or return of function is of low specificity and sensitivity. Changes in these values do not correlate with those in other parameters.

Normal glucose homeostasis and metabolism of lactic acid in the early post-transplant period is an important indicator of hepato-cellular function and prognosis.

Coagulation in porcine liver transplant recipients is not affected by the process of successful storage and transplantation. It is suggested that derangements in coagulation, seen clinically, is a complex of donor liver quality and/or pre-existing disease in the recipient.

# EPILOGUE.

The question posed has been answered affirmatively. To the complex interplay of factors determining successful post-transplant liver graft function is evidently added the procedure itself. A reference database for those changes due to transplantation of the liver, in the pig, has been set and several novel observations, which may have clinical relevance, have been made . It is my hope that this study will be a stone, albeit small, in the foundation of a better understanding of liver transplantation.

# REFERENCES.

1. Cordier G, Garnier H, Clot J-P et al. La greffe de foie orthotopique chez le porc. Premiers resultats. (Orthotopic grafting of the liver in pigs. First results)
Mem. Acad. Chir.(Paris),92:799,1966.
Glio Chirurgicae. 49-52. 1988.

2. Terblanche J, Peacock J, Bowes J, Hobbs K. The technique of orthotopic liver homotransplantation in the pig.
J Surg Res 1968 8: 151-160.

3. Hickman R, van Hoorn WA, Terblanche J. Exchange transplantation of the liver in the pig.
Transplantation 1977;24(4):237-242.

4. Dent D, Hickman R, Uys C, Saunders S and Terblanche J. The natural history of liver allo- and autotransplantation in the pig.
Br J Surg 1971;58:407-413.

5. Northover J, Terblanche J. A New look at the arterial supply of the bile duct in man and its surgical implications.
Br J Surg 1979;66:379-384.

6. Pienaar B, Lindell S, van Gulik T, Southard J and Belzer F. Revised method of orthotopic hepatic transplantation in canines.
Surgery, Gynecology & Obstetrics 1989;169:341-346.

7. Calne RY, Dunn DC, Herbertson BM et al. Preservation of the porcine liver.
Transpl Proc 1974;6(3):289-294.
8. Kamada N, Calne RY. Orthotopic liver transplantation in the rat.
Transplantation 1979;28(1):47-50.
9. Kamada N, Calne RY. A surgical experience with five hundred and thirty liver transplants in the rat.
Surgery 1983;93(1,1):64-69
10. van Wyk J, Liem DS, Eiseman B. Function of the cadaver liver.
Surgery 1965;58(1):120-130.
11. Kestens PJ, Farrelly JA, McDermott WV. A technique of isolation and perfusion of the canine liver.
J Surg Res 1961;1(1):58-63.
12. Abouna GM, Koo CG, Howanitz LF et al. Successful orthotopic liver transplantation after preservation by simple hypothermia.
Transpl Proc 1971;3(1):650-653.
13. Marino IR, DeLuca G. Orthotopic liver transplantation in pigs.
Transplantation 1985;40(5):494-498.
14. Hobbs K, Hunt A, Palmer D et al. Hypothermic low flow liver perfusion as a means of porcine hepatic storage for six hours.
Br J Surg 1968;55(9):696-703.

15. Skibba JL, Almagro A, Condon RE et al. A technique for isolation perfusion of the canine liver with survival.
J Surg Res 1983:125-132.
16. Toledo Peyrera LH.
Twenty four hour liver preservation by hypothermic intermittent perfusion.
Transplantation 1979;27:291-293.
17. Toledo Peyrera L, Simmons R, Najarian J.
Factors determining successful liver preservation for transplantation.
Ann Surg 1975;181:289-298.
18. Monden M, Fortner JG. Twenty four and forty eight hour canine liver preservation by simple hypothermia with prostacyclin.
Ann Surg 1982;196(1):38-42.
19. Benichou J, Halgrimson CG, Weil R et al. Canine and human liver preservation for 6 to 18 hours by cold infusion.
Transplantation 1977;24(6):407-411.
20. Calne RY, Dunn DC, Herbertson BM. Liver preservation by single passage hypothermic "squirt" perfusion.
Br Med J 1972;4:142-144.
21. Turner MD, Alican F. Successful 20 hour storage of the canine liver by continuous hypothermic perfusion.
Cryobiology 1970;6(4):293-301.

