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Nonfreezing cryopreservation – a possible means of improving long-term transplant function?

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Abstract Improving organ preservation techniques for transplantation is one of the most important goals of transplantation research. We have established a new, nonfreezing cryopreservation method to optimize the viability of rat kidneys for transplantation with up to 4 M dimethylsulphoxide (DMSO) in EuroCollins solution (EC) at −5°C to −15°C. We have confirmed the occurrence of a tubular and glomerular defect pattern that mediates acute tubular necrosis (ATN) and that may be a cause of major histocompatibility complex (MHC) independent immunological components of chronic transplant rejection. The extent of this defect [transplant survival and function, ^{31}P-NMR spectroscopy, histological defect index] in the nonfreezing cryopreserved groups (n = 22) is significantly (P = 0.017) lower than in the simple cold storage group (n = 12). Quality and localization of the lesions in kidney transplants can elucidate the context of organ preservation, progressive hyperfiltration defects, and the occurrence of graft failure without elevated frequency of acute rejection episodes. These results indicate that further efforts to provide higher pretransplant organ viability without using it to prolong cold storage intervals may provide better insight into MHC-independent factors of chronic transplant failure and may result in improved long-term transplant outcome.

Key words Preservation, kidney, rat · Cryopreservation, kidney, rat · Kidney, (cryo)preservation, rat

Introduction

Improving organ preservation techniques for transplantation is still one of the most important goals of transplantation research. Subzero storage of viable cells is presently restricted to some tissues and cell suspensions, whereas the gold standard for optimal short-term and long-term transplant function of bulky organs is hypothermic storage at temperatures of +4°C to +10°C. Organ storage at temperatures below this range or exceeding a "safe" storage interval is known to induce acute morphological lesions as well as chronic transplant deficiency.

For kidneys, many studies have demonstrated that precursors of chronic transplant rejection can already be observed in the early reperfusion phase [10, 20]. It has been shown in a rat kidney tubular necrosis model that ischemia can elevate the expression of m-RNA for cytokines [9]. Little information, however, is available about the role of hypothermia in this pathomechanism. The interdependence and similarity of ischemia and hypothermia effects under cold storage conditions makes it difficult to interpret the alterations observed. It has been clinically proven that acute tubular necrosis (ATN) after prolonged cold ischemia (> 24 h) can initiate multiple inflammatory processes and can, thereby, potentially induce acute rejection episodes [10, 20]. Insufficient preservation of cellular structure and function are also responsible for ATN [13, 28]. Clinical reports show how closely kidney transplants are associated

Table 1 Criteria for histological examination of preserved renal cortical tissue. Using these 20 criteria, we examined the perfusion-fixated sections of groups 1–3 with light transmission microscopy. Summing up the fraction of cells fitting criteria in one section (in %), we calculated one score (theoretical range 0–20) per tissue examined

Compartment	Criteria
Glomeruli	Bowman membrane thickening Sequestration of cells/slit membrane Massive mesangial/ juxtaglomerular cell hydrops
Proximal tubuli	Vacuolization Membrane defects Heterochromatin, karyolysis Loss of brush border Massive cell hydrops
Distal tubuli	Vacuolization Membrane defects Heterochromatin, karyolysis Massive cell hydrops
Collecting ducts	Vacuolization Membrane defects Heterochromatin, karyolysis Massive cell hydrops
Arterioles/capillary	Loss of endothelium Intimal disherent Luminar obstruction
Interstitium	Hydrops

with a higher frequency of acute rejection episodes after significant ATN. There is little doubt that frequent rejection episodes are an important cause of chronic transplant failure.

However, it is also true that, in many cases, chronic transplant failure occurs after prolonged cold storage intervals (> 24 h) without evident elevation of rejection episode frequency [13, 23]. Delayed graft function is a widely accepted predictor of deteriorated graft integrity and, therefore, an indicator of developing chronic transplant rejection. In this respect, it is quite interesting how histologically similar hyperfiltration defects in native kidneys with a reduced number of functioning nephrons are to the lesions found in kidneys [13] with chronic transplant failure [31]. The impact of hypothermia on renal cell viability is not fully understood, but there is clear evidence that even mild hypothermia can seriously damage nonischemic cells [17, 18]. Some mechanisms underlying the impairment of hypothermic function appear to be identical to defects due to normothermic hypoxia.

The objective of our study, therefore, was to learn about the early alterations found after cold storage in order to gain insight into the known [7, 24, 25, 32] nonfatal, cold ischemia-induced glomerular damage. The relevance of these alterations and the possibility of reducing their extent by lowering, the storage temperature and by using membrane-stabilizing cryoprotectants were examined.

During our investigation, our attention was drawn to a discrete, glomerular, accentuated lesion pattern in cold-stored rat kidneys. The extent of these storage-induced lesions was reduced by the use of the cryoprotectant dimethylsulphoxide (DMSO) in high concentrations. This phenomenon has been described by Khirabadi et al. [15, 16] in kidney transplants, and it was observed by Wang et al. [35] in subzero heart preservation experiments as well. Given the latest biophysical research on phospholipid bilayer properties and the effects on integral proteins, an explanation for the observed functional disorder in unprotected, cold-stored kidneys will probably emerge from further investigation. Close examination of these lesions has yielded evidence of parallels to progressive lesions following the pathomechanism of nephron hyperfiltration. Thus, we are tempted to conclude that temperature-dependent mechanisms propagating ATN in cold-stored organs may also be responsible for the induction of the observed glomerular lesions. This component may contribute to chronic transplant failure as well [3, 5, 11, 23]. In this context, a hypothermia-inducible component in the development of chronic kidney transplant failure [3, 23] and its potential prevention by DMSO protection are discussed.

Materials and methods

In order to investigate the preservation of parenchymatous organs at subzero temperatures, we developed a method for nonfreezing machine cryopreservation of rat kidneys with DMSO in EuroCollins (EC) solution for a period of 8 h. We then compared the results with that of a reference group with no cold storage after DMSO-free EC flushing. Since the ischemia tolerance of rat kidneys is about one-third that of human kidneys, we chose a storage period of 8 h to collect significant information.

