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Altered Distribution Pattern of Na^+-K^+-ATPase and Succinate Dehydrogenase Activities along the Nephron in Human Acute Post-Transplant Renal Failure

By *Heide Schmid, Adelheid Mall*

Pathologisches Institut der Universität Tübingen and

H. Bockhorn

Chirurgische Klinik und Poliklinik der Universität Tübingen

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Summary: The catalytic activities of Na^+-K^+-ATPase and succinate dehydrogenase, marker enzymes for active salt reabsorptive capacity of renal basolateral plasma membranes and for respiratory capacity of mitochondrial cristae membranes, were studied in the maintenance phase of human acute post-transplant renal failure. Biopsies of 4 kidney-allografts taken at transplantation operation and additionally at different post-transplantation periods, either with good function or in various stages of dysfunction, were compared with the unaffected part of a human kidney nephrectomized due to hypernephroma. In single nephron segments, Na^+-K^+-ATPase activity was determined after microdissection by microfluorometry, and succinate dehydrogenase activity was determined by a microphotometric procedure in stained cryosections.

In intraoperative and postoperative biopsies of a well-functioning allograft, both Na^+-K^+-ATPase and succinate dehydrogenase activities did not differ from those of normal renal tissue. In contrast, the catalytic activities were found to be decreased in the distal tubules of 2 anuric allografts when compared with their intraoperative controls. In addition, succinate dehydrogenase activity was reduced in distal tubules of a recovering allograft. Catalytic activities appeared to be unaffected in glomeruli, proximal tubules, and collecting ducts.

It is suggested that the predominant distal tubular alterations with regard to these parameters are a consequence of increased distal tubular vulnerability due to circulatory and metabolic conditions.

Verändertes Verteilungsmuster der Na^+-K^+-ATPase- und Succinat-Dehydrogenase-Aktivitäten entlang des Nephrons bei akutem Nierenversagen nach Nierentransplantation beim Menschen

Zusammenfassung: Die katalytischen Aktivitäten von Na^+-K^+-ATPase und Succinat-Dehydrogenase in der menschlichen Niere wurden in der Spätphase akuten Nierenversagens nach Nierentransplantation bestimmt. Die Enzyme dienten als Leitenzyme für die in den tubulären basolateralen Plasmamembranen lokalisierte Kapazität für aktiven Salztransport und für die Atmungskapazität der inneren Mitochondrien-Membranen. Biopsien von 4 Nierentransplantaten, intraoperativ und postoperativ zu verschiedenen Zeiten bei guter Funktion oder in verschiedenen Stadien gestörter Funktion, wurden im Vergleich zu dem normalen Teil einer menschlichen Niere mit Hypernephrom untersucht. In einzelnen Nephronsegmenten wurde die Na^+-K^+-ATPase-Aktivität nach Mikrodissektion mikrofluorometrisch und die Succinat-Dehydrogenase-Aktivität mikrophotometrisch in gefärbten Kryostat-Schnitten bestimmt.

Die Na^+-K^+-ATPase- und Succinat-Dehydrogenase-Aktivitäten unterschieden sich in den intraoperativen und in den postoperativen Biopsien eines funktionsfähigen Transplantats nicht von denen in normalem Nierengewebe. Verglichen mit den intraoperativen Kontrollen waren dagegen die katalytischen Aktivitäten in den distalen Tubuli von 2 anurischen Transplantaten abgefallen. In den distalen Tubuli eines Transplantats in

der Erholungsphase war die Succinat-Dehydrogenase-Aktivität ebenfalls vermindert. In den Glomeruli, proximalen Tubuli und Sammelrohren wurden unveränderte katalytische Aktivitäten gemessen.

Die bezüglich dieser Kenngrößen vorwiegend distal lokalisierten tubulären Veränderungen sind als Folge einer größeren Vulnerabilität dieser Nephronabschnitte auf Grund der Durchblutungs- und Stoffwechselverhältnisse anzusehen.

