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Effects of various HTK solution regimens on proteinuria after renal transplantation in dogs

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Abstract Investigations were carried out by means of an autologous, heterotopic model for kidney transplantation applied to dogs. Duration of cold ischemia was 48 h. Four experimental groups were arranged. During the first 20 min following revitalization of the transplanted kidney, group 1 (HTK solution/80 cm perfusion height) showed a significant glomerular and tubular malfunction. In group 2 (HTK solution/120 cm perfusion height), only four urinary proteins with molecular weights of 25 kDa, 67 kDa, 100 kDa and >100 kDa were found. The excretion of higher molecular proteins receded over the 20-min period of observation. In both group 3 (HTK/aspartate solution) and group 4 (HTK/tryptophan solution) the quantity of excreted glomerular and tubular protein was well above that of group 2. As opposed to the "Tryptophan" group, a complete restoration of renal function was observed in the "Aspartate" group after 4 weeks. In general, the "standard" HTK protective solution delivered with 120 cm perfusion pressure gave the most favorable results, with the lowest levels of proteinuria and a satisfactory recovery of renal function after revitalization.

Key words Proteinuria · Kidney transplantation · Kidney preservation · HTK solution

Dedicated to the memory of Prof. H. J. Bretschneider

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The most important clinical indicators of a disturbance of renal transplant function are a decrease in urinary excretion and an increase in serum creatinine. In addition, the characterization of excreted proteins can provide information about the localization of the renal dysfunction. Polyacrylamide gel electrophoresis (PAGE) has proven to be of particular value for the diagnosis of renal lesions [5, 9, 29, 30]. With the application of 2-D PAGE [3] or the Micro-PAGE techniques [37], only minimal amounts of proteinuria are needed to characterize the excreted proteins. Proteinuria can be classified according to its glomerular or tubular causes by analyzing the molecular weight of the involved proteins [1, 8, 21, 25, 33, 36]. Among the excreted proteins, plasma proteins of high or low molecular weight play a particularly important role as indicators of glomerular basement membrane integrity or of tubular reabsorptive capacity. Albumin (66.5 kDa) marks the borderline between the glomerular and tubular types of proteinuria. The electrophoretic pattern of excreted proteins in the dog is comparable to that of humans [2, 35].

Proteinuria following autologous kidney transplantation was examined in dogs. The following experiments examine the influence of HTK (histidine tryptophan ketoglutarate) solution on postischemic proteinuria. Specifically the perfusion pressure and the composition of the HTK solution was varied to elucidate their effects on proteinuria and transplant function in the first minutes after the reestablishment of

Table 1 Composition of kidney protective solutions

	HTK solution (mM)	HTK/ aspartate solution (mM)	HTK/ tryptophan solution (mM)
Sodium	15	15	15
Potassium	10	9	10
Magnesium	4	4	4
Chloride	50	18	50
Aspartate	–	32	–
Tryptophan	2	2	6
α-Keto-glutarate	1	1	1
His/His-HCl	180/18	180/18	180/18
Mannitol	30	30	30
Osmolality (mosmol/l)	310	310	310
pH at 8 °C	7.3	7.3	7.3
pO_2 at 37 °C (mmHg)	200	200	200

Table 2 HTK solution, 80 cm perfusion height: experimental data

Experiment no.	Perfusion volume (ml)	Urine excretion after recirculation of the kidney (ml/min)	Maximal post-operative plasma creatinine (mg/100 ml)
0	700	0.67	4.1
693	780	0.63	10.2
715	800	0.05	8.8
719	850	0.30	8.1
738	900	0.07	5.6
Mean	806	0.34	7.4
Median	800	0.30	8.1
Standard deviation	75	0.29	2.5

Table 3 HTK solution, 80 cm perfusion height: experimental data

Experiment no.	Perfusion volume (ml)	Urine excretion after recirculation of the kidney (ml/min)	Maximal post-operative plasma creatinine (mg/100 ml)
03	1200	1.60	7.0
04	1350	1.20	4.6
07	1000	0.95	6.5
7	1650	0.33	3.0
721	800	0.93	1.8
731	1400	0.16	7.7
747	1650	0.43	4.5
748	1100	0.78	1.8
Mean	1269	0.80	4.6
Median	1275	0.86	4.55
Standard deviation	270	0.48	2.3

circulation. An additional aim was to clarify whether the proteinuria occurring directly after transplantation allows conclusions to be made about long-term transplant function. For the most part the assessment of the pattern of proteinuria was considered to be more important than a precise identification or classification of the site of origin of each excreted protein.

