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Short-Term Perfusion and "Equilibration" of Canine Kidneys with Protective Solutions*

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Summary. Kidneys were perfused either with Euro-Collins-solution or with HTK-solution of Bretschneider. The perfusion pressure as well as the perfusion flow were measured during a six-minute perfusion. The perfusion resistance was higher in Euro-Collins-kidneys than during HTK-perfusion. The venous outflow of the kidney as well as the ureteral outflow was measured during each minute of the perfusion and has analysed for osmolality, and for sodium and potassium concentrations. In Euro-Collins-kidneys a complete "equilibration" of the extracellular space was not achieved, while during HTK-perfusion concentrations in the venous as in the tubular outflow, similar to those in the HTK-solution itself, could be reached. At the end of the different perfusions, tissue was analysed for biochemical parameters such as ATP, ADP, AMP and lactate as well as for morphological features. Lactate had increased and ATP had decreased during perfusion with Euro-Collins-solution, while ATP had not changed and lactate had decreased during perfusion with HTK-solution. Normal glomerular, tubular and dilated vascular structures can be seen after HTK-perfusion, while a glomerular and vascular contraction takes place during Euro-Collins-perfusion.

Key words: Kidney perfusion, Equilibration, HTK-solution, Euro-Collins-solution.

Introduction

Apart from the controversy whether to perfuse kidneys continuously [2, 11] or to "flush" kidneys prior to hypothermic storage with a protective solution such as the Euro-Collins- [9] or the Sacks-solution [39], it is not clear how much solution should be used for flushing, or for an initial short-term perfusion of kidneys to "equilibrate" the extracellular space of the kidney with the perfusate. Therefore, we perfused dog kidneys either with Euro-Collins- [9] or with HTK-solution of Bretschneider [5–7, 23, 25, 40] to study both the venous outflow and also the ureteral outflow, which we term "Kunstharn"-diuresis.

Only a complete "equilibration" of the extracellular space of the kidney, including the vascular *and* the tubular system, is able to fill the advantageously large extracellular space. Only by a complete equilibration of the whole extracellular space of the kidney, will the desired protective principles of buffering [5, 22], and "intracellular like"-composition [1, 8, 9, 13, 32, 39, 45], be realised. Therefore only by specification of how much solution should be used, can the protective ability of different solutions be compared.

The aim of this study was to perfuse kidneys for 6 min with the Euro-Collins-solution or with the HTK-solution and to measure the effects of perfusion, both during and after the 6 minute period with special regard to biochemical analyses (ATP, ADP, AMP, lactate) and also with regard to morphological features.

Materials and Methods

Experiments were performed on 49 kidneys of mongrel dogs of either sexes with a median body weight of 32 kg. After premedication with 90 mg piritramide[1] and 0.5 mg atrophine[2] by intramuscular injection, anaesthesia was induced after 30 min with 10–15 mg/kg body weight sodium thiopental[3] and continued with a combination of halothane[4] (0.4–1.5 Vol%) and N_2O/O_2 (80 : 20). Respiration was maintained via an endotracheal tube with a Dräger respirator AV1[5], with an end-expiratory CO_2 value of about 5.5% measured

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1 Dipidolor; Janssen GmbH, Düsseldorf, FRG
2 Atropinsulfat Drobena; Drobena Arzneimittel GmbH, Berlin, FRG
3 Trapanal; Byk Gulden, Konstanz, FRG
4 Halothan Hoechst, Hoechst AG, Frankfurt am Main, FRG
5 Dräger Respirator AV1, Dräger Werke, Lübeck, FRG

Table 1. Composition of kidney protective solutions used

	Euro-Collins-solution	HTK-solution of Bretschneider
Na^+	10	15
K^+	115	10
Ca^{++}	–	–
Mg^{++}	–	4
Cl^-	15	50
HCO_3^-	10	–
HPO_4^{--}	43	–
$H_2PO_4^-$	15	–
Glucose	198	–
Mannitol	–	30
Buffer	Bikarbonate, Phosphate	Histidine, Histidine-HCl
Osmolarity: calculated	406 mosmol/l	310 mosmol/l
pH	7.3 (8 °C)	7.3 (8 °C)
pO_2	~100 mmHg (37 °C)	~200 mmHg (37 °C)

(all concentrations are expressed as mmol/l)

continuously with a Datex CO_2[6] analyser. Fluid balance was maintained by 500–1,000 ml Tutofusin[7] and 500 ml glucose 5%[8] until kidney perfusion with the Euro-Collins-[9] or with the HTK-solution of Bretschneider[10] was started. The dogs received 1.250 I.U. heparin[11] about 20 min before perfusion. The arterial blood pressure was continuously monitored with a statham element (P 23 ID)[12] and an amplifier[13] via a catheter lying in the brachialis artery and was maintained around RR 120/80 mmHg at a heart rate of about 80/min. Arterial blood samples were taken through this catheter for controlling the acid-base-[14] and the Na^+/K^+-status[15] of the animal. During operation standard leads were used to record ECG[16].

After median laparotomy the kidneys were freed from the peritoneum and the v. cava inferioris and the aorta abdominalis were dissected proximal and distal to the origin of the left and right vv. renales and aa. renales. A kidney perfusion catheter (described in detail in ref. [27]) was inserted into the aorta abdominalis distal to the origins of the aa. iliaca communes and was threaded into the renal artery with a perfusion flow of 100 ml/min of the HTK-solution, *cooled to about 8 °C*, already being delivered by a peristaltic pump[17], and fixed in this position by a tourniquet. Immediately afterwards perfusion flow was raised to about 400–500 ml/min x 100 g_{ww} (for details see: Results, Fig. 1) and the renal vein was incised and clamped close to the v. cava within about 40 s to allow the perfusate to escape. If the kidneys were perfused with the Euro-Collins-solution, the perfusion flow was only raised after the venous incision because of its high potassium content (115 mmol/l) (Table 1) and the consequent danger of cardiac arrest. After 6 min of perfusion with the HTK- or with the Euro-Collins-solution the kidneys were excised within a minute and weighed after decapsulation. Tissue samples consisting of about 2/3 cortex and 1/3 medulla were taken immediately after perfusion, homogenized[18] in cold perchloric acid for determination of ATP[19], ADP[20] and AMP[20] as well as lactate [18]. For morphological features we used our perfusion fixation method described recently [24].