22. Toledo Peyrera LH, Chee M, Lillehei RC et al. Liver preservation by cold storage with hyperosmolar solution for 24 hours.
Cryobiology 1979;16:43-49.
23. Jablonski P, Douglas MC, Gordon E et al. Studies on the isolated perfused pig liver.
Br J Surg 1971;58(2):437-453.
24. Grundmann R. Basic concepts in organ procurement, perfusion and preservation for transplantation. 1982:93-120. In: Toledo Peyrera LH (Ed.): Fundamentals of Preservation Methods. Academic Press (New York).
25. Lambotte L. Use of hepatic cell membrane potential measurement to evaluate new methods of liver preservation.
Surgery 1973;74(4):509-518.
26. Iwatsuki S, Shaw B, Starzl T. Current status of hepatic transplantation.
Seminars in Liver Disease 1983;3(3):178-180.
27. Starzl T, Iwatsuki J, Shaw BW. Orthotopic liver transplantation in 1984.
Transpl Proc 1985;17(1):250-258.
28. Rolles K, Jamieson NV. Donor operation and preservation of the liver.
In: Calne RY.(Ed.): Liver Transplantation. Grune & Stratton (London) 1987. 141-156.

29. Bombeck CT, Biava C, Condon RE et al. Parameters of normal liver function in the isolated perfused bovine liver. In: Organ Perfusion and Preservation. Norman JC (Ed.): Appleton-Century-Crofts (New York). 1968:573-608.

30. Otte G, Wolff H, David H. Preservation damage in liver transplantation: electron microscopic findings. Transpl Proc 1984;16(5):1247-1248.

31. Searle R, Flaks B. An electron microscopic study of pig liver viability after extracorporeal storage. Br J Exp Path 1976;87:515-520.

32. Jamieson N, Sundberg R, Lindell S et al. A simplified technique for transplantation of the canine liver. Acta Chir Scan. 1988;154(9):511-516.

33. Fonkalsrud E, Ono H, Shafey O, Longmire W. Orthotopic canine liver homotransplantation without vena caval interruption. Surg Gynecol & Obstet 1967 125: 319-327.

34. Goodrich B, Welch H, Nelson J, et al. Homotransplantation of the canine liver. Surgery 1956 39: 244-251.

35. Monden M, Barters R, Fortner J. A Simple method of orthotopic liver transplantation in dogs. Ann Surg 1982 195: 110-113.

36. Welch C. A note on the transplantation of the whole liver in dogs. Transplant Bulletin 1955 2; 54-55.

37. Starzl T, Marchioro T, Porter K, et al. Factors determining short- and long-term survival after orthotopic liver homotransplantation in the dog.
Surgery 1965 58: 131-156.
38. Todo S, Kam I, Lynch S, Starzl T. Animal research in liver transplantation with special reference to the dog.
Semin Liver Dis 1985 5: 309-317.
39. Moore F, Smith L, Shoemaker W, Ceruker U and Dammin J. One stage homotransplantation of the canine liver after hepatectomy.
Transplant Bulletin 1959;6:103-107.
40. Moore F, Wheeler H, Demissianos H, et al. Experimental whole organ transplantation of the liver and of the spleen.
Ann Surg 1960;152:374-387.
41. Starzl T, Kaupp H, Brock D, Lazarus R and Johnson R. Reconstructive problems in canine liver transplantation with special reference to the postoperative role of hepatic venous flow.
Surgery, Gynecology & Obstetrics 1960;111:733-743.
42. Calne RY. Technique in the pig.
In: Calne RY.(Ed.): Liver Transplantation. Grune & Stratton (London) 1987. 9-16.
43. Starzl T. In: Experience in Hepatic Transplantation.
WB Saunders, Philadelphia,London and Toronto. 1969:475-479.