Table 4 HTK solution, 120 cm perfusion height: experimental data

Experiment no.	Perfusion volume (ml)	Urine excretion after recirculation of the kidney (ml/min)	Maximal post-operative plasma creatinine (mg/100 ml)
0–30	1200	2.05	5.8
6	1750	0.23	6.0
6/14	1550	0.50	3.1
6/24	1800	1.20	8.0
6/25	1100	0.25	2.8
6/29	1500	0.35	5.2
758	1300	1.45	3.3
764	1800	2.70	5.7
768	1200	0.90	5.3
Neboe 22	1350	1.15	7.2
Mean	1455	1.08	5.24
Median	1425	1.03	5.75
Standard deviation	265.5	0.82	1.85

Table 5 HTK solution, 120 cm perfusion height: experimental data

Experiment no.	Perfusion volume (ml)	Urine excretion after recirculation of the kidney (ml/min)	Maximal post-operative plasma creatinine (mg/100 ml)
0–31	1400	0.13	6.9
767	1000	3.68	1.4
769	2100	0.33	3.4
770	1600	0.23	4.0
771	1400	1.08	2.9
772	1300	0.55	8.7
773	1050	0.88	3.3
Mean	1407	0.98	4.4
Median	1400	0.55	3.4
Standard deviation	373	1.24	2.6

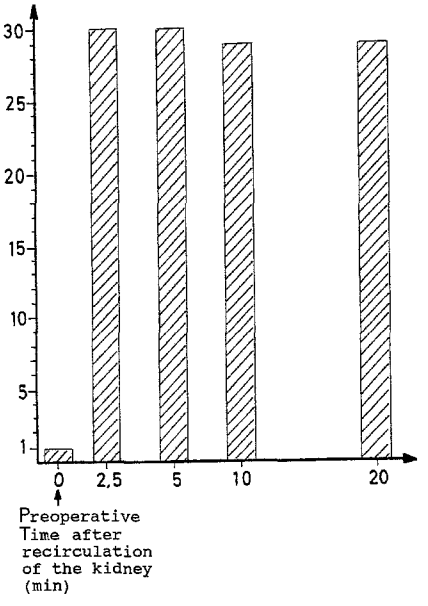

Fig. 1 Average rise in the urinary protein excretion expressed as a multiple of the preoperative baseline value. Gravity perfusion of the kidney with HTK solution from a height of 80 cm

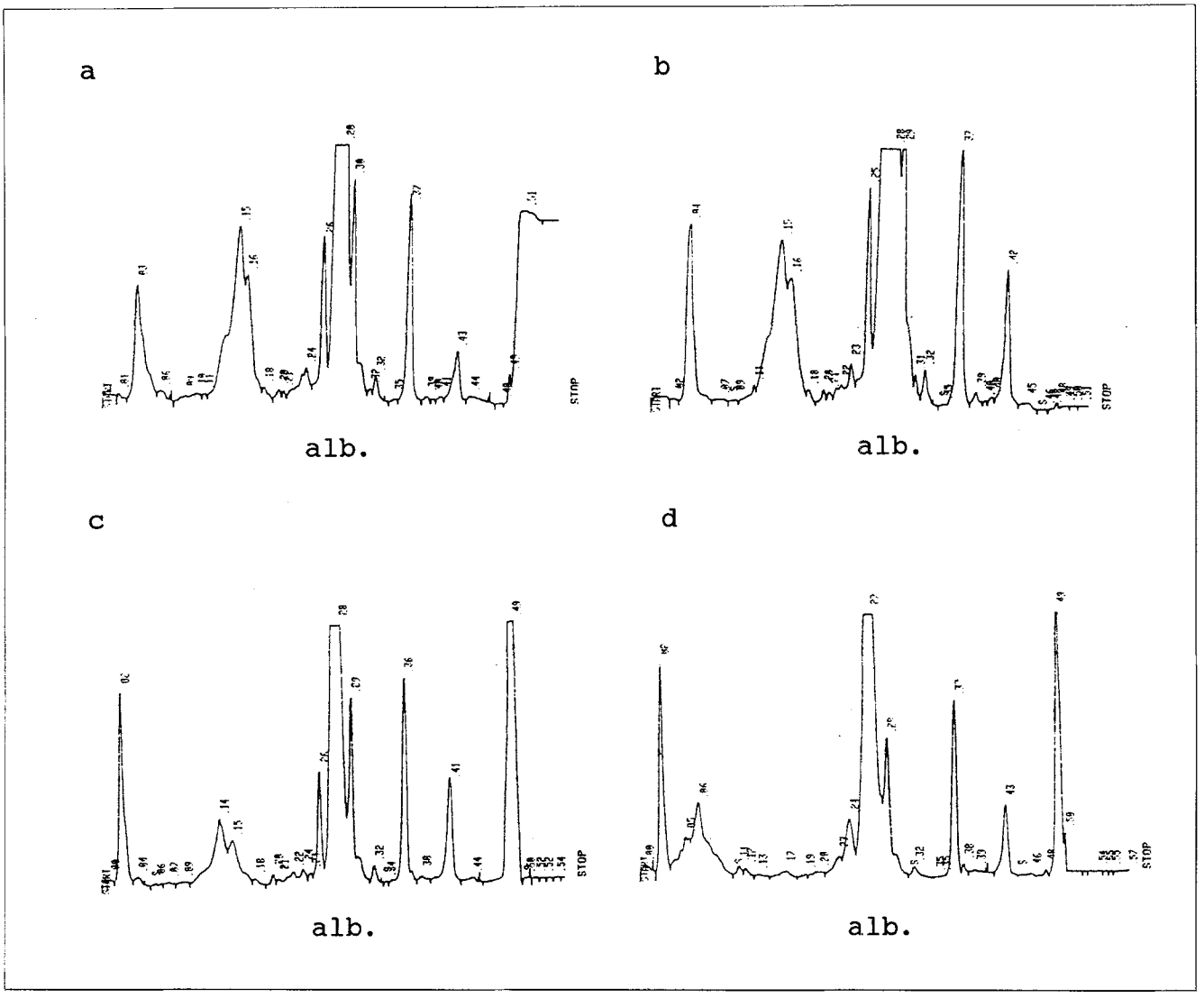

Fig. 2a–d Densitograms of urinary proteins obtained from dog No. 719 at **a** 2.5 min, **b** 5 min, **c** 10 min, **d** 20 min following revascularization of the renal transplant after cold ischemia of 48 h and perfusion of the kidney with HTK solution at 80 cm H_2O perfusion pressure. *alb.*, albumin