During perfusion of the kidneys perfusion pressure was measured at the tip of the catheter by a statham element (P 23 ID) connected to an amplifier. The flow was measured by calibrating the pump prior to and after perfusion and the perfusion resistance was calculated from the perfusion pressure and the perfusion volume. Samples of the perfusion fluid were taken from the tube[21] leading to the kidney, so-called "arterial" samples, and samples of the venous outflow were taken every minute of perfusion via a catheter in the renal vein and a glass syringe. Via the ureteric catheter, urine and so-called "Kunstharn" was collected during every minute of perfusion. The "arterial" and venous samples were analysed for Na^+, K^+ and for PO_2 (at 37 °C), from which by Fick's principal oxygen consumption could be calculated. Osmolality[22], Na^+ and K^+ were measured in the urine as well as urine volume.

Results

The arterial blood pressure was 100 mmHg +/– 10 before kidney perfusion with the Euro-Collins-solution. The renal blood flow was 450 ml/min x 100 g_{ww}. Therefore the organ resistance was 0.21 mmHg/ml/min x 100 g_{ww}. After one minute of perfusion the perfusion pressure rose to 130 mmHg, although the perfusion flow was reduced to 300 ml/min x 100 g_{ww}. The calculated perfusion resistance thereby climbed to 0.55 mmHg/ml/min x 100 g_{ww}. From the second minute of perfusion, the perfusion pressure fell to 110 mmHg with a constant flow of 300 ml/min x 100 g_{ww}. The perfusion resistance therefore was 0.4 mmHg/ml/min x 100 g_{ww} (Fig. 1, at left). Before HTK-perfusion, the arterial blood pressure was 94 +/– 2 mmHg, the renal blood flow was 440 ml/min x 100 g_{ww} and the resistance was 0.21 mmHg/ml/min x 100 g_{ww}. The perfusion pressure was in the first minute of HTK-perfusion 80 mmHg and the flow was 480 mmHg/ml/min x 100 g_{ww}. The perfusion resistance therefore was in the first minute 0.19 mmHg/ml/min x 100 g_{ww}. From the second minute on the perfusion pressure was about 115 mmHg. The flow was reduced to 400 ml/min x 100 g_{ww} in the second and to 360 ml/min x 100 g_{ww} in the third minute. Thereafter a constant flow was reached. The perfusion resistance was therefore 0.3 mmHg/ml/min x 100 g_{ww} during the last 3 min of perfusion (Fig. 1, at right).

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6 Datex Instrumentation OY; Espoo, Finland

7 Tutofusion; Pfrimmer & Co. GmbH, Erlangen, FRG

8 Glucose 5% Braun; B. Braun Melsungen AG, Melsungen, FRG

9 Euro-Collins-Lösung; Dr. E. Fresenius, Bad Homburg v.d.H., FRG

10 Kardioplegische Lösung HTK nach Bretschneider; Dr. Franz Köhler Chemie GmbH, Alsbach, FRG

11 Heparin-Natrium Braun 2.500 I.E./5 ml; B. Braun Melsungen AG, Melsungen, FRG

12 P 23 ID; Gould Statham Instr., Oxnard, USA

13 Vorverstärker Druck, Hellige GmbH, Freiburg im Breisgau, FRG

14 Corning Säure-Basen Analysator 352, Corning GmbH, Gießen, FRG

15 Na/K-Ionenanalyser 914, Corning GmbH, Gießen, FRG

16 Vorverstärker EKG, Hellige GmbH, Freiburg im Breisgau, FRG

17 Doppelpumpe 10200: Stöckert Inst., München, FRG

18 Ultra-Turax, Janke & Kunkel GmbH & Co. KG, LKA-Werk, Staufen, FRG

19 Testkombination ATP, Boehringer Mannheim GmbH, Mannheim, FRG

20 Testkombination ADP/AMP, Boehringer Mannheim GmbH, Mannheim, FRG

21 Tygon R3603, Labomatic GmbH, Sinsheim, FRG

22 Osmometer OM 801 Vogel, Gießen, FRG

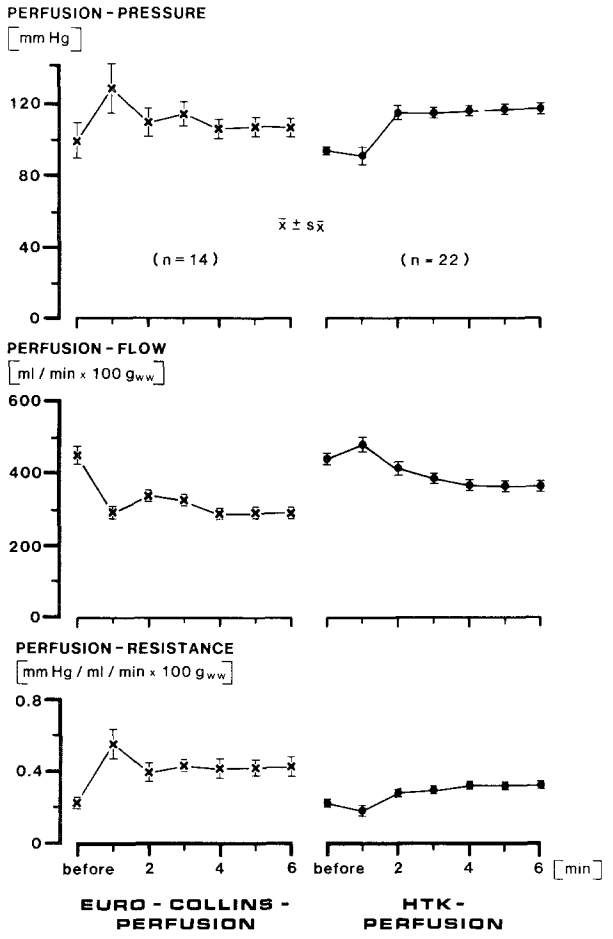


Fig. 1. Perfusion pressure, flow and resistance before and during kidney perfusion for 6 min either with Euro-Collins-solution (*left side*) or with HTK-solution (*right side*)