44. Terpstra ON, Schalm WS, Wiemar W. Auxiliary partial liver transplantation for end stage chronic liver disease.
N Eng J Med 1988;319(23):1507-1511.
45. Jamieson NV, Sundberg R, Lindell S et al. Preservation of the canine liver for 24-48 hours using simple cold storage with U.W. solution.
Transplantation 1988;46(4):517-522.
46. Pienaar B, Lindell S, van Gulik T, Southard J and Belzer F. 72 hour preservation of the canine liver by machine perfusion.
Transplantation 1989. Accepted for publication.
47 Kalayoglu M, Sollinger H, Stratta R, et al. Extended preservation of the liver for clinical transplant.
Lancet 1988; March: 617-619.
48. Starzl TE, Iwatsuki S, van Thiel DH. Evolution of liver transplantation.
Hepatology 1982;2:614-636.
49. Starzl TE, Klintmalm GBG, Porter KA et al. Liver transplantation with use of cyclosporin A and prednisone.
N Eng J Med 1981;305:266-269.
50. Slapak M, Wigmore RA, Maclean LD. Twenty four hour liver preservation by the use of continuous pulsatile perfusion and hyperbaric oxygen.
Transplantation 1967;5(4):1154-1158

51. Burdelski M, Oellerich M, Lamesch P et al. Evaluation of quantitative liver function tests in liver donors.
Transpl Proc 1987;19(5):3838-3839.
52. Annoni G, Chirillo G and Swannie D. Prognostic value of mitochondrial aspartate aminotransferase in acute myocardial infarction.
Clinical Biochemistry 1986;19(4):235-239.
53. Humphreys M. Fluid and Electrolyte Management in: Current Surgical Diagnosis and Treatment. Way L (Ed.) 1988:129-140.
Appleton Lange Norwalk Connecticut, California.
54. Ganong W. Review of medical physiology.
Lange Medical Publications. 1981. California.
55. Krause P. Statistical analysis of pH data.
Anaesthesia and Analgesia Current Researches 1978;57:143-144
56. Teschan P and Nace G. Renal function and renal failure. in Davis J. (ed). Clinical Surgery. Vol I.;1987:797-843.
C.V. Mosby & Co. St.Louis, Washington D.C.
57. Ellis R, Beeston J, Witherington S, Allen E and Keenan R. Liver transplantation: Effect of washing bank blood on intraoperative control of hyperkalaemia.
Transpl. Proc. 1987;19(S4#3):73-74.
58. Drucker P. Fluid therapy. in Davis J. (ed). Clinical Surgery. Vol I.;1987:847-859.
C.V. Mosby & Co. St.Louis, Washington D.C.

59. Schmidt E, Schmidt W and Otto P. Isoenzymes of malic dehydrogenase, glutamine oxalacetic transaminase and lactic dehydrogenase in serum in diseases of the liver.
Clin Chim Acta 1967;15:283-290.
60. Lindell S, Ametani M, Belzer F and Southard J. Hypothermic perfusion of rabbit livers: Effect of perfusate composition (Ca and Lactobionate) on enzyme release and tissue swelling.
Cryobiology 1989;26:407-412.
61. Williams A and Hoofnagle J. Ratio of serum aspartate to alanine amino transferase in chronic hepatitis. Relationship to cirrhosis.
Gastroenterology 1988;95(3):734-739.
62. Hayes P, Hussey A, Keating J et al. Glutathione S-transferase levels in autoimmune chronic active hepatitis: a more sensitive index of hepatocellular damage than aspartate transaminase.
Clin Chim Acta 1988;172(2-3):211-216.
63. Isselbacher K and Tisdale W. Diagnostic procedures in liver disease. in: Principles of Internal Medicine Wintrobe M, Thorn G, Adams R et al (Eds.) 1970;1526-1527.
McGraw-Hill Book Company. New York St.Louis etc.