Materials and methods

Thirty dogs of mixed race (average weight 24.5 kg) were prepared for transplantation by being placed under general anesthesia. First, a preoperative baseline urine sample was obtained for comparison with postoperative urinary protein values. The first operative stage consisted of removal of the left kidney; the extirpated kidney was then protected with one of four methods over the subsequent 48 h of cold ischemia (4 °C): group 1, HTK solution (Custodiol) at a perfusion pressure of 80 cm H_2O (five subjects); group 2, HTK solution at a perfusion pressure of 120 cm H_2O(eight subjects); group 3, HTK

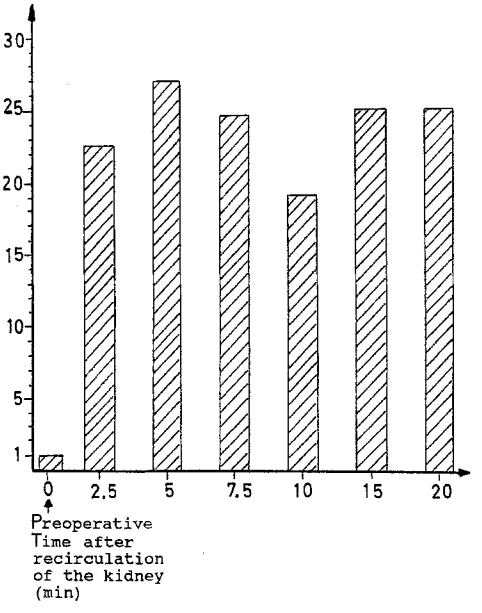

Fig. 3 Average rise in the urinary protein excretion expressed as a multiple of the preoperative baseline value. Gravity perfusion of the kidney with HTK solution from a height of 120 cm