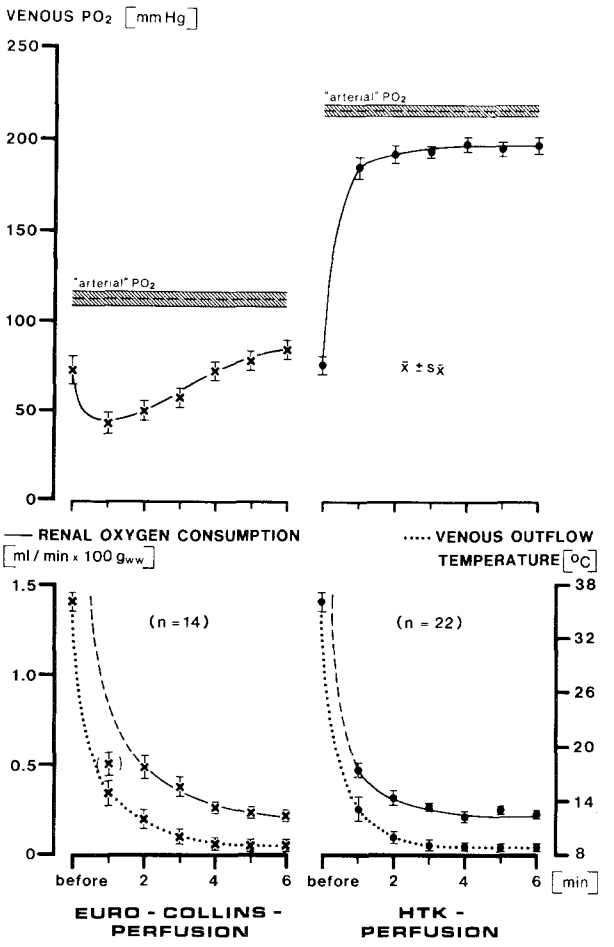


Fig. 2. "Arterial" and renal venous oxygen partial pressure (pO_2, measured at 37 °C), renal oxygen consumption and venous outflow temperature before and during kidney perfusion for 6 min either with Euro-Collins-solution (*left side*) or with HTK-solution (*right side*)

The oxygen partial pressure (pO_2, measured at 37 °C) was 110 mmHg in the Euro-Collins-solution. The venous pO_2 before perfusion with the Euro-Collins-solution was about 70 mmHg. After one minute of perfusion the venous pO_2 fell to 45 mmHg, and to 27 mmHg at about 10 °C, and then went up to 85 mmHg during the six minutes of perfusion (Fig. 2, left, upper panel). The renal oxygen consumption was calculated by the a-v O_2-content-difference and the perfusion flow (ml/min x 100 g_{ww}) (Fig. 1). Before kidney perfusion the oxygen consumption was between 5 and 6 ml/min x 100 g_{ww}. In the second minute of perfusion it was 0.5 ml O_2/min x 100 g_{ww} and fell to 0.2 ml/min x 100 g_{ww} (Fig. 1, left, lower panel). In the HTK-solution the pO_2 was between 210–220 mmHg (measured at 37 °C). Before perfusion of the kidney, the venous pO_2 was 75 mmHg and rose to 185 mmHg in the first minute of HTK-perfusion. During the second and the sixth minute of perfusion the venous pO_2 was between 190–200 mmHg. The renal oxygen-consumption was in the second minute of perfusion about 0.3 ml/min x 100 g_{ww} and from the fourth minute between 0.20 and 0.25 ml/min x 100 g_{ww} (Fig. 2, at right).

The osmolality of the Euro-Collins-solution was 350–360 mosmol/kg H_2O. Before perfusion the osmolality in urine was 450 mosmol/kg H_2O, which fell to 380 mosmol/kg H_2O within two minutes of perfusion and reached the same osmolality as the Euro-Collins-solution. The diuresis was between 2 and 3 ml/min x 100 g_{ww} before perfusion with Euro-Collins-solution. After 2 min of perfusion the diuresis was about 5.5 ml/min x 100 g_{ww} and reached values of 8–9 ml/min x 100 g_{ww} (Fig. 3, at left). The osmolality of the HTK-solution was between 290 and 300 mosmol/kg H_2O. Before perfusion the osmolality of urine was 310 mosmol/kg H_2O, which fell to 280 mosmol/kg H_2O within two minutes of perfusion and than rose to about 295 mosmol/kg H_2O similar to the value in the HTK-solution.

The diuresis was 5 ml/min x 100 g_{ww} before HTK-perfusion and the diuresis, one minute after perfusion was

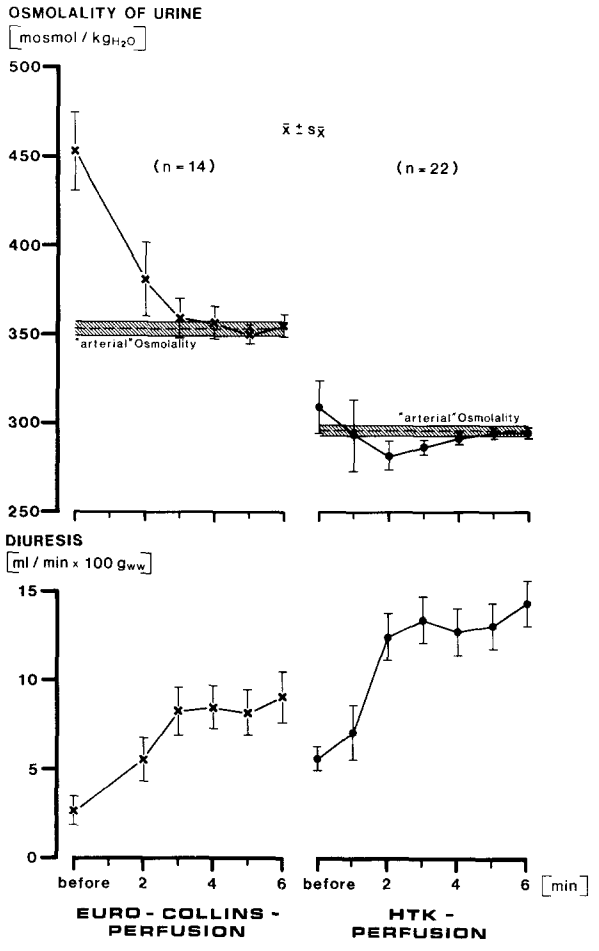


Fig. 3. "Arterial" and perfusion-urine osmolality and volume of the urine ("Kunstharn"-diuresis) before and during kidney perfusion for 6 min either with Euro-Collins-solution (*left side*) or with HTK-solution (*right side*)