Introduction

The pathogenesis of human acute renal failure appears to be as yet unknown. Many experimental models have been developed for the study of this dysfunction of the kidney by morphological and physiological methods (1). In acute renal failure induced by ischaemia, it was observed that the disturbance of excretory capacity is associated with the impaired efficiency of renal tubules in the reabsorption of the glomerular ultrafiltrate (2, 3). This is manifested by characteristic morphological alterations of the tubular cells (4) including a reduction in the area of plasma membranes and of mitochondrial membranes. This phenomenon has been described in the early phase of acute renal failure in animals in both qualitative (5) and quantitative terms (6) as well as in the maintenance phase of the human disease (7, 8) with the proximal tubules as the primary site of the lesions. On the other hand, functional disturbances have also been found in the distal part of the rabbit and rat nephrons (2, 3, 9).

The limited supply of oxygen and substrates due to ischaemia results in energetic depletion of the renal cells (6). The curtailed synthesis of ATP within the mitochondrial cristae membranes is followed by failure of ATP-driven salt transport, which depends on the action of Na^+-K^+-ATPase, and of other ATP-dependent metabolic reactions, resulting in functional and structural disintegration of the cells.

Biochemical analysis of characteristic enzymatic capacities of the different cell types within the nephron (10) makes it possible to assess functional alterations of the tubular system. In this study, the activities of Na^+-K^+-ATPase (ouabain-sensitive sodium- and potassium-activated magnesium-dependent adenosine triphosphate phosphohydrolase (EC 3.6.1.3)) and succinate dehydrogenase (succinate: (acceptor) oxidoreductase (EC 1.3.99.1)) were determined in single nephron segments from biopsies of human kidney-allografts in the maintenance and recovery phases of acute post-transplant renal failure. Na^+-K^+-ATPase serves as marker for the active salt reabsorptive capacity (11) located in the basolateral infoldings of the plasma membrane (12, 13), while succinate dehydrogenase, being an integral part of the mitochondrial inner membrane, serves as a marker for the capacity of complex II in the respiratory chain (14). The determinations should provide evidence for the location and degree of tubular alteration within the nephron, with respect to these transport and energy parameters, which are structurally (15) and functionally (16) closely related. Furthermore, it should be possible to correlate the results with clinical data on the function of the allografts.

Materials and Methods

Chemicals

All the chemicals were of analytical grade and obtained from Boehringer Mannheim, D-6800 Mannheim (enzymes, coenzymes, substrates), EGA-Chemie, D-7924 Steinheim/Albuch (tetranitro-blue tetrazolium chloride = 2.2′, 5.5′-tetra-(*p*-nitrophenyl)-3.3′-(3-dimethoxy-4-diphenylene)-ditetrazolium chloride), E. Merck AG, D-6100 Darmstadt, Sigma Chemical Co., St. Louis, U.S.A., Mo. 63178 (bovine serum albumin *Cohn* Fract. V, glycogen, phosphorylase a).

Tissue preparation

Biopsies were taken from 3 human kidney-allografts (A, B, C) (perfused with Euro-*Collins* solution and preserved on ice for up to 26–32 hours), incapable of diuresis after being transplanted and untreated with diuretics. The kidneys did not show any symptoms of cellular or humoral rejection at the time of biopsy. For control purposes, 2 biopsies were taken of a kidney-allograft (D) displaying diuresis immediately after transplantation (D_2, D_3), and a biopsy sample was taken from the unaffected part of a human kidney nephrectomized due to hypernephroma grade I (E). In addition, biopsies of kidney-allografts A, B, C, and D had been obtained at the time of the transplantation operation about 30 min after anastomosis (A_1, B_1, C_1, and D_1). The excretory function of the kidney-allografts at the time of biopsy is documented by the clinical data shown in table 1.

The tissue specimens were prepared for microdissection according to *Lowry & Passonneau* (17) by shock-freezing in liquid nitrogen immediately after the puncture and by subsequent freeze-cutting (Dittes-Duspiva, D-6900 Heidelberg) into sections of 16 µm thickness. The cryostat sections adjacent to those to be lyophilized were stained, the one with periodic acid/*Schiff*'s base reagent and the other for succinate dehydrogenase activity, thus serving for identification of the nephron segments within the lyophilized sections (18).

Determination of enzyme catalytic activities

The determination of specific Na^+-K^+-ATPase activity was performed on single microdissected nephron segments according to *Schmidt & Horster* (19) with slight variations for the use of smaller tissue specimens. Specific Na^+-K^+-ATPase activity was calculated by subtraction of ouabain-insensitive Mg^{2+}-ATPase activity from total ATPase activity, each determined in one half of an isolated tissue specimen and expressed in µmol · min^{-1} inorganic phosphate formed per g tissue dry weight at 37 °C.