A PC-controlled (486-based), pressure-constant, low-flow perfusion system was used for the gradual introduction of cryoprotectants into 34 rat kidneys of a PVG inbred strain, subdivided into three groups. In group 1 ($n = 12$), storage was at +4 °C in EC; in group 2 ($n = 12$), storage was at −5 °C in 2 M DMSO/EC; and in group 3 ($n = 10$), storage was at −15 °C in 4 M DMSO/EC. The perfusion and immersion temperature was lowered corresponding to the rising DMSO concentration. The individual flow properties of the en bloc kidneys thus determined the perfusate flow, as well as the DMSO loading rate and the cooling rate. The average introduction and removal rate was 30 mmol/min. During perfusion, we continuously monitored and analyzed perfusate flow, perfusion resistance, and urine flow and composition.

After anesthesizing the animal with 100 mg/250 g BM ketamine and 10 mg/250 g BM diazepam, the surgical procedures in all three groups followed the principles of en bloc kidney harvesting. When preparation of the en bloc kidney was completed, a perfusion catheter was used to gradually flush out the organs in situ from the inferior aortic artery with +4 °C EC. The suprarenal aorta was then ligated and the vena cava was opened. The donor animal was sacrificed by hemorrhage. The flushed en bloc kidney was

then excised under running perfusion cooling conditions and exposed to a cooling container maintained at a constant temperature. The mean en bloc harvesting time was 110 min.

The kidneys were then preserved according to one of the three different preservation protocols described above for 8 h. The individual preservation protocol was determined by a randomizing protocol unknown to the experimentor prior to surgery. After preservation, organ viability was assessed by two of the three assessment techniques described below. After separation of the en bloc kidneys via a workbench technique at +4 °C, the individual assessment technique for either kidney was determined by a second randomizing protocol, unknown to the experimentor at the time of separation. The mean separation time was 12 min.

Histological defect index (hdi)

After preservation and storage for 8 h and separation, the kidney was perfused with a preflush solution containing polyvinylpyrolidone (PVP), heparin, and procainhydrochloride for 2 min and with a fixation solution containing glutaraldehyde 25% (v/v), both at +4 °C for histopathological examination ($n = 12$: four kidneys from group 1, four kidneys from group 2, and four kidneys from group 3). The histopathological examination was done on subcortical and cortical microsections derived from biopsies of three different areas of the cryopreserved kidneys (upper pole, lower pole, and large convexity). The H & E-stained, perfusion-fixated microsections were examined by professional investigators using a catalogue of 20 criteria (Table 1) and light transmission microscopy at a magnification of 400 × and 1000 × (oil immersion), respectively. Specimens with noteworthy characteristics were embedded for electron transmission microscopy.

^{31}P-Nuclear magnetic resonance (NMR)

After preservation and storage for 8 h and separation, the kidney was flushed with phosphate-free solution similar to EC and continuously observed for ^{31}P-nuclear magnetic resonance (NMR) spectroscopic parameters in a Bruker AM-400 SWB ($n = 11$: four kidneys from group 1, four kidneys from group 2, and three kidneys from group 3). The kidneys were immersed in a phosphate-free TRIS-buffered variation of EC in an inner ∅ 12/∅ 15-mm container centered in an outer D_2O-filled ∅ 20-mm container in the HF-spool of the AM-400 tuned to 121.5 MHz for ^{31}P and 300.13 for ^{1}H. In transients of 1.02 s, 8000 data points are registered for 128 s per measurement. All standard peaks were registered and analyzed. The proportion of glycero-3-phosphocholine/phosphomonoesters (GPC/PME) was used as an indicator of phospholipid degradation and phospholipid loss since GPC is known to be the most important phosphodiester and catabolite of phospholipases active in hypothermic ischemia [27, 29, 38]. Total nucleotides (TN) and PME (TN/PME) signal integrals were used as an index for the proportion of the preserved nucleotide pool.

Early transplant function (tpl)

After preservation and storage for 8 h and separation, the kidney was transplanted and the graft was observed in situ at 1 h and 48 h posttransplantation (tpl, $n = 11$: four kidneys from group 1, four kidneys from group 2, and three kidneys from group 3). We analyzed the quality of organ perfusion, urine output and concentration, and potassium output at 1 h and 48 h post-transplantation. For that purpose we transplanted one of the separated kidneys into an anesthesized, left side-nephrectomized, recipient PVG rat. The surgical technique used was an end-to-side anastomosis of the graft vein to the inferior vena cava and of the graft artery to the infrarenal aortic artery. The graft vessels were carrying a Carrel patch. When the arterial and venous anastomoses were completed, we observed the graft for approximately 1 h while completing the ureteral anastomosis. The first urine specimen was drawn 1 h post-transplantation, directly from the graft ureter in cases of urine production. Then, the abdomen was closed with an all-layer running suture. Forty-eight hours post-transplantation, the animal was reanesthesized and the abdominal cavum reopened; if there was urine production, another urine specimen was drawn from the ureter and the graft was removed for histological examination.

Quality assurance

Using the two abovementioned randomizing protocols, we observed the following "pairs" in the 34 en bloc kidneys: tpl + score 6 pairs, score + NMR 6 pairs, tpl + NMR 5 pairs. All results were pooled in groups 1, 2, and 3 and tested for correlation and significant trends. Our criteria for overall significance was a P level below 0.05. to improve the quality of our statistical results, we included a reference group 0 with 17 "placebo" kidneys in our randomized experiments. In this group, the surgical procedures were identical, and the PC-controlled perfusion was maintained for 10 min. Then transplantation and histological procedures were performed to check our overall accuracy and the homogeneity of the standardized procedures, which were not biased by the cryopreservation maneuvers.