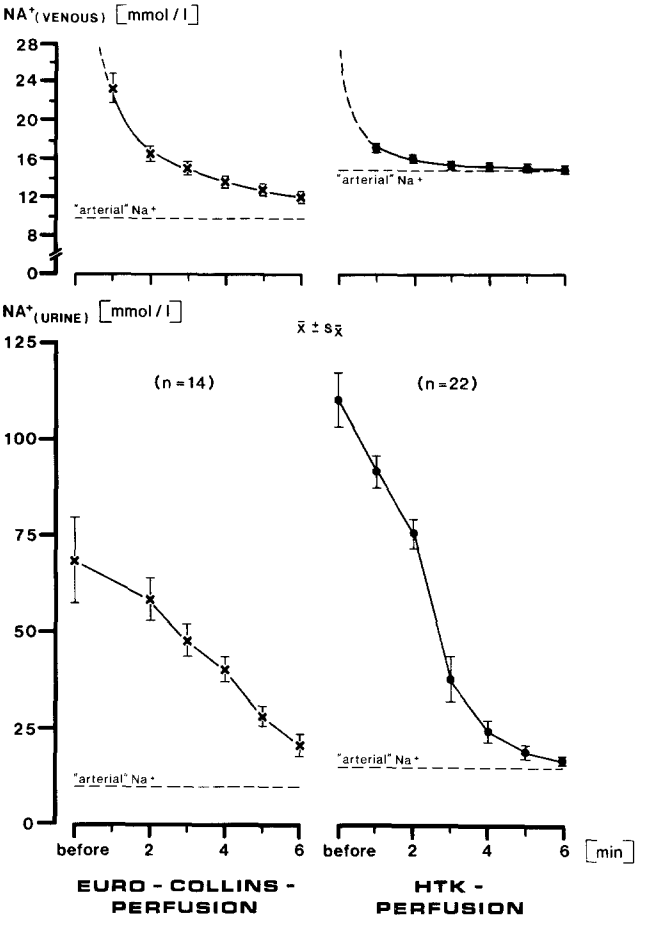


Fig. 4. "Arterial", renal venous and urine sodium concentration before and during kidney perfusion for 6 min either with Euro-Collins-solution (*left side*) or with HTK-solution (*right side*)

7 ml/min x 100 g_{ww} and thereafter increased to 12 and 14 ml/min x 100 g_{ww} (Fig. 3, right half).

The Na^+-concentration in the Euro-Collins-solution was 10 mmol/l. The venous Na^+-concentration before Euro-Collins-perfusion was 145 mmol/l and was 23 mmol/l after one minute of kidney perfusion with the solution. After two minutes the sodium-concentration goes down to about 16 mmol/l and reached values of 12 mmol/l within 6 min of perfusion.

The sodium concentration in the urine before perfusion was 70 mmol/l. After 2 min of perfusion with the Euro-Collins-solution the sodium concentration was 60 mmol/l and after 6 min of perfusion 20 mmol/l, thus two times higher than in the Euro-Collins-solution (Fig. 4, at left).

In the HTK-solution the sodium concentration was 15 mmol/l. After one minute of perfusion it was about 17 mmol/l rising to 15.2 mmol/l after perfusion for 6 min. The initial urinary sodium concentration was 110 mmol/l and was reduced to the "arterial" value of 15 mmol/l within 6 min.

The potassium concentration of the Euro-Collins-solution was 110 mmol/l, while in the HTK-solution it was 10 mmol/l. The renal venous K^+-concentration prior to perfusion was 3 mmol/l and increased to nearly 100 mmol/l within one minute of kidney perfusion with the Euro-Collins-solution. After 6 min the venous potassium concentration was similar to the potassium concentration in the Euro-Collins-solution (Fig. 5, upper left). The potassium concentration in the urine prior to perfusion was 40 mmol/l. It increased to 90 mmol/l within 6 minutes of perfusion (Fig. 5, at left).

The venous potassium concentration was 3 mmol/l before kidney perfusion with HTK-solution and was "equilibrated" within one minute of perfusion. The potassium concentration in the urine prior to perfusion was 24 mmol/l. After one minute of perfusion it reached an average of 26 mmol/l. After 3 min the perfusion "urine" was "equilibrated" with the potassium concentration of the HTK-solution (Fig. 5, right half).

The renal ATP-content was 10 $\mu mol/g_{dw}$, the ADP-content was 4 $\mu mol/g_{dw}$ and the AMP-content was 1.5