Tab. 1. Clinical data representing the function of human kidney-allografts (A, B, C, D) at the time of post-transplantation biopsy.

Biopsy	A_2 (post-transplantation anuria for 9 days)	B_2 (post-transplantation anuria for 14 days)	C_2 (post-transplantation anuria for 12 days, oliguria for 1 day, polyuria and normuria for 11 days)	D_2 (normuria, 7 days after transplantation)	D_3 (normuria, 20 days after transplantation)
Serum creatinine ($\mu mol \cdot l^{-1}$)	1070	416 (after haemodialysis)	336	133	133
Creatinine clearance ($ml \cdot min^{-1}$)	–	–	19	87	70
Urine volume per 24 h (ml)	–	–	2000	1250	2300
Mass density of urine ($kg \cdot l^{-1}$)	–	–	1.011	1.012	1.008
Urinary [Na^+] ($mmol \cdot l^{-1}$)	–	–	36	80	81
Urinary [K^+] ($mmol \cdot l^{-1}$)	–	–	56	14	32
Urinary [Cl^-] ($mmol \cdot l^{-1}$)	–	–	48	20	60

The cryostat sections to be stained for succinate dehydrogenase activity (20, 21) were incubated at 37 °C for 15 min in a reagent containing 50 mmol/l sodium phosphate buffer, pH 7.6, 50 mmol/l sodium succinate, 0.28 mmol/l tetranitro-blue tetrazolium chloride, 1 mmol/l KCN, 30 mmol/l $NaHCO_3$, 0.13 mmol/l $CaCl_2$, 0.25 mmol/l $MgCl_2$, and 0.20 mmol/l $AlCl_3$.

The approximately linear relationship of formazan precipitation with this incubation time permits the quantification of the deposits within single nephron segments by microphotometric measurement (Univar, Reichert-Jung, D-6907 Nußloch) (22). This was manually performed at 550 nm using a 40 × objective and a round measuring diaphragm to give a spot area of 6.13 μm diameter. Stained kidney sections were chosen for measurement which contained all available anatomical regions. The absorbance values of the individual nephron segments were averaged for each section. For expression in relative succinate dehydrogenase activities, the mean absorbance value of the cortical collecting ducts was taken as the reference unit for that of the other nephron segments obtained from the same section. In this way, any variations due to section thickness and unspecific formazan formation are cancelled, and measurements from sections stained at different times can be compared.

Differences between the means of enzyme activities were tested for statistical significance with *Student*'s t test for unrelated samples.

Results

Since normal human renal tissue is inaccessible for the investigation of enzyme activities, the unaffected part of a kidney nephrectomized due to hypernephroma (E) was considered to represent normal tissue, and this was supported by routine light microscopic diagnosis. The absolute values as well as the characteristic distribution pattern of specific Na^+-K^+-ATPase activity (highest activity in the distal tubules) agree with earlier data on human, rat, and rabbit nephrons (19) obtained with this technique. To support their active salt reabsorption, the distal tubules are equipped with a high mitochondrial respiratory capacity, as indicated by their relative succinate dehydrogenase activity.

Enzyme activities in biopsies of a functioning kidney-allograft (D), obtained at different periods after transplantation (D_2, D_3), were found to be not significantly different from those of normal renal tissue (E) (tabs. 2 and 3). The same holds true for the intraoperative biopsy of allograft D (D_1) as well as for the intraoperative biopsies of the remaining allografts (A_1, B_1, C_1) (tabs. 4 and 5). Thus, cold ischaemia up to 32 hours had no influence on the catalytic activities of these enzymes.

When postoperative samples with acute renal failure were examined, the characteristic distribution pattern of succinate dehydrogenase activity apparently disappeared (fig. 1). Quantification of the changes of both enzymes is depicted in tables 4 and 5. When intraoperative values were taken as controls, anuria for 9 days (A_2) was found to be associated with a significantly decreased (by 68%) specific Na^+-K^+-

Tab. 2. Specific catalytic activities of Na^+-K^+-ATPase in single nephron segments of human kidneys (inorganic phosphate, $\mu mol \cdot min^{-1} \cdot g^{-1}$ dry weight). Values are given as the mean ± standard deviation for the number of determinations indicated in parentheses. Abbreviations: G = glomerulus, PCT = proximal convoluted tubule, PST = proximal straight tubule, cTAL and mTAL = cortical and medullary thick ascending limb of *Henle*'s loop, DCT = distal convoluted tubule, cCD and mCD = cortical and medullary collecting duct.

