Oxygen delivery in perfused rat kidney: NADH fluorescence and renal functional state

HOLGER FRANKE, CLYDE H. BARLOW, AND BRITTON CHANCE
Johnson Research Foundation, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19174

F R A N K E, HOLGER, CLYDE H. BARLOW, AND BRITTON CHANCE. Oxygen delivery in perfused rat kidney: NADH fluorescence and renal functional state. Am. J. Physiol. 231(4): 1082-1089. 1976. - Changes in steady-state levels of reduced pyridine nucleotide (PN) recorded by continuous monitoring of surface fluorescence were correlated with changes in physiological function of perfused rat kidneys when subjected to anoxia, ischemia, hypothermia, variations in perfusion pressure, inhibition of Na-K ATPase, and uncoupling of oxidative phosphorylation. Biphasic responses of PN reduction and oxidation during ischemic cycles at varying temperatures and anoxic cycles at different perfusion pressures demonstrated the presence of two different cell populations in the kidney cortex, those with sufficient oxygen and those without. The magnitude of PN fluorescence change during ischemia increased with decreasing temperature demonstrating better tissue oxygenation during hypothermia. The measurement of mitochondrial NADH oxidation in the perfused kidney during transitions from CO anoxia to normoxia was made possible by flash photolytic activation of mitochondrial electron transport. The half-time for NADH oxidation (125 ms) was independent of the rate of oxygen delivery while the initial rate and extent of reaction was faster and steeper, respectively, at higher perfusion pressures, due to a better tissue oxygenation and faster CO washout.

During recent years the function of the isolated perfused mammalian kidney has improved markedly (4, 20, 23). However, most kidney preparations still suffer decreased functional ability relative to the in situ organ, especially when artificial plasma substitutes are used instead of albumin (12, 14, 26). Parameters such as the Na⁺ net transport, the fractional Na⁺ reabsorption, or the osmotic concentration ability are sharply reduced.

The limiting factor for the reduced function during the artificial perfusion is not clear. Some authors assume that it might be a lack of metabolic substrates, because it has been demonstrated that the function of the isolated perfused kidney could be improved by the addition of substrates to the perfusate in high concentrations (23, 26). On the other hand there is some evidence that the effectiveness with which oxygen is transported to the intracellular mitochondria from the capillary space may be the main reason for the reduced functional state (2, 17). A lack of extrarenal hormones has also been proposed (1, 4).

To examine further the basis for reduced function of the isolated perfused kidney, changes in the amount of reduced pyridine nucleotides (PN) in the kidney cortex were continuously monitored by surface fluorometry. The effect of ischemia at different temperatures and of the energy demand on the redox state of the cortex mitochondria were evaluated.

The kinetics of mitochondrial PN were studied using a specific activation of the mitochondrial electron flow caused by flash photolysis of the cytochrome a₃-CO compound in the presence of oxygen (6, 9). The metabolic transition employed in these experiments was from CO anoxia to normoxia. This method yields both information about the kinetics of PN oxidation in intact tissue and about the rate of O₂ diffusion from the capillary space to the intracellular mitochondria. The interpretation of such data is based on observations of PN and FP fluorescence from isolated mitochondria (6), perfused liver (25), and heart preparations (9).

METHODS

Perfusion technique. Male adult albino rats of the Wistar strain, weighing 180-220 g, were used throughout the experiments. Altromin laboratory chow and water was given ad libitum. The experiments were started with no special reference to the feeding cycle. The animals were anesthetized by ip injection of 150 mg Na thiobarbital/kg body wt. The operation was carried out as described elsewhere (12, 29). After starting the perfusion the kidney was excised and immersed in a thermostatically controlled bath filled with physiological saline at 37°C. In the experiments at lower temperatures (26, 20, 14°C) the temperature of the perfusate was reduced slowly to prevent a vasoconstriction induced by rapid temperature changes. The perfusion pressure was measured by a Statham element, the venous outflow recorded continuously using a custom-built electromagnetic flowmeter. The O₂ consumption was measured using two Clark-type electrodes (Beckman Instruments) in the arterial and the venous limb of the perfusion system.