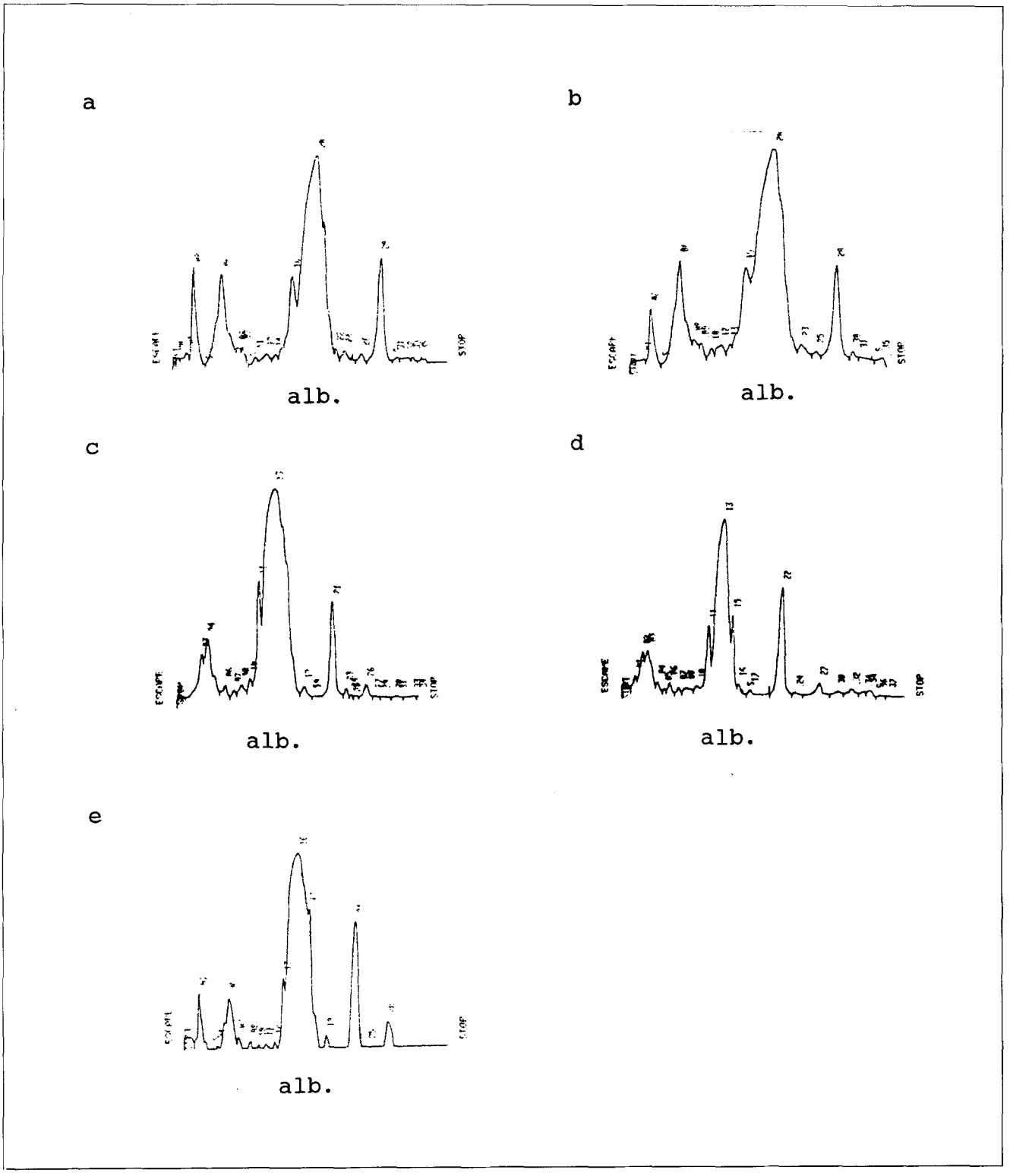

Fig. 4a–e Densitograms of urinary proteins obtained from dog No. 748 at **a** 4 min, **b** 6 min, **c** 10 min, **d** 15 min, **e** 17 min following revascularization of the renal transplant after cold ischemia of 48 h and perfusion of the kidney with HTK solution at 120 cm H_2O perfusion pressure. *alb.*, albumin

solution containing 32 mmol asparate at a perfusion pressure of 120 cm H_2O (ten subjects); group 4, HTK solution containing an additional 4 mmol tryptophan at a perfusion pressure of 120 cm H_2O (seven subjects). In group 3, asparate replaces the chloride component, since chloride can follow sodium into the cells during ischemia [18, 22]. Aspartate can bind calcium and magnesium, thereby increasing membrane stability under anaerobic conditions [8]. In group 4, the lipophilic tryptophan acts as a protective factor for the cell membrane, since it prevents the influx of histidine into the

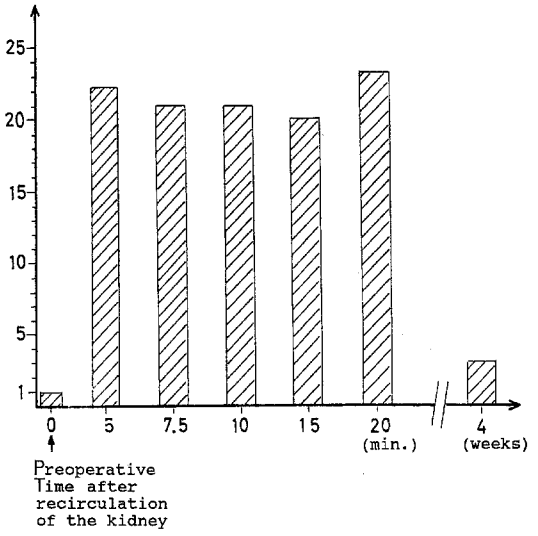

Fig. 5 Average rise in the urinary protein excretion expressed as a multiple of the preoperative baseline value.Gravity perfusion of the kidney with HTK/aspartate solution from a height of 120 cm

cell by inhibiting the amino acid transport system [22]. The total volume of HTK solution used during the protective perfusion of the kidney is referred to as the "perfusion volume" in Tables 2–5. After 48 h, in the second operative step, the right kidney was removed (in order to avoid an overlap of renal function) and the left kidney was implanted in the left iliac fossa with end-to-side anastomoses to internal iliac vessels. For the first 20 min after the vascular attachments were in place, the urine was diverted and collected (Tables 2–5). Urine samples for protein analysis were thereby taken directly from the ureter at 2.5, 5, 7.5, 10, 15 and 20 min after transplantation. The ureter was then implanted in the bladder. After a 4-week observation period, a final analysis of the urinary proteins was carried out. Total protein content was measured for each sample. In addition the protein in the samples was separated with one-dimensional sodium dodecyl-phosphate polyacrylamide gel electrophoresis (SDS-PAGE) with coomassie brilliant blue staining. The results were depicted as densitograms. The serum creatinine concentration was measured over the course of the 4-week postoperative period. For each group the highest value of the postoperative creatinine level is referred to as "maximal postoperative plasma creatinine", in Tables 2–5. The experimental groups were compared using variance analysis for a split-plot plan.

Results

In comparing the different groups' protective perfusion volume, the postoperative maximal creatinine concentrations and the urinary output using variance analysis, a significant difference was found in the protective perfusion volume among the groups ($P = 0.002$), whereas the differences in postoperative urine excretion ($P = 0.81$) and in postoperative maximal creatinine concentration ($P = 0.34$) were not significant (Tables 2–5).

Representative electrophoresis patterns for all experimental groups are shown in Figs. 1–5. The densitograms of the preoperative urine samples confirm that all dogs had physiological levels of albuminuria.

Urine samples from the experimental animals taken 2 days after unilateral nephrectomy yielded a total protein concentration ranging from a minimum of 0.08 mg/ml to a maximum of 0.56 mg/ml (median 0.27 mg/ml). After successful revitalization of the transplanted kidney, a sharp rise of the total protein content was measured in the excreted urine during the subsequent 20-min observation period (Fig. 1). With respect to the average initial values of proteinuria (see above), a 30-fold rise in total protein excretion was observed on average in the urine from the subjects of group 1 (HTK, perfusion pressure 80 cm H_2O) 2.5 min after the onset of recirculation. This elevated level of excretion did not appreciably diminish over the course of the 20-min observation period (Fig. 1).