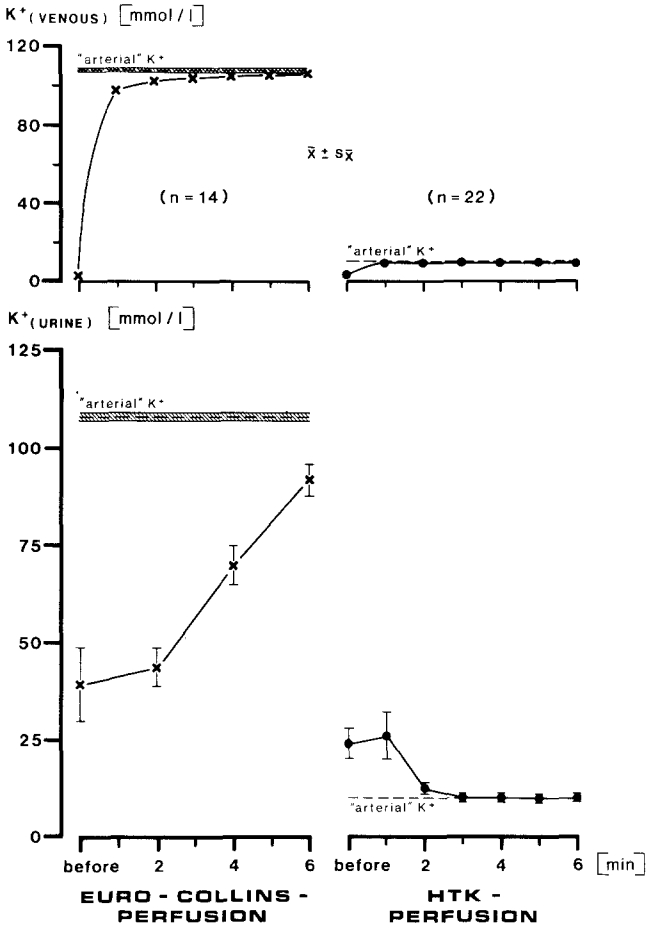
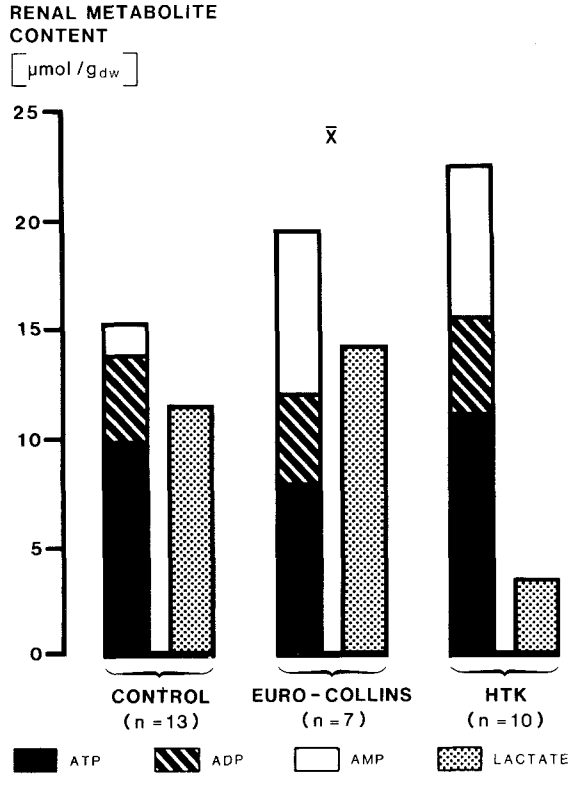


Fig. 5. "Arterial", renal venous and urine potassium concentration before and during kidney perfusion for 6 min either with Euro-Collins-solution (*left side*) or with HTK-solution (*right side*)



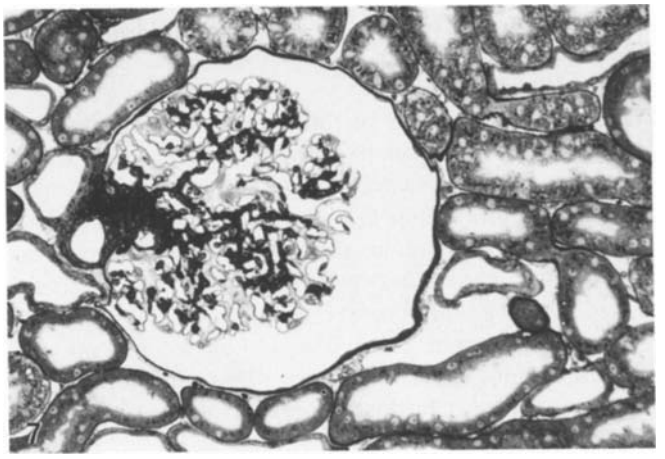


Fig. 7. Experiment 351, left kidney, silver impregnation (Movat) after perfusion fixation [24] immediately after perfusion for 6 min with Euro-Collins-solution; renal cortex: glomerules with retracted tuft of capillaries and enlarged glomerular capsule; focal vacuolation of the proximal tubulus ephithelia with partial loss of brush border. x 210

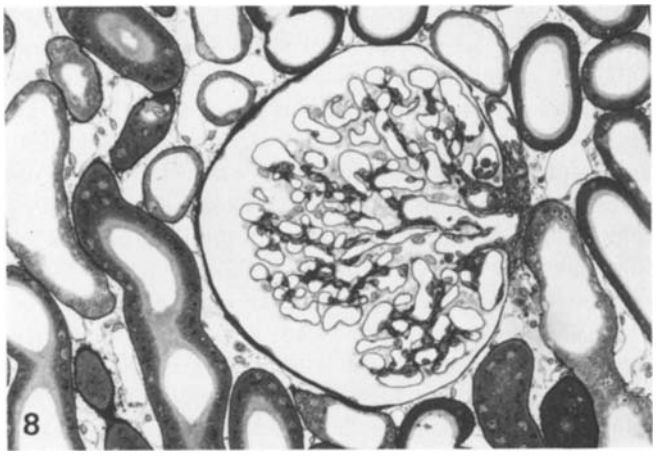


Fig. 8. Experiment 320, left kidney, silver impregnation (Movat) after perfusion fixation [24] immediately after protective perfusion for 6 min with HTK-solution; renal cortex: glomerulum with well unfolded tuft of capillaries. Proximal tubuli with homogenous epithelia and intact brush border. x 210

μmol/g_{dw} with anaesthesia at normothermia. The *T*otal-*A*denine-*N*ucleotides (TAN) were therefore about 15 μmol/g_{dw}. The renal lactate content was 11–12 μmol/g_{dw}. After perfusion with the Euro-Collins-solution the ATP-content averaged 8 μmol/g_{dw}, the ADP-content was 4 μmol/g_{dw} and the AMP-content was 7.5 μmol/g_{dw}. The TAN therefore was 19.5 μmol/g_{dw}. The renal lactate content was 14 μmol/g_{dw} after Euro-Collins-perfusion. The ATP-content in HTK-perfused kidneys was 11 μmol/g_{dw}, the ADP-content was 4.5 μmol/g_{dw} and the AMP-content

◀ Fig. 6. Renal content of ATP, ADP, AMP and lactate in control kidneys and in kidneys perfused for 6 min with Euro-Collins-solution or with HTK-solution

was 7 μmol/g_{dw}. The TAN was therefore 22 μmol/g_{dw}. The lactate content was 3.5 μmol/g_{dw} in HTK-perfused kidneys (Fig. 6).