Results

Quality assurance

The results of our reference group 0 were homogeneous. These organs showed excellent restoration of function, normal NMR spectra, and a regular histology. The results in groups 1, 2, and 3 were also homogeneous. The randomizing protocols were practical. We lost one donor animal due to problems related to the anesthesia.

Integrity of cryopreserved kidneys

Histological findings

The histological findings regarding the type and localization of the discrete lesions we found after cryopreservation were typical for hypothermic ischemic storage. Regular areas were found as well as altered areas. The focal aspect of damage due to organ storage was observed.

In group 2 the tubuli showed an almost intact morphology. We observed cell shrinkage and isolated disintegrated cells or karyolysis. In group 1 there seemed to be more tubular vacuolization and focal loss of brush border. In group 3 the high defect index for tubular cells

Table 2 Contribution of compartments to histological score in groups 1–3. The score shown in this figure is an absolute value (range 0–20) that transforms the histological examination according to Table 1 into an interval-scalable value

Compartment	Group 1 absolute (%)	Group 2 absolute (%)	Group 3 absolute (%)
Glomeruli	0.51 (26)	0.09 (8)**	0.25 (11)*
Proximal tubuli	0.67 (35)	0.56 (50)*	1.08 (47)
Distal tubuli	0.28 (14)	0.25 (22)*	0.45 (20)
Collecting ducts	0.24 (12)	0.14 (12)	0.3 (13)
Arterioles/capillary	0.09 (5)	0.06 (5)	0.16 (7)
Interstitium	0.15 (8)	0.03 (3)*	0.05 (2)*
Total	1.94 (100)	1.12 (100)*	2.29 (100)

* $P < 0.05$ (significantly lower score in minimally one feature of a compartment compared to group 1);
** $P < 0.01$ (highly significantly lower score in minimally one feature of a compartment compared to group 1)

Table 3 Function of rat kidneys after cold storage. The overall function of rat kidneys 1 and 48 h post-transplantation was satisfactory in group 1 (+4 °C, EC, $n = 4$), group 2 (−5 °C, EC with 2 M DMSO, $n = 4$) and group 3 (−15 °C, EC with 4 M DMSO, $n = 3$). However, function was restored most rapidly at the highest level in group 2

Parameters	Group 1 ($n = 4$)		Group 2 ($n = 4$)		Group 3 ($n = 3$)	
	1 h	48 h	1 h	48 h	1 h	48 h
Surviving recipient	4 100%	3 75%	4 100%	4 100%	3 100%	2 67%
Perfusion intact	3 75%	3 75%	4 100%	4 100%	3 100%	2 67%
Urine output intact	2 50%	3 75%	4 100%	4 100%	2 67%	2 67%
Concentration intact	0 0%	2 50%	3 75%	4 100%	1 33%	2 67%

was caused by the overall cell shrinkage observed. The difficult differentiation of the observed tubular defect pattern made us use electron transmission microscopy to determine the degree of tubular cell alteration between the three groups. In all tubular cell lesions, the brush border had a regular appearance. In all groups, focal membrane defects in proximal tubular cells were predominantly located in the basal area (Table 2). These lesions are generally situated in the basal cell membrane and its invaginations. In group 1 we found a no-reflow phenomenon in one of the four cases. The luminar side of the tubuli showed an almost intact morphology of the brush border and the mitochondria in all groups. Only in group 1 did we find a defect pattern of mitochondrial swelling and cell membrane lesions, again situated at the basal side of the tubular cells. This alteration was absent in groups 2 and 3.

The glomerular compartment showed slightly condensed capillary tufts and slightly dilated Bowman's capsules in all groups. The glomeruli in group 1 showed marked alterations compared to the ones found in groups 2 and 3 (Figs. 1, 2). The slit membranes, in particular, were found to be focally disintegrated and swollen in this region (Fig. 1). The mesangial cells were found to be intact, except for the altered areas of the focal glomerular damage in group. The vessels and capillaries of the cortex showed no significant damage in any group. The extraglomerular vessels, in particular, had a regular appearance.

Group 2 had a significantly lower hdi (1.12) than group 1 (hdi = 1.94, $P = 0.017$ at a 95% confidence level) and group 3 (hdi = 2.29, $P = 0.012$ at a 95% confidence level; Fig. 3, Table 2).

NMR findings

The retarded decay of TN/PME in groups 2 and 3, in contrast to the decay of TN/PME in group 1, indicated a significantly decelerated nucleotide metabolism. The extrapolated graph of TN/PME was associated with an extension of the safe cold storage period by 50% when high concentrations of DMSO were combined with mild subzero temperatures compared to simple cold storage in EC solution at +4 °C. The ΔGPC/PME (Fig. 4) showed a highly significant correlation to the histological score ($P_{hdi/GPC} = 0.0031$ at a 95% confidence level; Fig. 5) and transplant function, whereas ΔTN/PME ($P_{hdi/TN} = 0.85$ at a 95% confidence level) failed to predict the extent of cold storage-associated damage.

Early transplant function

Perfusion with EC solution did not lead to gross cellular defects in any group. The controlled introduction and removal of DMSO improved the flow properties of these cryoprotected organs. A significant enzymuria was not observed in any group. With an overall transplant function restoration of 9/11 (3/4 in group 1, 4/4 in group 2, 2/3 in group 3; Table 3), storage and transplantation were feasible. The revascularization of the kidney grafts had effect in 10/11 cases. Erythrocyte trapping was observed in one of four kidneys in group 1. After 48 h, kidney function was restored to a life-supporting extent in three of the four kidneys in group 1, in all four kidneys in group 2, and in two of three kidneys in group 3. The degree of potassium output restitution correlated significantly with the observed GPC/PME level ($P_{K+/GPC} = 0.042$ at a 95% confidence level). An immediate restoration of urine output and concentration of all kidneys was observed in group 2 only.