Segment	Kidney E (normal part of a kidney with hypernephroma)	Kidney-allograft D (functioning) Biopsy D_1 (intraoperative)	Biopsy D_2 (7 days after transplantation)	Biopsy D_3 (20 days after transplantation)
G	9.0 ± 6.2 (12)	16.2 ± 9.5 (7)	9.5 ± 11.8 (5)	11.5 ± 7.5 (5)
PCT	31.3 ± 13.8 (10)	27.8 ± 21.3 (8)	37.0 ± 24.2 (3)	34.2 ± 14.0 (11)
PST	31.5 ± 21.3 (10)	32.7 ± 16.0 (11)	30.2 ± 18.0 (9)	26.7 ± 13.5 (11)
mTAL	–	151.2 ± 62.7 (15)	142.8 ± 44.3 (9)	–
cTAL	161.3 ± 70.8 (15)	139.0 ± 60.3 (9)	125.3 ± 43.0 (9)	158.2 ± 71.0 (11)
DCT	195.8 ± 112.2 (7)	114.3 ± 49.2 (12)	128.2 ± 50.3 (8)	141.8 ± 82.5 (9)
cCD	67.2	31.7 ± 23.0 (8)	51.7 ± 23.7 (3)	47.8 ± 26.5 (8)
mCD	–	50.7 ± 30.2 (6)	38.8 ± 21.3 (8)	–

Tab. 3. Relative succinate dehydrogenase activities in single nephron segments of human kidneys ($A_{550\,segment} \cdot A^{-1}_{550\,cCD}$). Thirty absorbance readings per nephron segment were averaged to give the mean absorbance value ($A_{550\,segment}$) which was referred to that of the cortical collecting ducts ($A_{550\,cCD}$) from the same section. The data represent the mean ± standard deviation of this quotient obtained from the number of examined sections indicated in parentheses. In addition to table 2, the following abbreviations are used: cPST and mPST = cortical and medullary proximal straight tubule.