For the perfusion an osmotically and colloidosmotically isotonic solution of the following composition was used. NaCl 110.0 mM, KCl 5.0 mM, CaCl₂ 1.0 mM, MgCl₂ 0.3 mM, NaHCO₃ 25.0 mM, NaHPO₄ 0.8 mM, Na
pyruvate 5.0 mM, Na L-lactate 2.0 mM, n-glucose 5.0 mM, dextran 40 40.0 g/liter, 14C-labeled inulin 10.0 μCi/liter.

Dextran was selected as a plasma substitute, because much higher flow rates could be obtained with this colloid than with Pluronic-F-108 or hydroxyethyl starch at similar perfusion pressures (14). A high flow rate was necessary for flash photolysis, because the mixing time of the cytochrome oxidase-CO compound must be shorter than the time for the spontaneous dissociation of the aβ-CO complex (9).

Immediately before the perfusion the medium was passed through a Millipore filter with a mean pore diameter of 0.2 μm. The perfusate was not recycled (single pass system).

The perfusion pressure was adjusted by the pressure of the gas over the perfusate, normal perfusion pressure was 112 torr.

To alternate aerobic and CO-anaerobic perfusates, 95% O2 + 5% CO2 or 95% CO + 5% CO2 was equilibrated with the perfusate in duplicate vessels each at the same perfusion pressure.

The fluids were passed through two Millipore filters with a mean pore diameter of 3 μm installed in the perfusion line. Solenoid valves were used for changing from one medium to the other. The dead volume between the valves and the perfused kidney was less than 0.5 ml. Thus the new perfusate arrived at the organ within 1.5 s at flow rates of more than 20.0 ml/kg kidney wt·min. At a perfusion flow higher than 20 ml/min the estimated filling time for the capillaries was less than 45 ms assuming that the volume of this system in the rat kidney is about 0.015 ml. The duration of the CO exposure was 10–20 s. Thereafter the kidney was perfused with aerobic perfusate until the state of aerobic conditions was reached again. To switch over from normoxia to ischemia the perfusion flow was stopped with a pinch clamp.

Fluorometric techniques. Pyridine nucleotide oxidation-reduction changes were continuously monitored by measuring the fluorescence emission of reduced pyridine nucleotide from the surface of the kidney. The DC fluorometer (8) utilized a trifurcated fiber optics light pipe with 45 ± 4% of the fibers in two branches and 10 ± 1% of the fibers in the third branch with the fibers randomly mixed at the 4 mm diameter common end. The branches of the light pipe were arranged as in Fig. 1 for all experiments not requiring a laser. Reduced PN fluorescence emission (460 nm) was corrected for changes in tissue opacity by a 1:1 substraction of reflectance changes at 366 nm from the fluorescence (16, 21). Reflectance changes in the perfused organ were generally small so that uncorrected fluorescence emission was sufficient for many experiments. A procedure similar to that used for perfused heart (8) was followed to measure the kinetics of PN oxidation. Briefly the kidney was made anoxic with CO-saturated perfusate reducing mitochondrial PN and forming a CO complex with cytochrome c oxidase. When the oxygenated perfusate was resumed, dissolved CO was rapidly replaced by oxygen while CO covalently bound to cytochrome oxidase remained due to the stability of the cytochrome oxidase-CO complex (8). The photolability of this complex allowed the CO to be dissociated from cytochrome oxidase by a short laser flash (100 mJ, 585 nm, 0.4 μJ). The reduced cytochrome oxidase was then free to react with dissolved oxygen; PN oxidation rates were directly monitored from the rate of fluorescence decrease. In this experiment the initial state prior to the flash photolysis is the anaerobic state 5 and just after photolysis the active metabolic state (state 3) is obtained.