64. Comoda T, Kumegawa M, Yajima T et al. Induction of rat hepatic and intestinal alkaline phosphatase activity produced by bile from bile duct ligated animals.
American Journal of Physiology 1984;246:G393.
65 Hambraeus L. Metabolism of carbohydrates, fat and protein. in: Scientific Foundations of Surgery. Wells C, Kyle J and Dunphy J. (Eds.) 1976:577-591.
William Heinemann Medical Books Ltd. London. 1976
66. Felig P, Marliss E, Owen O and Cahill G. Blood glucose and gluconeogenesis in fasting man.
Archives of Internal Medicine 1969;123:293-302.
67. Mallette L, Exton J and Park C. Control of gluconeogenesis from amino acids in the perfused rat liver.
J Biological Chemistry 1969;244:5713-5723
68. Felig P. The glucose-alanine cycle.
Metabolism 1973;22:179-183.
69. Aldrete J, DeVine D and Gingrich T. Experience in anesthesia for liver transplantation.
Anesthesia Analgesia 1969;48:802-814.
70. Tullock W, Pinsky M, Shaw B and Martin D. Metabolic changes during the anhepatic phase of liver transplantation.
Anesthesia Analgesia 1984;61(Suppl.):A271.
71. Atchison S, Rettke S, Fromme G et al. Plasma glucose concentrations during liver transplantation.
Mayo Clinic Proceedings 1989;64:241-245.

72. Aggarwal S, Kang Y, Freeman J, Fortunato F and Pinsky M. Post-reperfusion syndrome : cardiovascular collapse following hepatic reperfusion during hepatic transplantation.
Transplant Proceedings 1987;19(S3):54-55.
73.K Ganong W. Review of medical physiology.
Lange Medical Publications. California. 1981:23-24,583-584.
74. Driscoll D, Bistrian B, Jenkins R et al. Development of metabolic alkalosis after massive transfusion during orthotopic liver transplantation.
Critical Care Medicine 1987;15(10):905-908.
75. Fath J, Estrin J, Balani K, Konstantinides, F, Najarian J, and Cerra F. Lactate metabolism during hepatic transplantation: Evidence for a perfusion sensitive population.
Transplantation Proceedings. 1985;17(1):284-285.
76. Kruse J, Syed A, Carlson R. Significance of blood lactate levels in critically ill patients with liver disease.
American Medical Journal 1987;83:77-82.
77. Cowan B, Burns H, Boyle P and Ledingham I. The relative prognostic value of lactate and haemodynamic measurements in early shock.
Anaesthesia 1984:39:750-755.

78. Moen J. Claesson K. Pienaar H. et al. Preservation of dog liver, kidney and pancreas using the Belzer-UW solution with a high sodium and low potassium content.
Transplantation. 1989;47(6):940-945.
79. Matheson G and McKenzie D. Breath holding during intense exercise: arterial blood gases, pH and lactate.
Journal of Applied Physiology.1988;64(5):1947-52.
80. Leavey J,Weil M and Rackow E. "Lactate washout" following circulatory arrest.
Journal of the American Medical Association. 1988;260(5):662-4.
81. Arieff A, Guisado R. Effects on the central nervous system of hypernatremic and hyponatremic states.
Kidney International 1976;10:104-109.
82. Marsden P and Skorecki K in: Body Fluid Homeostasis. Brenner B and Stein J. (Eds.) 1987;1-31.
Churchill Livingstone. New York, Edinburgh etc. 1987.
83. Rettke S, Janossy T, Chantigan R et al. Hemodynamic and metabolic changes in hepatic transplantation.
Mayo Clinic Proceedings. 1989;64:232-240.
84. Fortunato F, Kang Y, Aggarwal S, Freeman J and Pinsky M. Acid-base status during and after ortohtopic liver transplantation.
Transplantation Proceedings 1987;19{#4 S3}:59.
85. Carmichael F, Lindop M, Farman J. Anaesthesia for hepatic transplantation: Cardiovascular and metabolic alterations and management.
Anaesthesia Analgesia 1985;64:108-116.