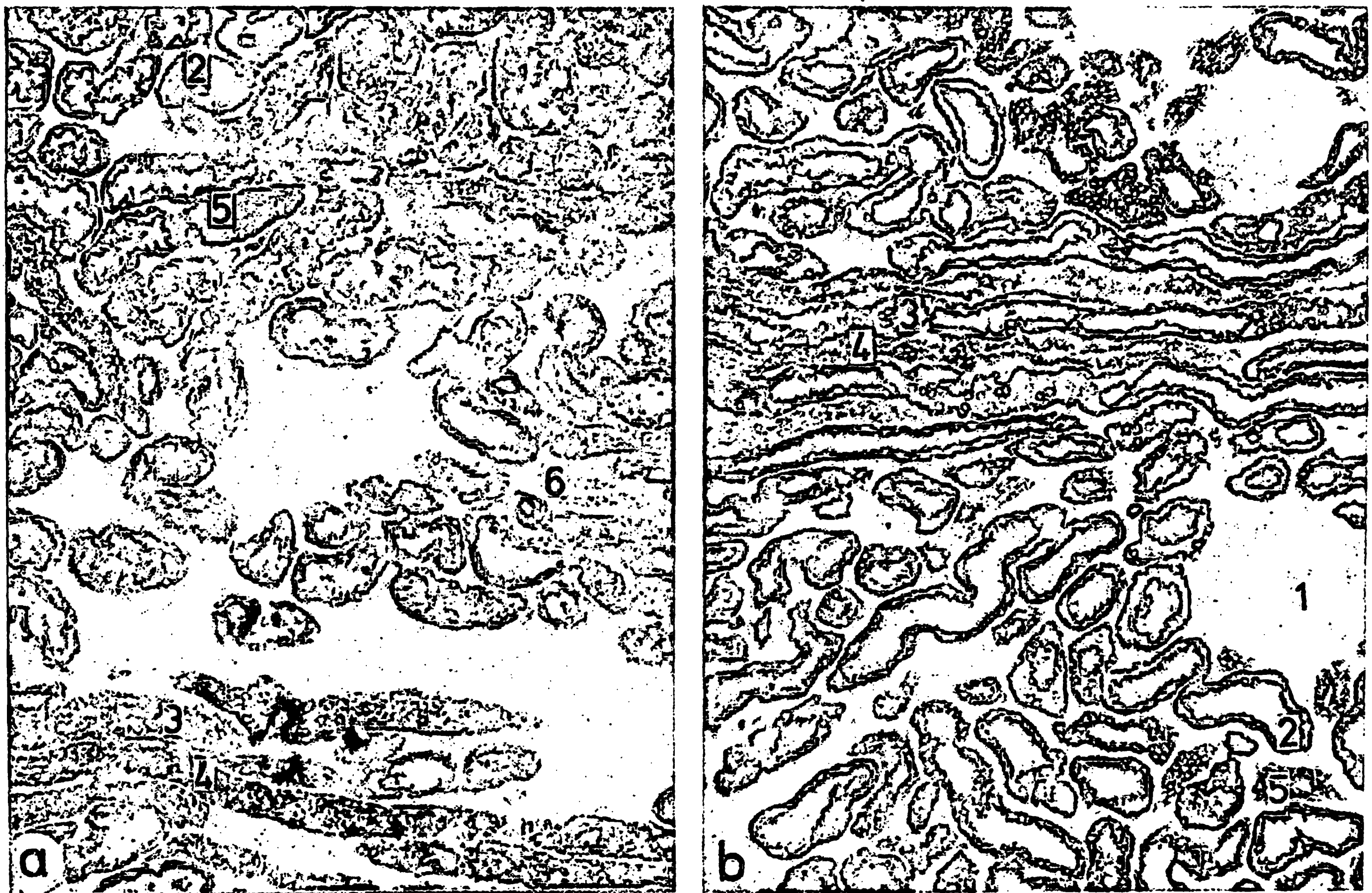
Segment	Kidney E (normal part of a kidney with hypernephroma)	Kidney-allograft D (functioning) Biopsy D_1 (intraoperative)	Biopsy D_2 (7 days after transplantation)	Biopsy D_3 (20 days after transplantation)
G	0.18 ± 0.02 (3)	0.15 ± 0.03 (5)	0.14 ± 0.02 (4)	0.15 ± 0.01 (2)
PCT	2.29 ± 0.30 (3)	2.22 ± 0.47 (6)	2.20 ± 0.12 (4)	2.34 ± 0.21 (2)
cPST	2.05 ± 0.20 (3)	2.30 ± 0.47 (5)	1.87 ± 0.15 (4)	2.26 ± 0.04 (2)
mPST	–	1.66 ± 0.04 (2)	1.58 ± 0.15 (2)	–
mTAL	–	3.17 ± 0.57 (3)	2.62 ± 0.11 (2)	–
cTAL	2.47 ± 0.39 (3)	2.97 ± 0.67 (5)	2.52 ± 0.23 (4)	2.80 ± 0.14 (2)
DCT	4.16 ± 0.76 (3)	3.91 ± 0.94 (4)	3.11 ± 0.27 (3)	3.20 ± 0.06 (2)
cCD	1.00	1.00	1.00	1.00
mCD	–	0.91 ± 0.07 (3)	0.91 ± 0.01 (2)	–

Tab. 4. Specific catalytic activities of Na^+-K^+-ATPase in single nephron segments of human kidney-allografts with acute insufficiency following transplantation (inorganic phosphate, $\mu mol \cdot min^{-1} \cdot g^{-1}$ dry weight). The data represent the mean ± standard deviation for the number of determinations given in parentheses. In addition to the abbreviations of table 2, the following indices for statistically significant differences are used: (a) = $p < 0.05$, (b) = $p < 0.01$, (c) = $p < 0.001$.