To further examine the relationship between changes in kidney function versus the PN redox level, specific inhibitors of renal processes were infused into the arterial limb of the kidney preparation. Ouabain (1 mg/ml) was used to inhibit the sodium-potassium (Na-K) ATPase system. FCP (pentachlorophenol, ~2 × 10−7 M) was used to uncouple mitochondrial oxidative phosphorylation. The maximum exposure time was 1 min and a 5 min recovery interval was afforded.

Functional control. The functional state of the isolated organ under different experimental conditions was determined in separate experiments for each experimental group under steady state conditions. All values were calculated per gram kidney wet weight and minute (g·min). The wet weight of the perfused organ was obtained from the dry weight (22.5 of the wet weight).

Analytical techniques and statistical controls. Sodium was determined by flame photometry in a Technicon AutoAnalyzer, 14C-labeled inulin activity for the determination of the glomerular filtration rate in a liquid scintillation counter (Packard Instruments, model 3008). All values given in the tables are means ± SE. For statistical analysis the Student t test for paired data was used.

RESULTS

The charts of the NADH fluorescence shown subsequently represent typical responses of the perfused kidney.

Effect of temperature. The changes in PN fluorescence during an ischemic transient were used to evaluate mitochondrial function in the kidney cortex during hypothermia. Cessation of perfusion resulted in a biphasic reduction of PN (Fig. 2). The initial phase, evidenced by a slow slow increase in PN fluorescence, lasted some 13
s at 37°C and increased in length as the temperature was lowered (Table 1). The duration of this initial phase was a function of the amount of O₂ dissolved in the perfusate and the rate of mitochondria metabolism at these temperatures.

The second phase was evidenced by a rapid reduction of PN resulting in a fluorescence increase of 28% at 37°C and increasing to 44% at 14°C. The size of fluorescence increase to presumably similar ischemic states at various temperatures yields insight regarding the initial states at these temperatures. Thus as temperature is lowered the mitochondrial electron transport chain becomes more oxidized.

The functional parameters for the perfused kidney at different temperatures are also presented in Table 1. Hypothermia to 14°C decreased urine flow 48.4% of the 37°C value. Perfusion rate was also lowered to 54.6% of the rate at 37°C. The active sodium reabsorption at 14°C was only 30.4% of that at 37°C. The fractional sodium reabsorption was also lowered even though the tubular sodium load had been diminished.

**Effect of pressure.** The responses of PN fluorescence during anoxic cycles were used to evaluate the effects of changes in perfusion pressure. Figure 3 illustrates typical fluorescence changes during CO anoxia and O₂ recovery at different perfusion pressures. The fluorescence changes are similar for CO and N₂ anoxia in that the PN reduction is biphasic as seen in studies with other organs. The initial phase represents a slow reduction of a small fraction of PN. As the perfusion pressure is lowered the rate of PN reduction increases (Fig. 3). This would suggest that this initial phase represents PN reduction in those mitochondria receiving insufficient O₂ from the perfusate.

The second phase of PN reduction represents the rapid reduction of the bulk of the cortex PN. As the perfusion pressure is increased the total percent fluorescence change on anoxia increases (Table 2). Thus the