The proteins excreted over the first 20 min were composed of low, middle and high molecular weight fractions (Fig. 2). Marked levels of albumin were present in all densitograms. Especially during the first few minutes after revitalization of the kidney, both the tubular (low molecular weight) and the glomerular (high molecular) components of the proteinuria were very high. A gradual reduction of the proteinuria was evident over the 20-min period of observation.

In group 2 (HTK, perfusion pressure 120 cm H_2O), the maximal average increase in total protein concentration in the first 5 min after the onset of recirculation was 27 times that of the normal value. At 10 min this diminished to a 19-fold increase (Fig. 3). A renewed increase in the proteinuria of 25 times that of the normal value was observed between 15 and 20 min. The total protein excretion was thus lower than in group 1, which also affected the protein fractions and their distribution on electrophoresis.

All subjects demonstrated an increase in glomerular protein excretion. However, in comparison with group 1, there was significantly less glomerular and tubular proteinuria. After the 6th min a pronounced albuminuria was seen, accompanied by the excretion of high molecular weight proteins (>67 kDa), with two predominant fractions of 100 kDa and >100 kDa. Furthermore, a band with a molecular weight of about 25 kDa was observed. With increasing time the albuminuria and the higher molecular weight fractions receded, whereas the low molecular fraction remained constant. This protein excretion pattern implies, on the one hand, a transitory structural lesion of the glomerular membrane which, however, appears reversible. On the other hand, an increased excretion or a selectively decreased tubular reabsorptive capacity may be inferred. This dog (Fig. 4) developed a maximal postoperative creatinine concentration of 1.8 mg% (Table 3).

In group 3 (HTK/aspartate) the total protein concentration reached an initial maximum after 5 min with a subsequent gradual decline up to 10–15 min. A second maximum occurred between 15 and 20 min after the onset of recirculation. However, the two peaks in the aspartate group were lower than those of group 2,