Segment	Kidney-allograft A Biopsy A_1 (intraoperative)	Biopsy A_2 (post-transplantation anuria for 9 days)	Kidney-allograft B Biopsy B_1 (intraoperative)	Biopsy B_2 (post-transplantation anuria for 14 days)	Kidney-allograft C Biopsy C_1 (intraoperative)	Biopsy C_2 (recovery from anuria, 24 days after transplantation)
G	12.7 ± 2.2 (4)	11.5 ± 9.7 (8)	8.5 ± 9.0 (5)	16.2 ± 7.5 (5)	8.7 ± 3.2 (6)	9.0 ± 7.5 (4)
PCT	37.3 ± 14.0 (9)	29.8 ± 20.2 (13)	15.3 ± 10.7 (8)	19.7 ± 19.2 (9)	23.8 ± 7.0 (6)	43.3 ± 20.8 (11) (a)
PST	23.7 ± 18.7 (5)	32.8 ± 12.3 (7)	28.8 ± 18.3 (13)	23.5 ± 17.8 (6)	31.0 ± 16.3 (8)	24.7 ± 12.2 (7)
mTAL	225.8 ± 64.5 (7)	72.7 ± 54.2 (9) (c)	221.7 ± 85.5 (5)	–	136.0 ± 46.5 (3)	–
cTAL	122.0 ± 52.5 (8)	73.5 ± 56.0 (11)	144.2 ± 52.3 (11)	83.0 ± 49.5 (14)	141.8 ± 62.0 (11)	107.7 ± 80.3 (18)
DCT	170.5 ± 82.0 (12)	139.0 ± 92.3 (14)	180.5 ± 65.3 (7)	110.0 ± 59.2 (17)	125.7 ± 52.0 (11)	95.2 ± 48.0 (13)
cCD	–	40.5 ± 21.2 (4)	65.2	38.3 ± 25.5 (10)	39.3 ± 17.5 (10)	41.5 ± 28.0 (8)
mCD	50.2 ± 32.5 (4)	45.8 ± 21.8 (5)	21.3 ± 20.0 (4)	–	–	–

Tab. 5. Relative succinate dehydrogenase activities in single nephron segments of human kidney-allografts with acute insufficiency following transplantation ($A_{550\,segment} \cdot A_{550\,cCD}^{-1}$). As in table 3, the mean absorbance values (A_{550}) originate from 30 readings per nephron segment, and the data represent the mean ± standard deviation of the mean absorbance in each segment relative to that in the cortical collecting ducts, with the number of examined sections in parentheses. In addition to the abbreviations of table 3, the following indices for statistically significant differences are used:
(a) = $p < 0.05$, (b) = $p < 0.02$, (c) = $p < 0.01$, (d) = $p < 0.001$.

Segment	Kidney-allograft A		Kidney-allograft B		Kidney-allograft C	
	Biopsy A_1 (intraoperative)	Biopsy A_2 (post-transplantation anuria for 9 days)	Biopsy B_1 (intraoperative)	Biopsy B_2 (post-transplantation anuria for 14 days)	Biopsy C_1 (intraoperative)	Biopsy C_2 (recovery from anuria, 24 days after transplantation)
G	0.14 ± 0.03 (3)	0.16 ± 0.01 (4)	0.20 ± 0.01 (3)	0.16 ± 0.01 (3) (c)	0.15 ± 0.02 (3)	0.21 ± 0.04 (3)
PCT	1.74 ± 0.32 (3)	1.73 ± 0.13 (4)	2.25 ± 0.15 (3)	1.93 ± 0.07 (3) (a)	1.79 ± 0.14 (3)	1.74 ± 0.32 (3)
cPST	1.72 ± 0.20 (3)	1.79 ± 0.12 (4)	2.24 ± 0.05 (3)	2.05 ± 0.06 (3) (b)	1.69 ± 0.14 (2)	1.58 ± 0.16 (3)
mPST	1.61 ± 0.19 (3)	1.49 ± 0.06 (2)	1.86 ± 0.21 (2)	–	1.31 ± 0.16 (2)	–
mTAL	2.44 ± 0.36 (3)	1.35 ± 0.02 (2) (b)	2.84 ± 0.35 (2)	–	2.16 ± 0.07 (2)	–
cTAL	2.05 ± 0.44 (3)	1.37 ± 0.07 (4) (a)	2.52 ± 0.25 (3)	1.00 ± 0.11 (3) (d)	1.84 ± 0.01 (2)	1.39 ± 0.14 (3) (b)
DCT	2.61 ± 0.51 (3)	1.52 ± 0.02 (3) (a)	3.24 ± 0.46 (3)	1.39 ± 0.07 (3) (c)	2.60 ± 0.20 (3)	1.78 ± 0.27 (3) (b)
cCD	1.00	1.00	1.00	1.00	1.00	1.00
mCD	0.87 ± 0.07 (3)	0.78 ± 0.01 (2)	0.84 ± 0.11 (2)	–	0.69 ± 0.01 (2)	–