**TABLE 1. Effects of temperature on kidney function and NADH fluorescence**

<table>
<thead>
<tr>
<th></th>
<th>37°C</th>
<th>37 → 28°C</th>
<th>37 → 20°C</th>
<th>37 → 14°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urine flow, ml/g min</strong></td>
<td>0.33 ± 0.02</td>
<td>0.13 ± 0.01*</td>
<td>0.15 ± 0.001*</td>
<td>0.16 ± 0.004*</td>
</tr>
<tr>
<td><strong>Perfusion, ml/g min</strong></td>
<td>20.5 ± 0.17</td>
<td>13.8 ± 0.44*</td>
<td>12.9 ± 0.38*</td>
<td>11.2 ± 0.15*</td>
</tr>
<tr>
<td><strong>GFR, %</strong></td>
<td>2.19 ± 0.03</td>
<td>3.84 ± 0.14*</td>
<td>3.3 ± 0.07*</td>
<td>1.98 ± 0.08*</td>
</tr>
<tr>
<td><strong>G.S. consumption, ml/g min</strong></td>
<td>0.77 ± 0.02</td>
<td>4.2 ± 0.01*</td>
<td>3.35 ± 0.007*</td>
<td>0.3 ± 0.07*</td>
</tr>
<tr>
<td><strong>Na reabsorption, pmol/g min</strong></td>
<td>67.6 ± 2.3</td>
<td>46.0 ± 0.8*</td>
<td>32.4 ± 1.2*</td>
<td>20.6 ± 1.44*</td>
</tr>
<tr>
<td><strong>Fractional Na reabsorption, % of tub load</strong></td>
<td>60.1 ± 0.41</td>
<td>75.6 ± 0.82*</td>
<td>60.3 ± 1.15*</td>
<td>45.5 ± 2.17*</td>
</tr>
<tr>
<td><strong>O₂ consumption, ml/g min</strong></td>
<td>0.159 ± 0.004</td>
<td>0.134 ± 0.003*</td>
<td>0.116 ± 0.003*</td>
<td>0.09 ± 0.004*</td>
</tr>
<tr>
<td><strong>ΔF&lt;sub&gt;NADH&lt;/sub&gt;, %</strong></td>
<td>28</td>
<td>40</td>
<td>48</td>
<td>44</td>
</tr>
<tr>
<td><strong>Induction time, s</strong></td>
<td>13</td>
<td>18</td>
<td>30</td>
<td>65</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 20); perfusion pressure: 112 Torr. * Significantly different from control values at 37°C. † ΔF<sub>NADH</sub>, %: change in fluorescence during the rapid phase upon ischemia. ‡ Induction time: time from clamping the arterial limb until the beginning of the rapid phase.

**FIG. 2.** PN fluorescence response to ischemia at a perfusion temperature of 37°C. (R: reflectance at 366 nm; PN: fluorescence emission at 450-500 nm; PN<sub>corr</sub>: corrected PN fluorescence PN - R.)

**FIG. 3.** Responses of PN fluorescence to CO anoxia and O₂ recovery at perfusion pressures of 155 torr (A) and 80 torr (B). Upper figures illustrate spontaneous NADH oxidation during O₂ recovery from CO anoxia; lower figures illustrate the photolysis activated NADH oxidation following laser flash.
initial redox state of the mitochondrial electron transport system continues to become more oxidized with increasing perfusion pressure even at a relatively high pressure of 155 torr.

When oxygenated perfusate is reintroduced into the CO anaerobic kidney, the aerobic PN steady state redox level is reached in a few seconds. There is a period after CO is admitted during which a significant fraction of the tissue exists in an environment containing sufficient oxygen to support electron transport but cytochrome oxidase fails to become reoxidized due to bound CO. The CO may be removed by flash photolysis as evidenced by the activation of the rate of PN oxidation in Fig. 3 following the laser flash. The half time for this photolysis-activated PN oxidation was 125 ms and was independent of the perfusion pressure (Fig. 4). The short half time for PN oxidation and its freedom from the rate of O₂ delivery substantiates the concept that the intrinsic rate of oxidation of the mitochondrial PN pool in the kidney cortex was being measured. On the other hand, the rate of O₂ delivery, as governed by the perfusion pressure, affects both the extent and initial rate of the photolysis-activated PN oxidation (Fig. 5).

The function of the kidney at different perfusion pressures is given in Table 2. With increasing pressure both urine flow and perfusion rate rose. Moreover, it is remarkable that the fractional Na reabsorption remained practically constant in spite of the raised tubular Na load above a perfusion pressure of 125 torr (Table 2). It was possible to raise the Na net transport to an amount of 138 μmol/g/min by increasing the perfusion pressure up to 155 torr. The relationship between the Na net transport and the O₂ consumption appeared to be linear.