86. Martin D, Marquez J, Kant Y, Shaw B and Pinsky M. Liver transplantation: Hemodynamic and electrolyte changes seen immediately following revascularisation.
Anaesthesia Analgesia 1984;63:284.
87. Kost G, Jammal M, Ward R and Safwat A. Monitoring of ionized calcium during human hepatic transplantation.
American Journal of Clinical Pathology 1985;86(1):62-70.
88. Schanne FAX, Kane AB, Yonug EE, Farber JL. Calcium dependance of toxic cell death: A final common pathway.
Science 1979;206:700-702.
89. Thomas C and Reed D. Current status of calcium in hepato-cellular injury.
Hepatology 1989;10(3):375-38.
90. Reinhart P, Van De Pol E, Taylor W et al. An assessment of the calcium content of rat liver mitochondria in vivo.
Biochemistry Journal 1984;218:415-420.
91. Macfarlane R. An enzyme cascade in the blood clotting mechanism and its function as a biochemical amplifier.
Nature 1964;202:498-499.
92. Davie E, Ratnoff O. Waterfall sequence for intrinsic blood clotting.
Science 1964;145:1310-1312.

93. Poller L. (ed.) in: Recent advances in blood coagulation #4. 1985.
Churchill Livingstone. London.
94. Saito H, Hamilton H, Tavill A, Goodnough L and Angell L. Synthesis and releases of Hageman factor (XII) by the isolated rat liver.
Journal of Clinical Investigation 1983;72:948-954.
95. Amphlett G, Kisiel W and Castellino F. The interaction of $Ca^{2+}$ with human Factor IX.
Archives of Biochemistry and Biophysics.1981;208(2):576-585.
96. Bach R, Nemerson Y and Konigsberg W. Purification and characterization of bovine tissue factor.
Journal of Biological Chemistry. 1981;256:8324-8331.
97. van Dieijen G, Tans G, Rosing J and Hanuker H. The role of phospholipid and factor VIIIa in the activation of bovine F X.
Journal of Biological Chemistry. 1981;256:3433-3442.
98. Bontempo F, Lewis J, Van Thiel D et al. The relation of preoperative coagulative findings to diagnosis, blood usage, and survival in adult liver transplantation.
Transplantation. 1985;39:532-536.
99. Flute P, in: Clinical organ transplantation. R.Calne (Ed.) 1971;388-394.
Blackwell Scientific Publications, Oxford and Edinburgh.

100. Calne R, in: Liver transplantation. R.Calne (Ed.) 1987;247-248.
Grune and Stratton Ltd., London.
101. Porte J, in : Hemostatic disorders in orthotopic and auxillary liver transplantation. 1989;23.
Ph.D thesis. Erasmus University Rotterdam.
102. Howland W, Castro E, Fortner J and Gould P. Hypercoagulability: Thromboelastographic monitoring during extensive hepatic surgery.
Archives of Surgery 1974;108:605-608.
103. Stock P, Estrin J, Fryd D et al. Factors influencing early survival after liver transplantation.
American Journal of Surgery 1989;157:215-219.
104. Ladenson J, Lewis J and Boyd J. Failure of total calcium corrected for protein, albumin and pH to correctly assess free calcium status.
Journal of Clinical Endocrinology and Metabolism 1978;46(6):986-993.
105. Rosenberg D. in: The molecular basis of diseases. Stamatoyannopoulos G, Nienhuis D, Leder P and Majerus P. (Eds.) 1987:535.
W.B. Saunders & Co. Philadelphia, Toronto etc.
106. Robertson WG. Chemistry and biochemistry of calcium.
In: Calcium in Human Biology. Nordin B.E.C. (Ed.) Springer-Verlag Berlin Heidelberg. 1988.