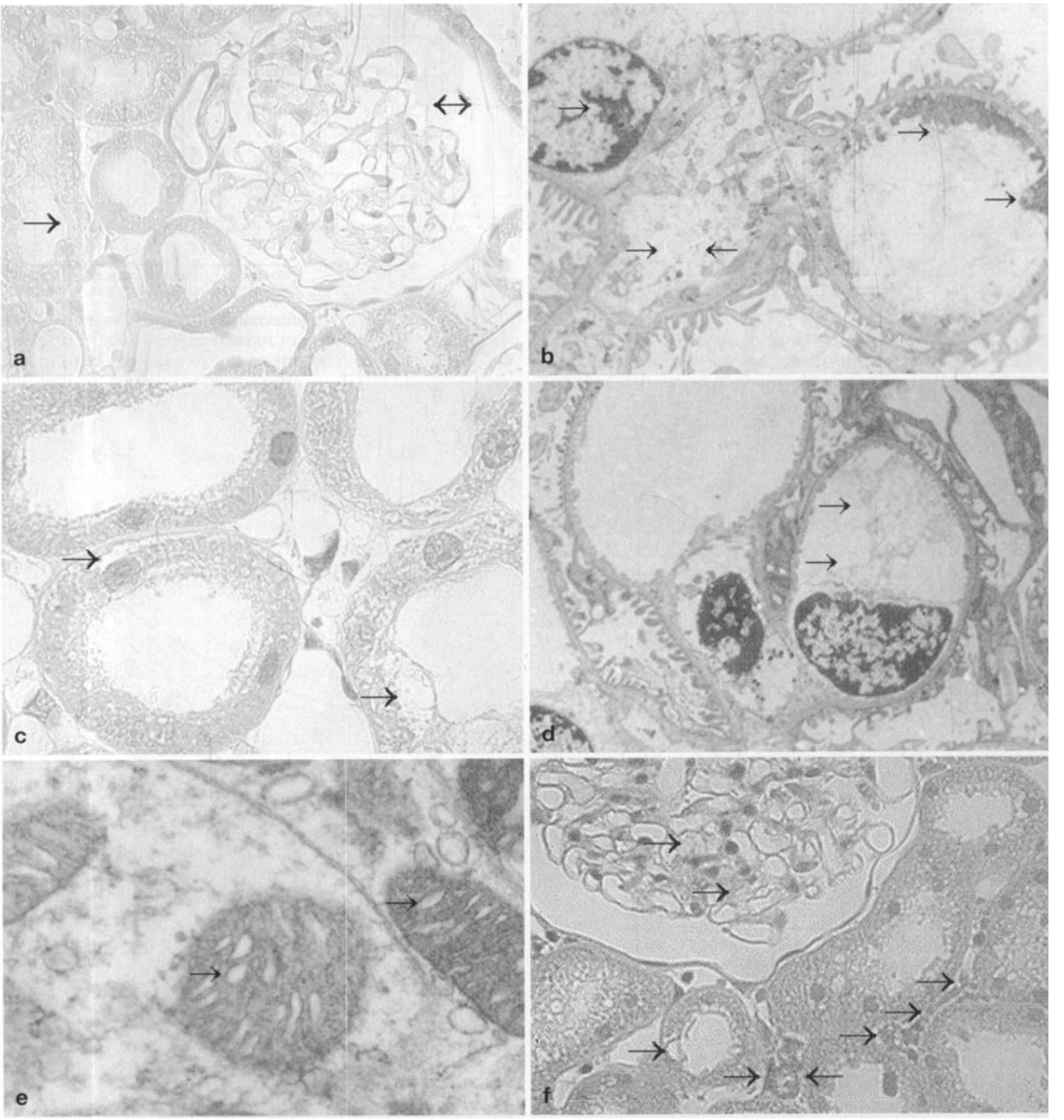

Fig. 1 a–f Observations in kidneys cold-stored in EC at 4 °C for 8 h: **a** high transparency of mesangial cells and tubular cells in S2 and S3, hydrops of glomeruli (× 400); **b** mesangial cell lesions, accumulation of heterochromatin, thickening of basement membrane, slit membrane lesions (× 8000); **c** discrete vacuolization of brush border, basal loss of coherence of tubular cells (× 1000); **d** necrobiosis of mesangial cells, sequestration of cell humps into capillaries (× 8000); **e** hydrops of basal mitochondria, amorphous deposits (× 50000); **f** kidney 48 h post-transplantation: tubular necrosis, mesangial transparency enhancement, and erythrocyte trapping in vasa recta, detritus in tubuli (× 400)