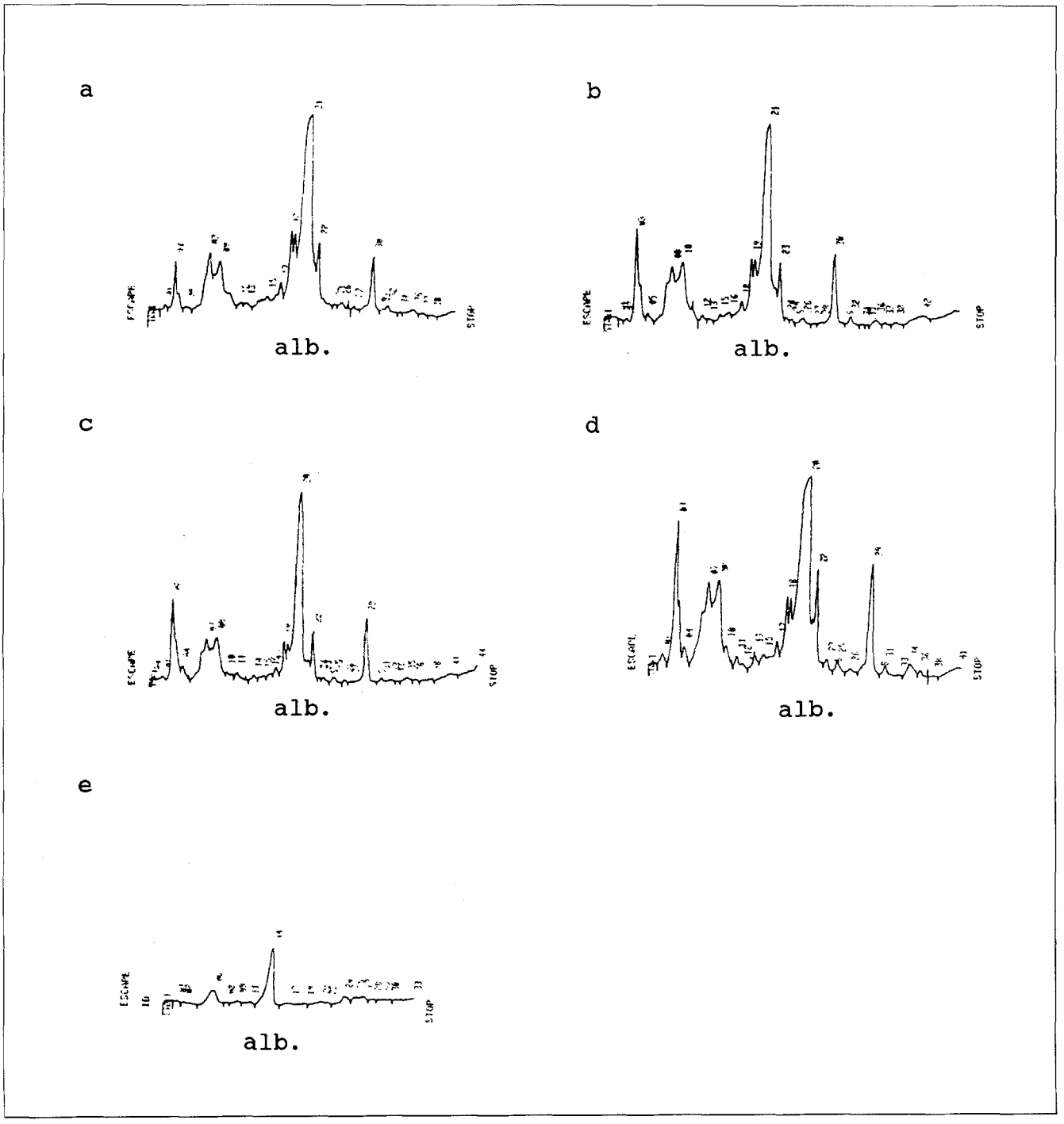

Fig. 6a–e Densitograms of urinary proteins obtained from dog No. 758 at **a** 6 min, **b** 10 min, **c** 15 min, **d** 20 min following revascularization of the renal transplant after cold ischemia of 48 h and perfusion of the kidney with HTK/aspartate solution at 120 cm H_2O perfusion pressure. **e** 26 days after transplantation. *alb.*, albumin

with a 22-fold increase (at 5 min) and a 23-fold average increase (at 20 min; HTK, perfusion pressure 120 cm H_2O), where the values were 27- and 25-fold, respectively. At the end of the 4-week observation period the total urinary protein in group 3 was on average 3 times higher than the initial value (Fig. 5). With the exception of two subjects, a normalization of total protein excretion was observed. These two subjects demonstrated an eight fold increase in proteinuria. This accounts for the increase in the average total urinary protein.

In the initial urinary specimens an increased albuminuria was observed with an excretion of high molecular weight proteins down to 67 kDa. In addition, a pronounced excretion of a molecule with an M_r of 25 kDa that increased over the observation period was found (see preceding group). There was also a

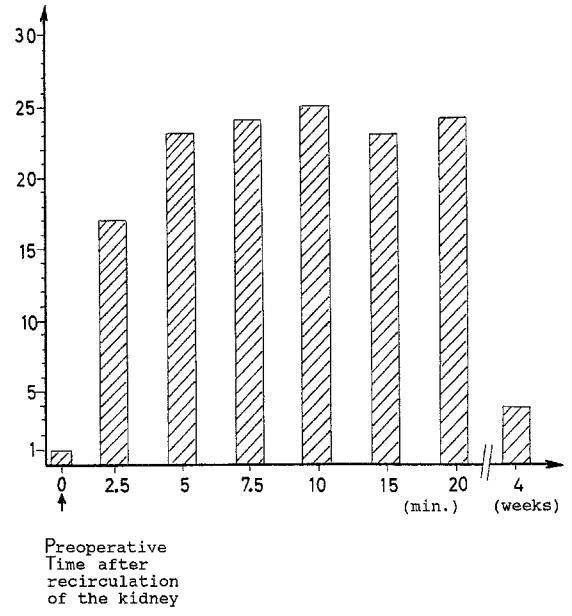

Fig. 7 Average rise in the urinary protein excretion expressed as a multiple of the preoperative baseline value. Gravity perfusion of the kidney with HTK/tryptophan solution from a height of 120 cm

reduced reabsorptive capacity of molecules near the albumin band.

In the electrophoreses of group 3 (Fig. 6), six high molecular weight proteins besides albumin were prominent: three with a molecular weight of 70–75 kDa, two with a molecular weight of 100–110 kDa and one with a molecular weight of >120 kDa. The permeability of two proteins with molecular weights of 55 kDa and 25 kDa was increased. With time the excretion of high molecular weight proteins rose. The most pronounced lomerular proteinuria occurred at the end of the 20-min, postrevitalization observation period. The lesser component of tubular proteins also increased. However, by the 4th postoperative week the electrophoresis of the urine proteins showed a completely normal pattern.

In comparison to the previous groups, protection of the kidneys with the HTK-tryptophan solution led to clear differences in the increased postoperative total protein excretion. As can be seen in Fig. 7, a maximum level of proteinuria was reached in 10 min, followed by a minimal decline through the 20th min of observation. In contrast to groups 2 and 3, a curve with two definite peaks was not observed. The values of the average increase in urinary protein excretion in the tryptophan group were lower than in groups 1 and 2, but higher than in the third group, protected with the aspartate solution.

In group 4 (HTK/tryptophan), evidence of an increasing permeability of large molecules up to 67 kDa (Fig. 8) was found. Additionally, a reduced tubular reabsorption of low molecular weight proteins and a marked albuminuria was present. In the low molecular weight range, electrophesis again demonstrated the previously described bands at 14 kDa and 25 kDa. In the 4th week of observation the 25-kDa band was still evident.

Four protein molecules with molecular weights of 70, 75, 100 and 110 kDa were excreted in the urine with increasing magnitude until the 10th min due to a lesion of the glomerular basement membrane. Similar proteins have been found in previous experiments. For the first 10 min of the observation period, the results suggest advantages for the HTK/tryptophan protective regimen in comparison to the other groups. However, as the observation period continued, proteins with molecular weights of 25, 80 and 120 kDa and higher were found in the urine. After 15 min the most marked excretion occurred, suggesting that, as opposed to groups 2 and 3, an increasing defect in the integrity of the glomerular basement membrane as well as an increasing tubular disorder took place. Whereas all subjects in group 3 (HTK/aspartate) had normal levels of proteinuria at the 4th postoperative week, normal levels were only apparent in four of the subjects of group 4 (HTK/tryptophan) (Fig. 9). The three other subjects of group 4 showed an increased level of albumin excretion.