107. Chernow B, Zaloga G, McFadden E et al. Hypocalcemia in critically ill patients.
Critical Care Medicine 1982;10:848-851.
104. McClean F and Hastings A. A biological method for the estimation of calcium ion concentration.
Journal of Biological Chemistry 1934;107:337-350.
109. McClean F and Hastings A. The state of calcium in the fluids in the body. I. The conditions affecting the ionization of calcium.
Journal of Biological Chemistry 1935;108:285-322.
110. Marshall R. Plasma fractions. in :Calcium, Phosphate and Magnesium Metabolism. Nordin B.E.C (Ed.) 1976.p.162-185.
111. Raman A. The calcium fractions of normal serum.
Clinical Biochemistry 1971;4:141-146.
112. Moore E. Studies with ion exchange calcium electrodes. III. The state of of serum calcium in patients with cirrhosis.
Gastroenterology 1971;60:43-54.
113. Gray T, Buckley B, Sealey M, Smith S, Tomlin P and McMaster P. Is calcium important for hemodynamic stability during liver transplantation?
Transplantation Proceedings 1985;17(1):290-292.
114. Southard J and Belzer F. Control of canine kidney cortex slice volume and ion distribution at hypothermia by lactobionate.
Cryobiology 1980;17:540-548.

115. Southard J and Belzer F. Principles of solid-organ preservation by cold storage.
Transplantation 1988;45:673-676.
116. Otamiri T. Oxygen radicals, lipid peroxidation, and neutrophil infiltration after small-intestinal ischemia and reperfusion.
Surgery 1988;105(5):593-596.
117. Pienaar B, Lindell S, Southard J and Belzer F. What is the best oxygen level in perfusate for longterm perfusion preservation of the liver?
S. Afr. J. Surg. 1989;27(4):156.
118. Pittinger C, Pong Moon Chang and Faulkner W. Serum ionised calcium: Some factors influencing its level.
Southern Medical Journal 1971;64(10):1211-1215
119. Carlon G, Howland W, Goldiner P et al. Adverse effects of calcium administration.
Archives of Surgery 1978;113(7):882-885.
120. Drop L. Ionized calcium, the heart, and hemodynamic function.
Anesthesia Analgesia 1985;64:432-451.
121. Kancir C, Petersen P, Madsen T and Olesen A. In vivo and in vitro ionized calcium variations induced by acute respiratory acid base disturbances.
Clin Chim Acta 1988;175:307-314.

122. Peoples J. The role of pH in altering serum ionized calcium concentration.
Surgery 1988;104:370-374.
123. Kahn D, Hickman R, Dent D and Terblanche J. For how long can the liver tolerate ischaemia?
European Surgical Research 1986;18(5):277-282.
124. Retkke S, Chantigan R, Janossi T et al. Anesthesia approach to hepatic transplantation.
Mayo Clinic Proceedings 1989;64(2):224-231.
125. Schaer H and Bachmann U. Ionized calcium in acidosis: Differential effect of hypercapnic and lactic acidosis.
British Journal of Anaesthesia 1974;46:842-848.
126. Kahn R, Jascott D, Carlon G et al. Massive blood replacement: Correlation of ionized calcium, citrate,.and hydrogen ion concentration.
Anesthesia and Analgesia 1979;58(4):274-278.
127. Quamme G. Effect of calcitonin on calcium and magnesium transport in the rat nephron.
American Journal of Physiology 1980;238:573-576.
128. Read A. The liver cell and surgery. in: Scientific Foundations of Surgery. Wells C, Kyle J and Dunphy J. (Eds.) 1976:545-553.
William Heinemann Medical Books Ltd. London. 1976