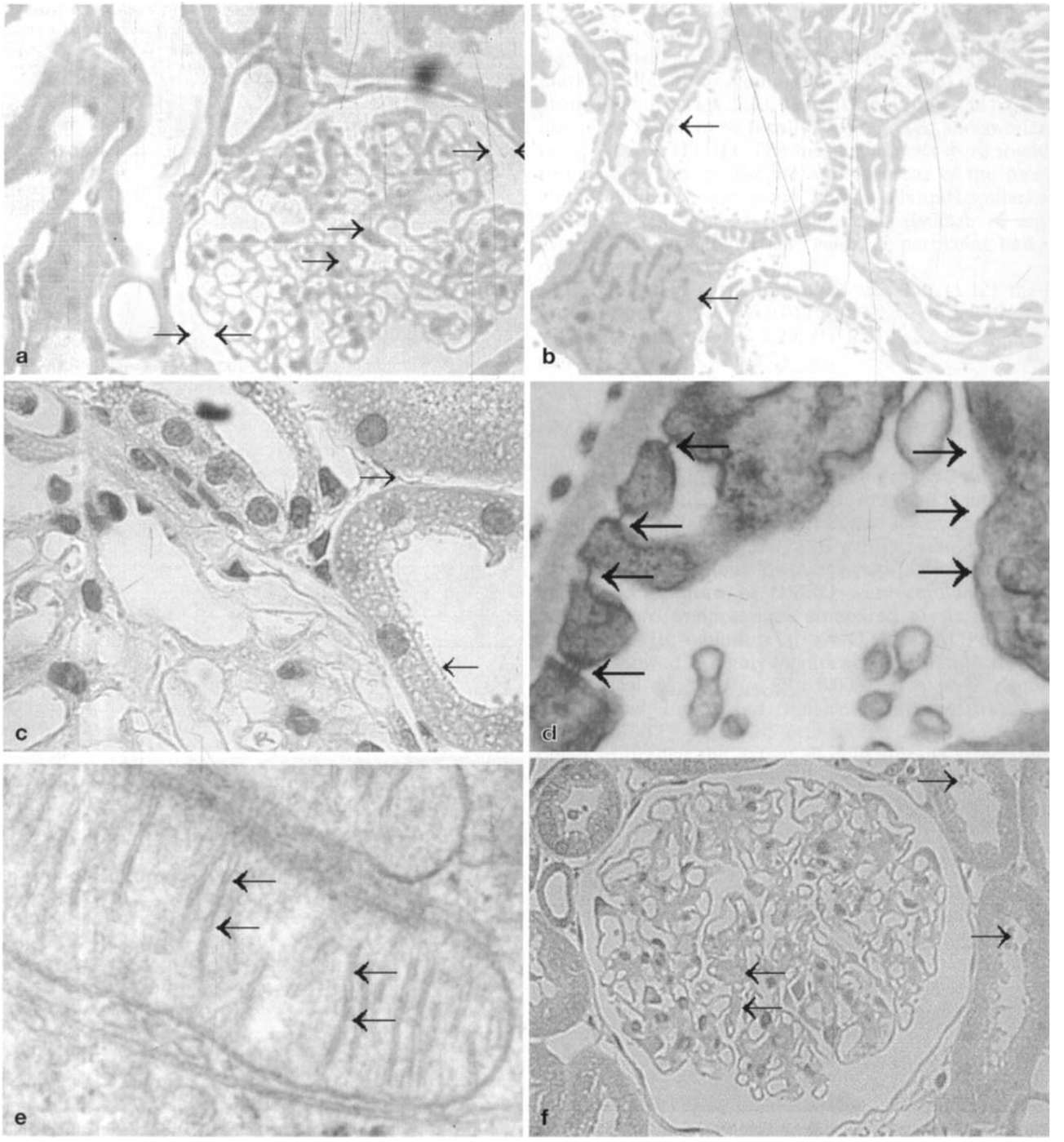

Fig. 2a–f Observations in kidneys cold-stored in EC/2 M DMSO at −5 °C for 8 h: **a** normal staining of mesangial cells, tubulus cells, little hydrops of glomeruli (× 400); **b** basement membrane regular, no gross lesions in podocytes or mesangial cells (× 8000); **c** intact brush border, no loss of basal coherence, few vacuoles (× 1000); **d** intact mesangial cells, slit membrane intact, vessels of Malpighi bodies intact (× 45000); **e** no hydrops of basal mitochondria, no amorphous deposits (× 85000); **f** kidney 48 h post-transplantation: little tubular necrosis, no enhanced mesangial transparency, no erythrocyte trapping in vasa recta, little detritus in tubuli (× 400)

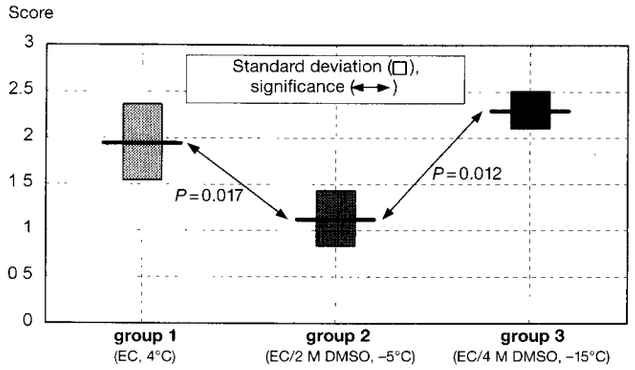

Fig. 3 Indices for alteration, according to histological criteria in groups 1–3 (hdi-score). In contrast to group 2, the total scores in groups 1 and 3 reveal a significant structural deterioration detectable by light transmission microscopy

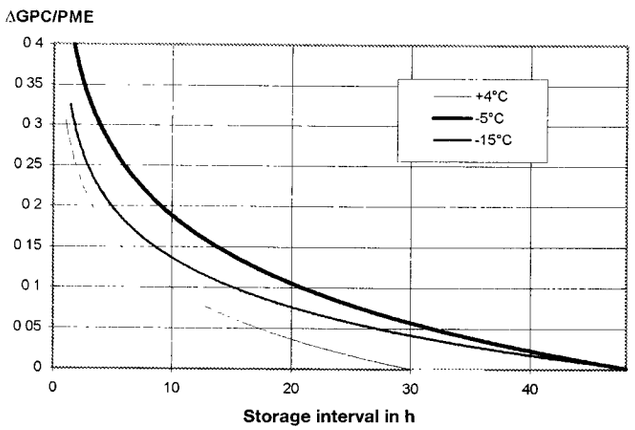

Fig. 4 ΔGPC/PME of groups 1–3, an index for metabolic phospholipid balance. Using phosphodiester, glycero-3-phosphocholine, and phosphomonoester balance, conclusions can be drawn with regard to the global phospholipid metabolism of an organ. Based on the well-known kinetics, the logarithmic curves shown have been extrapolated.