Kidneys, which were perfusion-fixed immediately after the Euro-Collins-perfusion showed by light microscopy arterial contraction and a retraction of glomerular capillaries with an enlarged volume between the capsule and the capillaries. The proximal tubule demonstrated flat cells with an intact brush border beside enlarged cells with vacuoles and a loss of the brush border (Fig. 7).

The HTK-perfused kidneys showed dilated afferent and efferent arterioles, free capillaries within the glomerulum and intact and widely dilated proximal and distal tubules (Fig. 8).

Discussion

Short-term perfusion of kidneys with subsequent hypothermic storage is a simple technique for kidney preservation. Nevertheless, initial protective perfusion of kidneys is not standardized. The duration and volume of perfusion vary. Different protective solutions rely on different principles for optimum effect [9]. This is the reason for different and not totally comparable results of experimental or clinical kidney preservation. Therefore, we examined the perfusion of canine kidneys on the one hand with the Euro-Collins-solution and on the other hand with the HTK-solution of Bretschneider. The composition of the solutions differs with respect to potassium concentration by a factor of 11. There are differences in principle with respect to the buffer used, its concentration and with respect to the osmotic source, i.e. glucose or histidine. The composition of both solutions used is similar with respect to sodium-concentration and both solutions are calcium-free.

The myocardium possesses, in contrast to the kidney, myoglobin as an oxygen store and possesses as a further energy store phosphocreatine [3–5, 14, 16, 43]. While myoglobin covers oxygen demand at normothermia for 5–10 s, phosphocreatine is able to maintain energy supply for 1–3 min. Thus the myorcadium, can resist a short-term lack of oxygen, while the kidney, which possesses no oxygen store beside physically dissolved oxygen and no phosphocreatine, is vulnerable to short-term hypoxia which leads to a decrease of ATP [3, 14, 43]. Therefore an effective kidney protective method *has to allow* high perfusion rates, ab initio without generating cardiac problems. A potassium concentration of more than 110 mmol/l as in the Euro-Collins- or the Sacks-solution is in our opinion not usuable for in situ protection of kidneys. Further, it is also probably not optimal for transplantation of kidneys, as the high potassium concentration leads to a higher perfusion resistance (Fig. 1) possibly by a contraction of glomerular capillaries (Fig. 7). Only after the second and third minute of perfusion is a venous pO_2 outflow from the kidney of more than 50 mmHg reached. Therefore a critical low oxygen partial pressure in the tissue of the kidney may have existed during the first two to three minutes of perfusion leading to a reduction of ATP and an inrease of lactate (Fig. 2 and 6). Using HTK-solution as perfusate, perfusion resistance is not changed during introduction of the perfusate.

Prevention of smooth muscle contraction is in principle possible by Na^+- and Ca^{++}-withdrawal. But an increase of potassium to 115 mmol/l as in the Euro-Collins-solution leads to a long duration of depolarisation [12]. During further perfusion, the Na^+-reduction to 10 mmol/l and the Ca^{++}-withdrawal by the Euro-Collins-solution became effective, by cancelling excitation contraction coupling. Probably the perfusion resistance was thereby reduced to 0.4 mmHg/ml/min x 100 g_{ww} (Fig. 1).

The renal oxygen consumption, which depends mainly on Na^+-resorption [10], was also reduced to 0.2 ml/min x 100 g_{ww}. This value is very similar to values published by Levy [34], although in his experiments kidneys were perfused by blood, with a higher sodium concentration. Probably all oxygen-consuming transport processes [15–17, 21, 35, 36, 41], mainly Na^+-reabsorption [10] cease below 10 °C [34, 37], thus only oxygen consumption necessary for maintaining structure can be measured.

By reducing or nearly preventing Na^+-reabsorption, by Euro-Collins as well as by HTK-solution the concentration gradients [19, 20, 29, 30, 31, 44, 46] existing normally from kidney cortex to medulla, were washed out. The osmolality in the perfusion urine became similar to the osmolality of the respective protective solution during perfusion so that by a complete "equilibration" of the extracellular space of the kidney an abolition of the countercurrent system of the kidney took place, by reduced Na^+-reabsorption and by washing out osmotic gradients. During HTK-perfusion the sodium-concentration was "equilibrated" in the venous outflow within 3–4 min and in the tubular-collecting-duct system within 6 min under optimal conditions before and during perfusion, i.e. high preperfusion diureses [26] and a sufficiently high perfusion pressure was [27] followed by a high perfusion diuresis. Urea, necessary for the concentration effect of the countercurrent multiplication system [33], was washed out within 4 min of HTK-perfusion (not shown).

Using the advantageously large extracellular space of the kidney the vascular system must be equilibrated with the protective solution, and in addition the perfusate must also equilibrate with the ramified tubular and collecting duct system of the kidney. For example, a buffered solution can protect tubulus cells against damaging acidosis [22] from the luminal side as well as from the vascular side. Therefore, sufficient perfusion diuresis has to be attained, which is possible at a perfusion pressure of 80–100 mmHg. During HTK-perfusion, a perfusion diuresis of about 13 ml/min x 100 g_{ww} was reached, corresponding to about 56 l for 300 g human kidneys over 24 h. These are values which can be reached in the absence of colloids in the HTK-solution, as the net utrafiltration pressure is doubled.

According to results of Kehrer et al. [26], the amount of perfusion diuresis depends on preperfusion diuresis and the higher the preperfusion diuresis and the lower the preperfusion osmolality in the urine, the lower is the perfusion resistance on kidney perfusion with the HTK-solution [28]. Whether this is also correct for perfusion of kidneys with the Euro-Collins-solution with an osmolality of about 340–360 mosmol/kg H_2O is uncertain [38, 42, 45]. A furosemide dose of 0.1 mg/kg body weight is adequate to induce preperfusion diuresis of about 5 ml/min x 100 g_{ww} under these reported circumstances of anesthesia and hydration. Therefore, prior to a protective perfusion with the HTK-solution, concentration gradients were almost washed out by a preperfusion diuresis. Nevertheless, sodium reabsorption is supposed to be not completely inhibited prior to protective perfusion and therefore the decrease of osmolality in the perfusion urine during the second and third minute of perfusion with the HTK-solution (Fig. 3, at right) might have been a sign of a washout of the fluid from the ascending part of Henle's loop, and not of histidine reabsorption [17]. During further perfusion nearly all transport processes [15] cease due to cooling the kidney below 10 °C and also due to the electrolyte composition of the solution, thus resulting in an equilibrium of osmolality, sodium and potassium concentration of the venous and ureteral outflow with the HTK-solution.