a b

Fig. 1. Cryostat sections (16 μm thick) of kidney-allograft B stained for succinate dehydrogenase activity. Magnification × 100.
a = intraoperative biopsy (B_1) with highest enzymatic activity in thick ascending limbs of *Henle*'s loop and distal convoluted tubules;
b = biopsy after post-transplantation anuria for 14 days (B_2) with decreased enzymatic activity in thick ascending limbs of *Henle*'s loop and distal convoluted tubules, and increased interstitial volume.
1 = glomerulus, 2 = proximal convoluted tubule, 3 = proximal straight tubule, 4 = cortical thick ascending limb of *Henle*'s loop, 5 = distal convoluted tubule, 6 = cortical collecting duct.

ATPase activity ($p < 0.001$) in medullary thick ascending limbs of *Henle*'s loop, whereas relative succinate dehydrogenase activity was decreased to 55% ($p < 0.02$) in the medullary part, to 67% ($p < 0.05$) in the cortical part of these segments, and to 58% ($p < 0.05$) in distal convoluted tubules. This phenomenon was found to be even more pronounced in anuria persisting for 14 days (B_2). Specific catalytic Na^+-K^+-ATPase activities were reduced to 58% ($p < 0.01$) in cortical thick ascending limbs of *Henle*'s loop and to 61% ($p < 0.05$) in distal convoluted tubules. In this biopsy, relative succinate dehydrogenase activities were lowered to 40% ($p < 0.001$) in cortical thick ascending limbs of *Henle*'s loop and to 43% ($p < 0.01$) in distal convoluted tubules. Distal tubular decrease of specific Na^+-K^+-ATPase activity in acute renal failure is characterized by lowered total ATPase activity but unaltered ouabain-insensitive Mg^{2+}-ATPase activity. In contrast, glomeruli, proximal convoluted and straight tubules as well as collecting ducts appeared to be unaffected with respect to both enzyme activities. Functional recovery from anuria of kidney C, 24 days after transplantation (C_2), was manifested by insignificantly altered distal tubular Na^+-K^+-ATPase activities but slightly diminished relative succinate dehydrogenase activities, by 24% ($p < 0.02$) in cortical thick ascending limbs of *Henle*'s loop and by 32% ($p < 0.02$) in distal convoluted tubules. Interestingly, Na^+-K^+-ATPase activity was found to be higher in proximal convoluted tubules of this biopsy. Unfortunately, samples B_2 and C_2 did not contain medullary structures.

In summary, the characteristic distribution pattern of both enzymes appears to be altered in the advanced stage of human acute post-transplant renal failure in parallel with anuria and functional recovery, respectively.

Discussion

This is the first report of changes in enzyme activities in defined nephron segments during acute renal failure in man. Only two reports exist on Na^+-K^+-ATPase activity in human nephron (19, 23). The absolute activities and distribution pattern found in the present work are similar to those previously reported, and to those reported from animal studies (19). The distribution of succinate dehydrogenase activity recently described in human nephron (22) was confirmed in this study. It differs from that of the rabbit nephron (10) mainly by the finding of highest activity in distal convoluted tubules. This may indicate a species-dependent difference in the mitochondrial content of distal convoluted tubules.

The intraoperative biopsies of allografts A, B, C, and D displayed normal activities of Na^+-K^+-ATPase and of succinate dehydrogenase as compared with the unaffected part of hypernephroma-bearing kidney E, although allografts A, B, and C had developed post-transplantation anuria. This finding agrees with that of *Pfaller* (6) showing only slightly altered membrane areas of the basolateral interdigitations and mitochondrial cristae in the early phase of acute renal failure in rats induced by renal arterial clamping with subsequent blood reflow. This can be explained by the finding that, under normal metabolic conditions, rat kidney protein fractions containing Na^+-K^+-ATPase subunits and mitochondrial proteins have apparent half-life times in the range of 60–86 hours (24, 25). Therefore, changes in enzyme activity may be too small to be detected early after ischaemia.