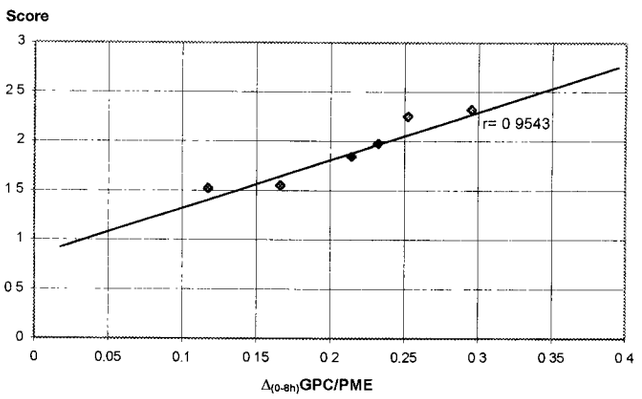

Fig. 5 Correlation of histologically detectable (membrane) lesions to GPC/PME deficiency after preservation of rat kidneys for 8 h. The good correlation of phospholipid markers to the histological score indicates the reliability of GPC/PME as a viability predictor with $P = 0.0031$, whereas the comparable TN/PME-to-score ratio was associated with an $r = 0.1002$ and $P = 0.8503$

Discussion

This study shows, in accordance with the results of other studies [15, 16, 35, 36], that the preservation of parenchymatous organs at subzero temperatures using cryoprotectants in high concentrations is feasible.

In all groups, focal membrane defects in proximal tubular cells were found to be predominantly located in the basal area (Table 2). These lesions, which are generally situated in basal cell membrane and its invaginations, as described by Linner et al. [21], were found exclusively in group 1. We observed a no-reflow phenomenon in one of the four cases in group 1. Electron transmission microscopic findings and the better initial function of the tubuli in groups 2 and 3 ruled out that the defect index determined by light transmission microscopy underscored the tubular defects of group 1. On the other hand it overscored groups 2 and 3 (Figs. 1, 2). The observed morphology of tubular mitochondria helped to explain the delayed restoration of function in group 1. Because of the evident correlation of ΔGPC/PME with organ function parameters, one must assume that kidney transplant function after reperfusion is determined by the integrity of cellular phospholipid membranes and phospholipid metabolism resources and the enzymes located in these membranes, as well as by cellular nucleotide resources prior to reperfusion.

We were able to confirm that after conventional cold storage at 4 °C, discrete hypothermia-associated defects can be found in nephrons [17, 18]. These defects, located in glomeruli, are well known and have been described [2, 7, 24, 25, 32], but information about their relevance is less clear-cut. Discrete membrane lesions in proximal convoluted tubules [2, 7, 21, 32] were observed. The impact of these primarily nondeleterious hypothermia-associated lesions on postreperfusional transplant function is subject to speculation. These microscopic lesions in mesangial cells [33], other glomerular structures [20], and the basal micro-invaginations [21] of proximal tubular cells, as well as the postulated loss of microenvironment integrity of integral membrane proteins and their function [29], are likely to determine the quality and quantity of kidney function and the restoration of energy resources in transplants. The importance of these findings should be evaluated in light of new immunological findings [9, 10, 12, 19, 20, 22]. Comparing our histological observations with the ^{31}P analyses, we found a better correlation of phospholipid indicators to glomerular struc-

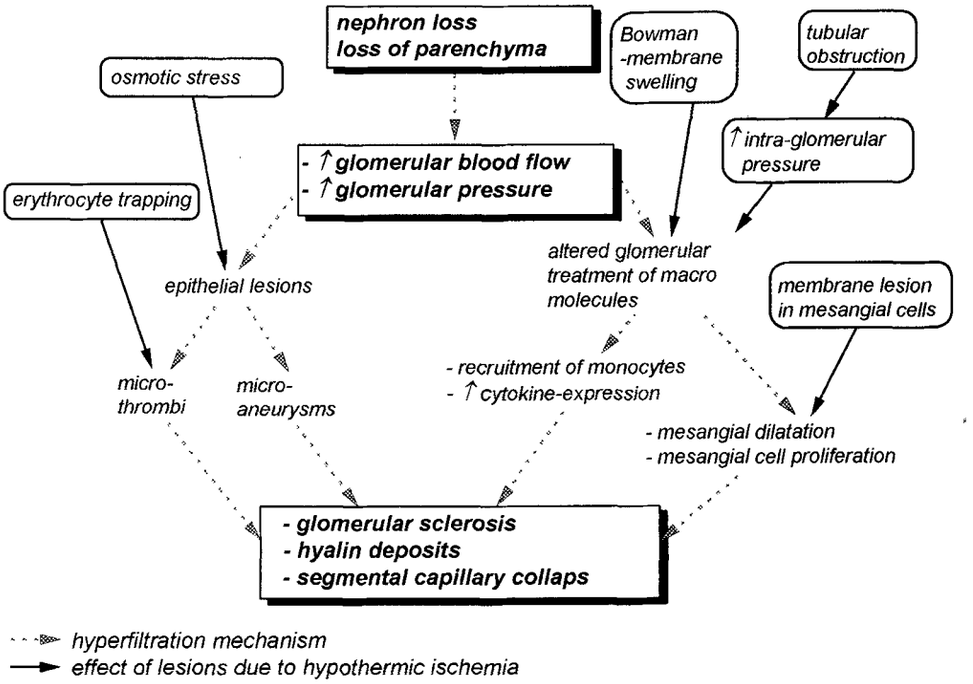

Fig. 6 Lesions after hypothermic ischemia and pathogenetic model of progressive renal damage by hyperfiltration (modified [10])

ture (GPC/PME-to-hdi ratio: $r = 0.9543$ and $P = 0.0031$) and to glomerular function (GPC/PME to K^+-diuresis ratio: $r = 0.8275$ and $P = 0.0421$), whereas the comparable TN/PME-to-hdi ratio was $r = 0.1002$ and $P = 0.8503$, and the TN/PME-to-K^+-diuresis ratio was $r = 0.0737$ and $P = 0.8900$. This supports the theory that nondeleterious hypothermia-induced structural alterations of phospholipid bilayer membranes, the site of nucleotide regeneration, rather than the cellular nucleotide deficiency per se, determine the viability of a cold-stored organ, and to a greater extent than one would expect.