During kidney perfusion with Euro-Collins-solution, an equilibrium with the osmolality of the Euro-Collins-solution occurred, but the sodium as well as the potassium concentration in the ureteral outflow was not equilibrated to the concentration of the respective electrolyte in the solution. If total osmolality was in equilibrium with the Euro-Collins-solution, but Na^+ and K^+ were not, another ingredient of the solution would have to be reabsorbed or rediffused. The highest concentration of such another substance was glucose with nearly 200 mmol/l. As we have shown [22], the glucose content of dog kidneys, perfused with Euro-Collins-solution, was about 800 $\mu mol/g_{dw}$, i.e. 16 times higher than in unperfused kidneys.

Kidneys contain according to Gerlach [14], Bergström [3] and Thorn [43], 8–12 $\mu mol/g_{dw}$ ATP and about 5–10 $\mu mol/g_{dw}$ lactate. These values are comparable to our results given in Fig. 6 as control values. At the end of perfusion of kidneys with the Euro-Collins-solution, ATP changed little to about 8 $\mu mol/g_{dw}$, ADP was similar to control, values AMP increased to about 7.5 $\mu mol/g_{dw}$ and lactate rose to about 11.5 $\mu mol/g_{dw}$. This increase of lactate and the small decrease in ATP may have been related to the low oxygen pressure of about 100 mmHg at 37 °C in the Euro-Collins-solution, which was 60 mmHg pO_2 at 10 °C because of the changed solubility of oxygen in the solution. The venous oxygen pressure, reached in the first and second minute of protective perfusion, was only 25 to 30 mmHg at 10 °C. The other possible explanation was, that energy turnover was stimulated during the first to third minute of perfusion by the potassium and the glucose content of the solution.

The ATP-content of HTK-protected kidneys was 11 μmol g_{dw}, ADP was not changed relative to control, AMP was 7 $\mu mol/g_{dw}$, thus TAN was about 22 $\mu mol/g_{dw}$. Lactate content decreased to 3.5 $\mu mol/g_{dw}$. The oxygen consumption at the end of protective perfusion was about 0.2 ml/min x 100 g_{ww} (Fig. 2). Thus the energy turnover at the end of "nephroplegia" was decreased similar to the effect of Euro-Collins-solution. As the HTK-solution allowed higher perfusion rates at the beginning of protective perfusion and contained an oxygen pressure of about 200 mmHg, there was a complete transfer from an *aerobic-blood* perfusion to an *aerobic-protective* perfusion with HTK-solution, which led to an optimal preparation at the beginning of ischemic stress.

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References

1. Andrews PM, Coffey AK (1982) Factors that improve the preservation of nephron morphology during cold storage. Lab Invest 46:100–120
2. Belzer FO, Ashby BS, Dunphy JE (1967) Twenty-four hour and 72-hour preservation of canine kidneys. Lancet II:536
3. Bergstrom J, Collste H, Groth C, Hultman E, Melin B (1971) Water, electrolyte and metabolite content in cortical tissue from dog kidneys preserved by hypothermia. Proc Eur Dial Transplant Assoc VIII:313–321
4. Bretschneider HJ (1964) Überlebenszeit und Wiederbelebungszeit des Herzens bei Normo- und Hypothermie. Verh Dtsch Ges Herz-Kreislaufforsch 30:11–34
5. Bretschneider HJ, Hübner G, Knoll D, Lohr B, Nordbeck H, Spieckermann PG (1975) Myocardial resistance and tolerance to ischemia: Physiological and biochemical basis. J Cardiovasc Surg 16:241–260
6. Bretschneider HJ (1980) Myocardial protection. Thorac Cardiovasc Surg 28:295–302
7. Bretschneider HJ, Gebhard MM, Preusse CJ (1984) Cardioplegia. Principles and problems. In: Sperelakis N (ed) Physiology and pathophysiology of the heart. Martinus Nijhoff, Boston, pp 605–616
8. Coffey AK, Andrews PM (1983) Ultrastructure of kidney preservation: varying the amount of an effective osmotic agent in isotonic and hypertonic preservation solution. Transplantation 35:136–143
9. Collins GM, Bravo-Shugarman M, Terasaki PD (1969) Kidney preservation for transportation. Lancet II:1219–1222
10. Deetjen P, Kramer K (1961) Die Abhängigkeit des O_2-Verbrauches der Niere von der Na^+-Rückresorption. Pfluegers Arch 273:636–650
11. Downes G, Hoffmann R, Huang J, Belzer FO (1973) Mechanism of action of washout solutions for kidney preservation. Transplantation 16:46–53
12. Fleckenstein-Grün G, Fleckenstein A (1980) Calcium-Antagonismus, ein Grundprinzip der Vasodilatation. In: Fleckenstein