The results obtained during the maintenance phase of human acute renal failure demonstrate that the total capacities of active salt reabsorption and succinate-dependent respiration appeared to be predominantly diminished in thick ascending limbs of *Henle*'s loop and distal convoluted tubules. These changes paralleled the period and degrce of dysfunction of the allografts, although they do not give evidence of the true transportive and energetic status of the renal cells.

In contrast to the present finding of unchanged proximal tubular enzyme catalytic activities in the advanced stage of acute renal insufficiency, several authors described severe functional and morphological alterations in proximal tubular cells in the early phase of experimentally induced ischaemic acute renal failure in animals (2, 5, 6, 9, 26). The failure to detect, if any, slight activity changes may be explained on a methodologic basis. Epithelial flattening due to proximal tubular brush border loss described in experimentally induced acute renal failure (2, 5, 6, 26) and in the human disease (7, 8) may obscure the detection of slight activity changes of both enzymes, because tissue dry weight and cellular volume, respectively, served as reference units. The increased Na^+-K^+-ATPase activity in proximal convoluted tubules of biopsy C_2 may be explained on this basis. Furthermore, while succinate dehydrogenase activity is clearly detectable, the low values of specific Na^+-K^+-ATPase activity in proximal tubules are near the detection limits, and are therefore an unreliable index of change. Analogously, the variations of analytical procedures may account for discrepancies between significant changes of these catalytic activities in distal tubules of biopsies A_2 and C_2. However, they might also reflect different rates of

enzyme degradation and regeneration, respectively, which become manifest in these stages of renal insufficiency.

In accordance with the present findings, early and late functional and morphological alterations of the distal tubules have been observed by various authors (2, 3, 6, 8, 9). The results of the present study are best comparable with the morphological analysis of acute renal failure of the rat following kidney transplantation, which results in slight changes in proximal tubules and severe changes in distal tubules (27, 28). The authors ascribed this specific pattern to normothermic ischaemia following insufficient blood reflow of medullary regions, the mechanism of which is unknown. This "no-reflow" phenomenon has also been observed after renal ischaemia due to temporal renal artery occlusion (29). Apart from the existence of this phenomenon, cold ischaemia and reperfusion of the transplant per se could induce cellular energy depletion, particularly of the medullary thick ascending limbs of *Henle*'s loop, as demonstrated by *Alcorn* et al. (30) in a normothermically perfused isolated kidney preparation. The measurement of the redox state of cytochrome aa_3 in such a preparation gave evidence of near-hypoxic conditions at this site of the nephron (31) due to circulatory conditions (32) and high transport activity (33). The present finding that the medullary thick ascending limbs of *Henle*'s loop of biopsy A_2 are most affected with regard to both enzymes supports this interpretation.

Despite this interpretation, the difference in the severity of the effect on medullary proximal straight tubules and collecting ducts on the one side and thick ascending limbs of *Henle*'s loop on the other side remains unexplained. In the cortical labyrinth and medullary rays, the same discrepancy exists for proximal tubules, collecting ducts and distal tubules. The special vulnerability of distal tubules found in the present study suggests that distinct metabolic characteristics of the different nephron segments (10) may contribute to this pattern of tubular alterations. The high glycolytic capacity of collecting ducts (18, 34) poorly equipped with mitochondria appears to protect them against ischaemic insults, as demonstrated by unchanged Na^+-K^+-ATPase and succinate dehydrogenase activities. On the other hand, the capacity for anaerobic glucose utilization cannot account for the different viability of proximal and distal tubular cells which are both highly dependent on oxidative energy (10). The high glycolytic capacity of distal tubules (18, 34, 35) seems too low for maintaining functional and structural integrity of the cells.

In conclusion, biochemical changes in human acute post-transplant renal failure can be correlated with some of the known morphological and pathophysiological findings. Thus, they may form the basis for a future pathobiochemical interpretation of acute renal failure. However, it remains to be investigated, whether the profile change of Na^+-K^+-ATPase and succinate dehydrogenase activities found in the maintenance phase of human acute post-transplant renal failure is a general phenomenon of ischaemia-induced renal insufficiency in man.

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Dr. Heide Schmid
Pathologisches Institut der Universität
Liebermeisterstraße 8
D-7400 Tübingen