The extent of the glomerular lesions observed in unprotected kidneys is significantly reduced by the application of DMSO in hypothermia at −5 °C. The protective properties of DMSO with respect to hypothermia-induced disruption of phospholipid membranes and consecutive disturbance of membrane-bound enzymes may be responsible for this phenomenon. Hypothermic storage is known to lead to defects in cells, even at temperatures of 8 °C–25 °C, in the absence of cryoprotective agents [17, 18]. At these temperatures, the toxicity of protective agents (e.g., butylated hydroxytoluene, DMSO) is so great [19, 38] that the net benefit is limited. Storage at subzero temperatures is much more useful because the ischemic metabolism is retarded and the resealing process of phospholipid bilayers is highly accelerated by DMSO [18]. Phospholipid membrane fluidity is improved, and the disturbance of the susceptible microenvironment of membrane-bound enzymes is prevented [38, 39]. The observed excellent early function associated with the protected kidneys may be interpreted as an improved basis for short- and long-term transplant function of kidney grafts. Protection against the activity of phospholipases, by reducing the level of cellular free phosphodiesters as a substrate, seems to be less relevant. Significant activity of these enzymes is found even at temperatures as low as −196 °C [29, 34]. However, the viability of surviving cells is significantly improved, and the demand for postreperfusional repair is reduced [10].

The conclusion from our findings and the observation that, some 9–18 h after perfusion of an underproportioned parenchymal mass [5, 6], structural alterations begin that signal renal hypertrophy and hyperplasia [30] a link between the extent of pretransplant viability loss due to cold storage defects, and the onset of hyperfiltration is not ruled out yet. This might explain the occurrence of morphological alterations due to the release of cytokines, growth factors, and proinflammatory eicosanoids [20] from damaged mesangium (Fig. 1) that become apparent [33] very early [2, 7, 24, 25]. As a natural consequence, a cellular level transformation of myocytes in a proliferative form, migration of myocytes into the intima, and a fibroblast activation are observed in these cases.

Our good results, which minimize the pretransplant loss of viability of kidney grafts using nonfreezing cryopreservation with DMSO, are in total accordance with the results of other investigators [15, 16, 35, 36]. Once again, they emphasize the importance of pretransplant viability in preventing hyperfiltration and its inevitable

effects and, essentially, in determining short-term and long-term transplant survival [1, 4, 6], independent of histocompatibility and adequate immunosuppression [14, 26]. Given the fact that hyperfiltration is involved in chronic transplant rejection, the latest findings on direct and MHC-mediated inflammation processes in transplanted organs [9, 10, 13, 20] and our own results suggest a rationale for a contemporary approach to modification of the treatment of kidney grafts [1, 2, 5, 6, 8, 9, 13, 14, 20]. This might include with respect to pretransplant viability, modification of the storage method by (1) further reduction in storage, (2) an optimal low storage temperature, and/or (3) optimized low-flow perfusion for DMSO application. This would most likely lead to (1) stabilization of phospholipid-bilayer membranes of the cells, (2) protection of the integral membrane proteins, (3) a reduction in catabolic metabolism, and (4) a higher poststorage nephron mass. As a result, one would achieve (1) a lowered immunogenity, (2) improved restoration of function after transplantation, (3) reduced hyperperfusion/hyperfiltration, and (4) a longer half-life of kidney grafts.

In this context, it has to be emphasized how important preservation of pretransplant viability at the highest level is. Immunological donor-recipient matching aside, even with the help of more complicated and costly procedures [5], maximum preservation of viable nephrons is most likely to prevent hyperfiltration [37] and the inevitable effects, essentially determining short-term and long-term transplant survival [3, 6, 8, 11, 23]. The recent tendency of Eurotransplant to reduce the emphasis on HLA matching [26] of kidneys in favor of non-immunological factors is based not only on the latest immunological study results [9, 13] but also on new psychosocial aspects, as well as on the encouraging results with living related and unrelated organ donation, encouraging "fair organ donation". These efforts might result in enhanced graft survival but cannot be achieved without further experimental research. The social and financial benefits of further improving the half-life of kidney grafts can pay off the higher expenses for organ storage far better. Better functioning kidney grafts may, in the long run, even help to solve such problems as dialysis capacity and the organ shortage.