129. McGeown M. Calcium and phosphorus metabolism and the parathyroid glands. in: Scientific Foundations of Surgery. Wells C, Kyle J and Dunphy J. (Eds.) 1976:131-140.
William Heinemann Medical Books Ltd. London. 1976
130. Gamelli R. Nutritional support: Parenteral alimentation. in Clinical Surgery I. J.Davis (Ed.) 1987:877-908. The C.V. Mosby Company. St.Louis Washington, D.C Toronto 1987.
131. Jahn W. Transmembrane water and ion shifts following experimental induced endocytotic fluid uptake in the isolated perfused rat liver. In: Membrane alterations as basis of liver injury. Popper H, Bianchi L and Reutter W. (Eds.)1976:267-276.
Proceedings of the first Basel Liver Week. 1976. Falk Symposium 22. M.T.P Press Lancaster.1977.
132. Jenkins R, Benotti P, Bothi A et al in: Liver Transplantation.
Surgical Clinics of North America 1985;65:103.
133. Litwin M, Panico F, Rubin C,Harken D and Moore F. Acidosis and lactic acidemia in extracorporeal circulation: The significance of perfusion flow rate and the relation to preperfusion respiratory alkalosis. Annals of Surgery 1959;149:172-178.
134. Pienaar B, Roncone A, Schwartz I and Hickman R. Is the pig liver a good model for preservation studies?. Proceedings of the South African Transplantation Society Congress: August 1989.

135. Gordon R, Iwatsuki S, Esquivel C et al. in: Liver Transplantation. Cerilli G.J (Ed.) 1988:511-534. J.B. Lippincott Company.Philadelphia.

136. Southard J. Viability assays in organ preservation. Cryobiology 1989;26:232-238.

137. Pichlmayr R, Broelsch C, Wonigeit K et al. Experiences with liver transplantation in Hannover. Hepatology 1984;4(1):56S-60S.

138. Mouridsen H. The extravascular retention of albumin in wound tissue and its contribution to the postoperative relative hypoalbuminaemia in rabbits. Clinical Science 1969;37:431-441.

139. Gutenberger J, Miller D, Dibbins A and Gitlin D. Hypogammaglobulinemia and hypoalbuminemia in neonates with ruptured omphaloceles and gastroschisis. Journal of Paediatric Surgery 1973;8(3):353-359.

140.Vaziri N and Forthal D. Hypoalbuminemia after renal transplantation. Archives of Surgery 1980;115:286-289.

141. Vierling J. Epidemiology and clinical course of liver diseases: Identification of candidates for liver transplantation. Hepatology 1984;4(1):84S-89S.

142. Dahn S, Jacobs L, Mitchell R and Kirkpatrick J. The significance of hypoalbuminemia following injury and infection. American Surgeon 1984;51(6):340-343.

143. Mallet S, Kang Y, Freeman J, Aggarwal S, Gasior S and Fortunato F. Prognostic significance of blood glucose levels during liver transplantation.
Anesthesiology 1987;67(Suppl.):A313
144. DeWolf A, Kang Y, Todo S et al. Glucose metabolism during liver transplantation in dogs.
Anesthesia and Analgesia 1987;66:76-80.
145. Khoury G, Kaufman R and Musich J. Metabolic and electrolyte changes during massive blood transfusion in liver transplantation.
Anesthesia 1985;63(Suppl.):A265.
146. Halliday J, Sheil R, Hansley W, Bookallie J and Witherspoon G. Biochemical aspects of experimental hepatic allotransplantation.
Archives of Surgery 1974;108:75-80.
147. Kirby R, McMaster P, Clemens D et al. Orthotopic liver transplantation: Postoperative complications and their management.
British Journal of Surgery 1987;74:3-11.
148. Kang Y, Martin D, Marquez et al. Intraoperative changes in liver transplantation.
Anesthesia and Analgesia 1985;64:888-896.
149. Ritter M, Owen J, Bowie E et al. Evaluation of preoperative hematology-coagulation screening in liver transplantation.
Mayo Clinic Proceedings 1989;64:216-223.