Discussion

The initial 20 min following kidney transplantation are of particular relevance to postoperative function of the organ [17, 34].

In order to obtain information about the function of the transplanted kidney in the first minutes after recirculation, the total protein content was measured and a PAGE of urinary proteins was carried out on the urine collected during the first 20 min following renal recirculation. The results of the qualitative measurements of the proteinuria showed that group 2 (HTK/aspartate) had the lowest values. This is correlated with the highest levels of primary urinary excretion occurring after recirculation. In contrast, the qualitative protein analysis (PAGE) as well as the comparison of the postoperative maximal creatinine concentration suggest a better renal function in groups 2 and 4. No clear conclusion as to the function of the transplanted kidney can be drawn from the quantitative measurements of the proteinuria.

PAGE can be used to both uncover as well as to localize the site of ischemic damage [3, 36, 37]. A good correlation between the histopathology, clinical parameters and the electrophoresis results has been postulated [26]. After kidney transplantation, some level of proteinuria is to be expected [6, 10, 11, 19, 20, 23, 32]. In the literature a predominantly tubular proteinuria following renal transplantation has been described

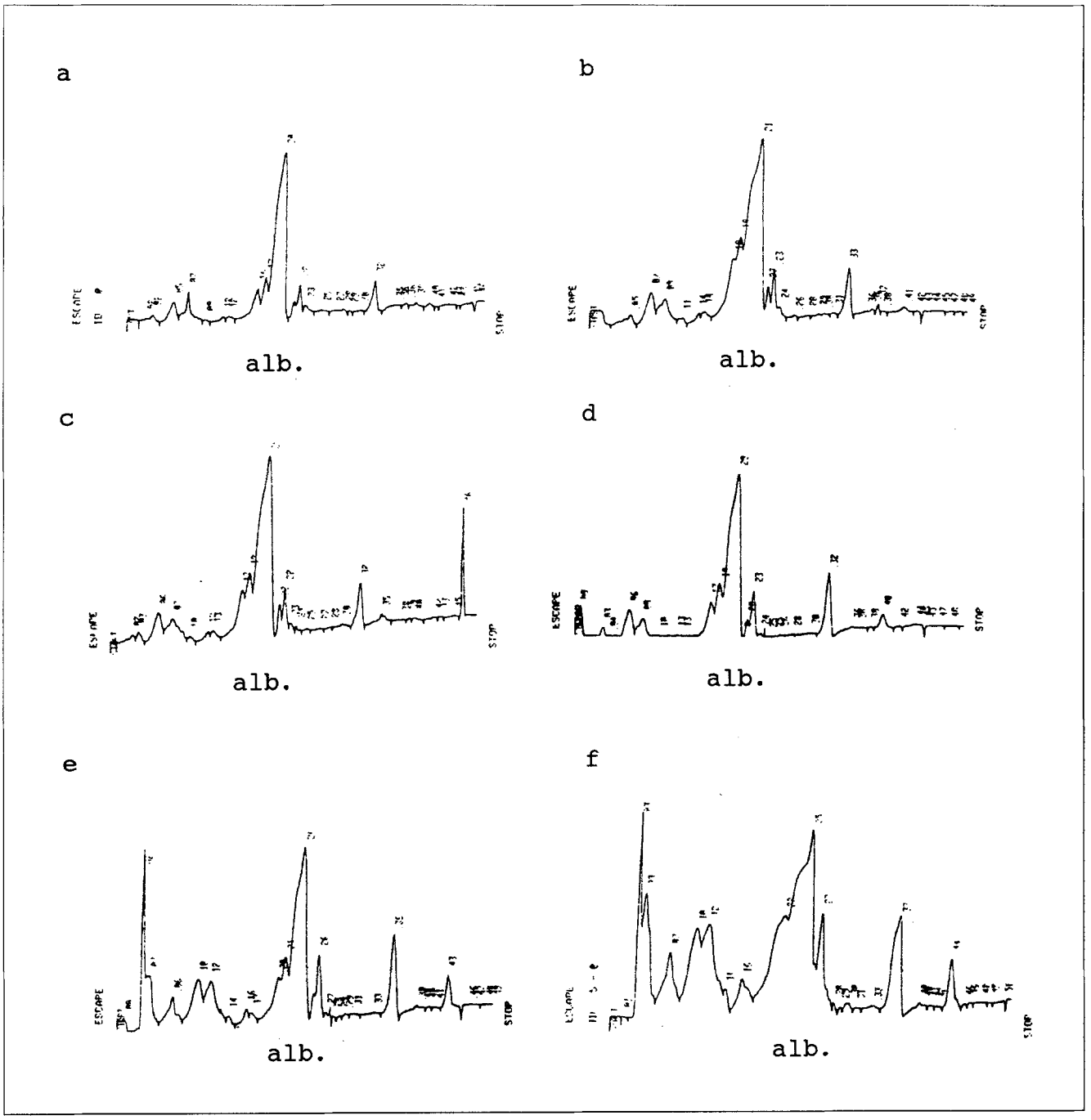

Fig. 8a–f Densitograms of urinary proteins obtained from dog No. 771 at **a** 2.5 min, **b** 5 min, **c** 7.5 min, **d** 10 min, **e** 15 min, **f** 20 min following revascularization of the renal transplant after cold ischemia of 48 h and perfusion of the kidney with HTK/tryptophan solution at 120 cm H_2O perfusion pressure. *alb.*, albumin