A, Roskamm H (Hrsg), Calcium-Antagonismus. Springer, Berlin Heidelberg New York
13. Flores J, Dibona DR, Beck CH, Leaf A (1972) The role of cell swelling in ischemic renal damage and the protective effect of hypertonic solute. J Clin Invest 51:118–126
14. Gerlach E, Bader W, Schwoerer W (1961) Über den Stoffwechsel säurelöslicher Phosphorverbindungen in der Rattenniere. Pfluegers Arch 272:407
15. Greger R (1985) Ion transport mechnism in thick ascending limb of Henle's loop of mammalian nephron. Physiol Rev 65: 760–797
16. Guder WG, Wirtensohn G (1981) Renal turnover of substrates. In: Greger R, Lang F, Silbernagel S (eds) Renal transport of organic substances. Springer, Berlin Heidelberg New York
17. Günther R, Silbernagel St (1981) Renal handling of L-Histidine studied by continuous microperfusion and free flow micropuncture in the rat. Pfluegers Arch 389:137–142
18. Gutmann J, Wahlefeld AW (1974) L-(+)-Lactat, Bestimmung mit Lactat-Dehydrogenase und NAD. In: Bergmeyer HU (Hrsg) Methoden der enzymatischen Analyse, Aufl 3, Bd II. Chemie Verlag, Weinheim, S 1510–1514
19. Hargitay B, Kuhn W (1951) Das Multiplikationsprinzip als Grundlage der Harnkonzentrierung in der Niere. Z Elektrochemie 55:539–558
20. Jamison RL, Bennett CM, Berliner RW (1967) Countercurrent multiplication by the thin loops of Henle. Am J Physiol 212: 357–366
21. Kallerhoff M, Hölscher M, Kläß G, Bretschneider HJ (1981) The equilibration proceedings at a 12' long perfusion of kidneys with HTP-solution of Bretschneider. Pfluegers Arch 389: R43
22. Kallerhoff M, Hölscher M, Kehrer G, Kläß G, Bretschneider HJ (1985) Effects of preservation conditions and temperature on tissue acidification in canine kidneys. Transplantation 39: 485–489
23. Kallerhoff M, Kehrer G, Siekmann W, Blech M, Gebhard MM, Helmchen U, Bretschneider HJ (1985) Experimentelle Anwendung der kardioplegischen Lösung HTK nach Bretschneider für eine in-situ-Protektion von Nieren. In: Harzmann R (Hrsg) Experimentelle Urologie. Springer, Berlin Heidelberg New York, S 180–188
24. Kallerhoff M, Blech M, Kehrer G, Kleinert H, Schnabel PhA, Siekmann W, Helmchen U, Bretschneider HJ (1985) A new method for perfusion fixation of dog kidneys. Pfluegers Arch 405 (Suppl 2):R33
25. Kallerhoff M, Blech M, Kehrer G, Kleinert H, Siekmann W, Helmchen U, Bretschneider HJ (1986) Postischemic renal function after kidney protection with the HTK-solution of Bretschneider. Urol Res 14:271–278
26. Kehrer G, Gebhard MM, Kallerhoff M, Siekmann W, Blech M, Helmchen U, Bretschneider HJ (1984) The influence of glucose premedication on perfusion resistance, perfusional diuresis, and equilibration of the dog kidney during perfusion with Bretschneider's cardioplegic solution HTK in standardized anaesthesia. Pfluegers Arch 400:R22
27. Kehrer G, Kallerhoff M, Probst R, Siekmann W, Blech M, Bretschneider HJ, Helmchen U (1985) Construction and experimental application of a catheter for selective arterial kidney perfusion in situ. Urol Res 13:85–89
28. Kehrer G, Blech M, Kallerhoff M, Kleinert H, Bretschneider HJ (1985) Influence of preischemic perfusion resistance on postischemic renal function. Pfluegers Arch 405:R33
29. Kokko JP, Rector FC (1972) Countercurrent multiplication system without active transport in inner medulla. Kidney Int 2:214–223
30. Kramer K, Thurau K, Deetjen P (1960) Hämodynamik des Nierenmarks. I. Mitteilung. Capillare Passagezeit, Blutvolumen, Durchblutung, Gewebehämatocrit und O_2-Verbrauch des Nierenmarks in situ. Pfluegers Arch 270:251–269
31. Kuhn W, Ryffel K (1942) Herstellung konzentrierter Lösungen aus verdünnten durch bloße Membranwirkung. Hoppe Seylers Z Physiol Chem 276:145–178
32. Leaf A (1970) Regulation of intracellular fluid volume and disease. Am J Med 49:291
33. Levinsky NG, Berliner RW (1959) The role of urea in the urine concentrating mechanism. J Clin Invest 38:741–748
34. Levy MN (1959) Oxygen consumption and blood flow in the hypothermic perfused kidney. Am J Physiol 197:1111
35. Mason J, Beck F, Dörge A, Rick R, Thurau K (1981) Intracellular electrolyte composition following renal ischemia. Kidney Int 20:61
36. Pfaller W (1981) Morphologic analysis of tubular transport. In: Greger R, Lang F, Silbernagel S (eds) Renal transport of organic substances. Springer, Berlin Heidelberg New York
37. Preusse CJ, Gebhard MM, Bretschneider HJ (1981) Myocardial "Equilibration Processes" and myocardial energy turnover during initiation of artificial cardiac arrest with cardioplegic solution – Reasons for a sufficiently long cardioplegic perfusion. Thorac Cardiovasc Surg 29:71–76
38. Ruedas G (1980) Changes in flow resistance in kidney vessels of dogs by hypothermic hyperosmotic perfusion. Urol Int 35:81–90
39. Sacks SA, Petritsch PH, Kaufmann JJ (1973) Canine kidney preservation using a new perfusate. Lancet II:1024–1028
40. Siekmann W, Blech M, Kallerhoff M, Kehrer G, Kleinert H, Bretschneider HJ, Helmchen U (1985) Morphologische Befunde nach zweistündiger kompletter Nierenischämie unter Anwendung verschiedener Protektionsverfahren. Verh Dtsch Ges Pathol 69:612
41. Silbernagel S (1981) Renal transport of amino acids and oligopeptides. In: Greger R, Lang F, Silbernagel S (eds) Renal transport of organic substances. Springer, Berlin Heidelberg New York, pp 93–117
42. Southard JH, Rice MJ, Ammetani MS, Belzer FO (1985) Effects of short-term hypothermic perfusion and cold storage on function of the isolated-perfusion dog kidney. Cryobiology 22:147–155
43. Thorn W, Heimann J, Müldener B, Gereken G (1957) Beitrag zum Stoffwechsel von Leber, Niere, Herz und Skelettmuskulatur in Asphyxie, Anoxie und bei Hypothermie. Pfluegers Arch 265:34–54
44. Ullrich KJ, Drenckhahn FO, Jaransch KH (1955) Untersuchungen zum Problem der Harnkonzentrierung und -verdünnung. Über das osmotische Verhalten von Nierenzellen und die begleitende Elektrolytanhäufung in Nierengewebe bei verschiedenen Diuresezuständen. Arch Ges Physiol 261:62–77
45. Wesson LG, Colburg JE, DeGutman A, Elsasser W, Dunn St (1979) Extracellular fluid of the kidney preserved by the Collins technique. Transplantation 27:380–383
46. Wirz H (1953) Der osmotische Druck des Blutes in der Nierenpapille. Helv Physiol Acta 11:20–29

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