References

1. Anderson S, Brenner BM (1988) Intraglomerular hypertension: implications and treatment. Annu Rev Med 39: 243–253
2. Andrews PM, Coffey AK (1982) Factors that improve the preservation of nephron morphology during cold storage. Lab Invest 46: 100–120
3. Azuma H, Nadeau K, Mackenzie HS, Brenner BM, Tilney NL (1997) Nephron mass modulates the hemodynamic, cellular, and molecular response of the rat renal allograft. Transplantation 63: 519–528
4. Banafsche R, Bretan PN, Garovoy M (1993) Improved long term graft survival in pediatric donor kidney transplant recipients using quadruple immunosuppression. Urol 149: 390A
5. Banafsche R, Pomer S, Motzkau D, Jansen S, Staehler G, Bretan PN (1996) Verbesserung des Organüberlebens bei der Transplantation von Kindernieren durch die Optimierung des Spender-Empfänger-Größenverhältnisses. Urologe A 35: 18–25
6. Chertow GM, Milford EL, Mackenzie HS, Brenner BM (1996) Antigen independent determinants of cadaveric kidney transplant failure. JAMA 276: 1732–1736
7. Coffey AK, Andrews PM (1983) Ultrastructure of kidney preservation: varying the amount of an effective osmotic agent in isotonic and hypertonic preservation solutions. Transplantation 35: 136–143
8. Eschwege P, Randrianjohany A, Trifa M, Alexandre L, Blanchet P, Decaux A, Richard C, Cherpentier B, Benoit G (1996) Impact of weight difference between donor and recipient on the function of the transplanted kidney. Prog Urol 6: 257–259
9. Goes N, Urmson J, Vincent D, Ramassar V, Halloran PF (1996) Effect of recombinant human insulin-like growth factor-1 on the inflammatory to acute renal injury. J Am Soc Nephrol 7: 710–720
10. Grady RW, Novick AC (1996) Hyperfiltration of renal injury: pathophysiology and clinical implications. Urology 47: 273–283
11. Heemann UW, Azuma H, Tullius SG, Schmid C, Philipp T, Tilney NL (1996) Infections and reduced functioning kidney mass induce chronic rejection in rat kidney allografts. Clin Nephrol 46: 34–38
12. Hughes AK, Stricklett PK, Padilla E, Kohan DE (1996) Effect of reactive oxygen species on endothelin-1 production by human mesangial cells. Kidney Int 49: 181–189
13. Isoniemi H, Kyllönen L, Eklund B, Höckerstedt K, Salmela K, von Willebrand E, Ahonen J (1995) Acute rejection under triple immunosuppressive therapy does not increase risk of late first cadaveric renal allograft loss. Transplant Proc 27: 875–877
14. Kahan BD (1996) The three fates of immunosuppression in the next millenium: selectivity, synergy, and specificity. Transpl Int 9: 527–534
15. Khirabadi BS, Fahy GM, Ewing LS, Saur J, Meryman HT (1994) 100% survival of rabbit kidneys chilled to −32 °C after perfusion with 8 M cryoprotectant at −22 °C (abstract). Cryobiology 31: 597
16. Khirabadi BS, Fahy GM, Ewing LS (1995) Survival of rabbit kidneys perfused with 8.4 M cryoprotectant (abstract). Cryobiology 32: 543
17. Kruuv J, Glofcheski DJ (1993) Further evidence for two modes of hypothermia damage. Cryobiology 30: 313–321
18. Kruuv J, Glofcheski DJ, Lepock JR (1995) Evidence for two modes of hypothermia damage in five cell lines. Cryobiology 32: 182–190
19. Laver AJ, Hibbs M, Smith RAG (1995) Activation of complement by DMSO and ethanol and its inhibition by soluble complement receptor type 1. Biochem Soc Trans 23: 167S

20. Lemström K, Koskinen P, Häyry P (1995) Molecular mechanisms of chronic renal allograft rejection. Kidney Int 48: S2–S10
21. Linner JG, Livesey SA, Harrison DS, Steiner AL (1986) A new technique for removal of amorphous phase tissue water without ice crystal damage: a preparative method for ultrastructural analysis and immunoelectron microscopy. J Histochem Cytochem 34: 1123–1135
22. Lopez-Neblina F, Paez AJ, Toledo-Pereyra LH (1995) Modulation of neutrophil infiltration through nitric oxide in the ischemic rat kidney. Transplant Proc 27: 1883–1885
23. Modlin C, Goldfarb D, Novick AC (1996) Hyperfiltration nephropathy as a cause of late graft loss in renal transplantation. World J Urol 14: 255–264
24. Neagu S, Chirculescu AR, Ranga V, Popescu F (1983b) Morphological aspects of the rat kidney preserved by cold storage. I. Glomerular morphometric changes. Morphol Embryol Bucur 29: 121–125
25. Neagu S, Gabrielescu E, Codorean E, Chirculescu AR (1983c) Morphological aspects of the rat kidney preserved by cold storage. III. Ultrastructural aspects. Morphol Embryol Bucur 29: 279–282
26. Opelz G (1992) Collaborative transplant study – 10-year report. Transplant Proc 24: 2342–2355
27. Podo F, Carpinelli G, Proietti E, Belardelli F (1993) ^{31}P magnetic resonance studies of cytokine-inducedalterations in tumor cells transplanted into syngenic mice. In: Pomer S, Hull WE (eds) Magnetic resonance in nephrourology: clinical and experimental applications. Springer, Berlin Heidelberg New York, pp 14–24
28. Pomer S (unpublished observation)
29. Rauchova H, Ledvinkova J, Kalous M, Drahota Z (1985) The effect of lipid peroxidation on the activity of various membrane-bound ATPases in rat kidney. Int J Biochem Cell Biol 27: 251–255
30. Remuzzi A, Mazerska M, Gephardt GN, Novick AC, Brenner BM, Remuzzi G (1995) Three-dimensional analysis of glomerular morphology in patients with subtotal nephrectomy. Kidney Int 48: 155–162
31. Rennke HG (1986) Structural alterations associated with glomerular hyperfiltration. In: Mitch WE, Brenner BM, Stein JM (eds) The progressive nature of renal disease (Contemporary issues in nephrology, vol 14). Churchill Livingstone, New York, pp 111–131
32. Schabel J, Pressler H, Holzhausen HJ, Pauer HD, Langkopf B (1985) Morphologische Untersuchungen an DMSO-perfundierten Nieren. Z Urol Nephrol 78: 659–665
33. Schlöndorff D (1996) Roles of the mesangium in glomerular function. Kidney Int 49: 1583–1585
34. Sumida S, Watanabe T, Hayano Y (1995) Morphological and physiological integrity of red cells cryopreserved for 25 years (abstract). Cryobiology 32: 544
35. Wang T, Xiao X, Zhang C, Hicks GL (1995) Nonfreezing storage of the isolated rat heart at –10 °C Cryobiology 32: 543
36. Yang X, Zhu Q, Layne JR, Claydon M, Hicks GL, Wang T (1993) Subzero nonfreezing storage of the mammalian cardiac explant: I. Methanol, ethanol, ethylene glycol, and propylene glycol as colligative cryoprotectants. Cryobiology 30: 366–375
37. Yoshida Y, Fogo A, Shiraga H, Glick AD, Ichikawa L (1988) Serial micropuncture analysis of single nephron function in subtotal renal ablation. Kidney Int 33: 855–867
38. Ytrehus K, Hegstad AC (1991) Lipid peroxidation and membrane damage of the heart. Acta Physiol Scand S599: T81–91
39. Yu ZW, Quinn PJ (1994) Dimethyl sulphoxide: a review of its applications in cell biology. Biosci Rep 14: 259–281