[10, 24, 33]. Frey et al. [12] describes a mixed glomerular-tubular type of proteinuria. In the present study evidence of predominantly glomerular and to a lesser extent tubular lesions was observed in the first 20 min after revascularization. When perfusion pressure was raised to 120 cm H_2O, these disturbances of renal function were reduced. Use of the original HTK solution resulted in the least pronounced mixed glomerular-tubular type of proteinuria in comparison to group 3 (HTK/aspartate) and 4 (HTK/tryptophan). Of particular note is that in group 2, the glomerular lesions were diminishing within the first 20 min after revitalization. In contrast, no regression was observed in the other groups within 20 min. With use of the original HTK solution only proteins with a molecular weight up to 110 kDa were excreted in the urine. In contrast, addition of tryptophan and aspartate resulted in the excretion of proteins with a molecular weight above 110 kDa as well as proteins of 55 kDa. This provides evidence for a better stability of the basement

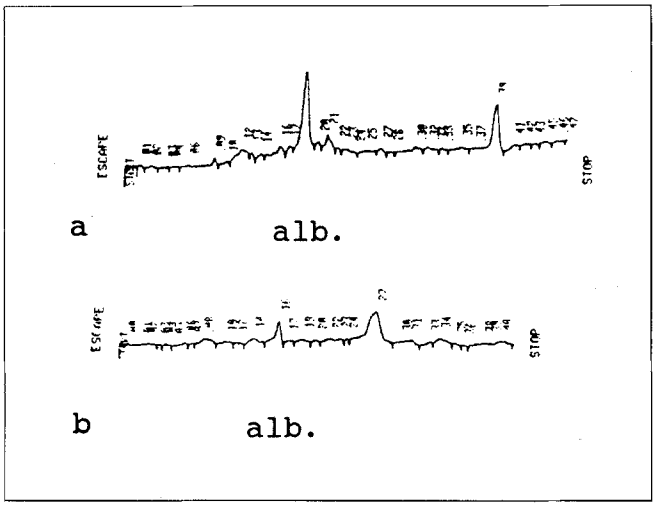

Fig. 9 Densitograms of urinary proteins obtained from dog No. 771 at **a** preoperative, **b** 28 days post-transplantation (perfusion of the kidney with HTK/tryptophan solution at 120 cm H_2O perfusion pressure). *alb.*, albumin

membrane, retention of structure and preservation of renal function in group 2. It has to be considered, however, that use of the original HTK solution leads to a reduction in renal blood flow as well as a decrease in diuresis during the 15–30 min following recirculation [16]. This may in itself explain the observed decrease in proteinuria.

In all test groups, a reduced tubular reabsorption of proteins of about 25 kDa was observed; this did not improve over the 20-min observation period. This low molecular weight protein is probably a secreted sexual hormone that is normally excreted in small amounts. This assumption is supported by the observation that the male dogs demonstrated a more intense low molecular weight electrophoretic band than the female dogs. The diagnostic relevance of this finding is therefore questionable.

By analysis of the excreted proteins it is clear that in all groups the proteinuria due to glomerular lesions is more pronounced than that due to tubular lesions. There are conflicting results in the literature concerning the duration of proteinuria after renal transplantation [7, 12, 28]. Also unclear is what influence a particular protective method has on the duration of proteinuria [4]. An additional point of discussion is the comparison of creatine clearance and proteinuria between patients with long-standing renal transplants and normal individuals. Some investigators found no significant difference between these two groups [13, 15, 27], while others found increased amounts of urinary protein in patients with renal transplants [14, 31]. A normalization of the level of urinary protein 3–6 months following renal transplantation has been reported [23]. However, 50% of patients with satisfactory levels of initial postoperative proteinuria show complete normalization of these values after only 3 weeks [7].

Frey et al. [12] reported that after at most 43 days the tubular fraction of proteinuria disappears despite a persistence of the glomerular fraction. In contrast, our results demonstrate a complete reversal of both the tubular and glomerular components at 4 weeks. In the HTK/tryptophan group a minor degree of tubular protein excretion with normal levels of serum creatinine was observed at 4 weeks while the creatinine concentration was not significantly different from those of the other groups.

Although the HTK/aspartate group in the initial 20 min after recirculation showed the least favorable results in the quantitative protein analysis in comparison to groups 2 and 4, the long-term function of the transplant normalized as judged by the quantitative and qualitative urinary protein analysis. This suggests that a measurable glomerular and tubular proteinuria occurring after recirculation is not always combined with poorer long-term renal function of the transplanted kidney, and vice versa.

In conclusion, a satisfactory primary postoperative urinary excretion after 48 h of cold ischemia can be achieved using the HTK solution. This is accompanied by an initial glomerular and tubular proteinuria. After 4 weeks, excretion of protein is within normal limits.

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