150. Oshiba S and Anga T. Purification and characterization of the biliary plasminogen activator bilokinase.
Journal of Biological Chemistry 1983;258(1):622-628.
151. Goldsmith M. Liver transplantation: big business in blood.
Journal of the American Medical Association 1983;250:2904-2905.
152. Perkins H, May R and Belzer F. Cause of abnormal bleeding after transplantation of pig liver stored by a perfusion technique.
Archives of Surgery 1970;101:62-68.
153. Bracher M, Hickman R, Pienaar H, Jacobs P, Bird A and Terblanche J. Is heparin the cause of the coagulopathy which may complicate liver grafting?
Surgery, Gynecology & Obstetrics. In press 1990.
154. Plevak D, Halma G, Forstrom L et al. Thrombocytopenia after liver transplantation.
Transplant Proceedings 1988;20(Suppl. 1):630-63354.
155. Porte R, Blankenstein J, Knot E et al. A comparative study on changes in haemostasis in orthotopic and auxilliary liver transplantation in pigs. in: Hemostatic disorders in orthotopic and auxilliary liver transplantation. R.J. Porte (Ph.D thesis).
Erasmus Universiteit, Rotterdam. 1988.

156. Hutchison D, Genton E, Porter K et al. Platelet changes following clinical and experimental hepatic homotransplantation.
Archives of Surgery 1968;97:27-33.
157. Schalm S, Terpstra J, Achterberg J et al. Orthotopic liver transplantation: An experimental study on mechanisms of hemorrhagic diathesis and thrombosis.
Surgery 1975;78:499-507.
158. Calne R, Williams R and Rolles K. Liver transplantation in the adult.
World Journal of Surgery 1986;10:422-431.
159. Tilney N and Strom T. Chemical manipulation of the immune responses. in: Organ Transplantation and Replacement. Cerilli G. (Ed.) 1988;118-136.
J.B.Lippincott & Co. London, Mexico City, New York etc.
160. Hickman R, Bracher M, Tyler M et al. The acute effects of total hepatectomy upon plasma pancreatic hormones, adrenaline and noradrenaline and coagulation profile.
Digestive Diseases and Sciences. In press 1991.
161. Mouridsen H and Faber M. Accumulation of serum-albumin at the operative wound site as a cause of postoperative hypoalbuminaemia.
Lancet 1966;October 1:723-725.

162. Relman S. Lactic acidosis. In: Acid-base and Potassium Homeostasis, Brenner B and Stein J. (Eds.);65-100.
Chuchill Livingstone, New York, Edinburgh and London. 1978.
163. Sebastian A, Hulter H and Rector F. Metabolic alkalosis. In: Acid-base and Potassium Homeostasis, Brenner B and Stein J. (Eds.);101-136.
Chuchill Livingstone, New York, Edinburgh and London. 1978.
164. Pienaar H, Roncone A, Schwartz I and Hickman R. Simple cold storage of the porcine liver.
Transplantation. In press 1991.
165. Terblanche J, Peacock J, Bowes J et al. The use of the pig as experimental animal for orthotopic liver homotransplantation.
British Journal of Surgery 1967;54:231.
166. Calne R, White H, Yoffa D et al. Observations of orthotopic liver transplantation in the pig.
British Medical Journal 1967;2:478-480.
167. Kahn D, Hickman R, Terblanche J and Child P. Transhepatic changes in insulin and glucagon following partial hepatectomy in the pig.
Surgery, Gynecology & Obstetrics 1984;158:475-481

168. Marquez J, Martin D, Virgi M et al. Cardiovascualr depression secondary to ionic hypocalcaemia during hepatic transplantation in humans.
Anesthesiology 1986;65:457-461.
169. Rettke S, Chantigian R, Janossy T et al. Anaesthesia approach to hepatic transplantation.
Mayo Clinic Proceedings 1989;64:224-331.
170. Sheil A, Thompson J, McCaughan et al. Determinants of successful liver transplantation.
Transplantation Proceedings 1990;XXII(5):2144-2146.
171. Cofer J, Clintmalm G, Howard K et al. A comparison of UW with Eurocollins preservation solution in liver transplantation.
Transplantation 1990;49:1088-1093.
172. Bittard H, Benoit G Ecoffey C et al. Renal allograft blood flow study between University of Wisconsin and Eurocollins perfused kidneys.
Transplantation Proceedings 1990;22:390-391.