Effect of ouabain and PCP. Infusion of specific metabolic modifiers into the isolated kidney was used to evaluate further the relationships between changes in kidney functions and PN fluorescence changes. Kidneys perfused with ouabain (1 mg/ml) exhibited alterations in important functional parameters (Table 3). While the urine flow rose to 118% of the control, U/P₁₅ was reduced from a control value of 2.19±1.55. Na net transport was reduced to 46.9% of control while the fractional Na reabsorption dropped from 60.1 to 34.2%. PN fluorescence increased by 7% upon initiation of ouabain infusion (Fig. 6) and returned to normal within 3 min following removal of ouabain from the perfusate. The changes in functional parameters reflect the Na-K ATPase inhibition by ouabain. The lowered rate of ATP hydrolysis shifts the mitochondrial respiration of the

![FIG. 4. Effect of perfusion pressure on initial rate of NADH oxidation in perfused rat kidney during O₂ recovery from CO anoxia.](http://ajplegacy.physiology.org/)

![FIG. 5. Effect of perfusion pressure on half times of PN oxidation in perfused rat kidney during O₂ recovery from CO anoxia.](http://ajplegacy.physiology.org/)

![TABLE 2. Effects of perfusion pressure on kidney function and rates of NADH oxidation](http://ajplegacy.physiology.org/)

<table>
<thead>
<tr>
<th>Perfusion Pressure, Torr</th>
<th>155</th>
<th>125</th>
<th>112</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine flow, ml/g.min</td>
<td>0.49 ± 0.09</td>
<td>0.44 ± 0.01</td>
<td>0.33 ± 0.02</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Perfusion flow, ml/g.min</td>
<td>24.7 ± 0.3</td>
<td>22.3 ± 0.10*</td>
<td>20.5 ± 0.17</td>
<td>16.0 ± 0.36*</td>
</tr>
<tr>
<td>U/P₁₅</td>
<td>2.71 ± 0.14</td>
<td>2.24 ± 0.02*</td>
<td>2.19 ± 0.02*</td>
<td>2.49 ± 1.07*</td>
</tr>
<tr>
<td>Glomerular filtration rate, ml/g.min</td>
<td>1.28 ± 0.02</td>
<td>0.98 ± 0.02*</td>
<td>0.77 ± 0.02*</td>
<td>0.38 ± 0.02*</td>
</tr>
<tr>
<td>Na reabsorption, pmol/g.min</td>
<td>119 ± 3.12</td>
<td>66.5 ± 2.06*</td>
<td>67.6 ± 2.3*</td>
<td>41.6 ± 2.76*</td>
</tr>
<tr>
<td>Fractional Na reabsorption, % of load</td>
<td>67.7 ± 1.2</td>
<td>63.3 ± 0.34</td>
<td>60.1 ± 0.41*</td>
<td>82.8 ± 3.2*</td>
</tr>
<tr>
<td>O₂ consumption, ml/g.min</td>
<td>0.23 ± 0.004</td>
<td>0.19 ± 0.002*</td>
<td>0.16 ± 0.004*</td>
<td>0.13 ± 0.004*</td>
</tr>
<tr>
<td>ΔPN, %†</td>
<td>No flash</td>
<td>16.0 ± 0.36*</td>
<td>11.0 ± 0.41*</td>
<td>8.25 ± 0.63*</td>
</tr>
<tr>
<td></td>
<td>Flash</td>
<td>37.9 ± 0.004</td>
<td>31.0 ± 0.004</td>
<td>26.3 ± 0.004*</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 20); perfusion pressure: 112 Torr; temperature: 37°C. *Significantly different from control values. † ΔPN, %: rate of oxidation of NADH during O₂ recovery from CO anoxia.

![TABLE 3. Effects of ouabain, PCP, and anoxia on kidney function and NADH fluorescence](http://ajplegacy.physiology.org/)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ouabain</th>
<th>PCP†</th>
<th>95% CO + 95% N₂</th>
<th>95% CO + 95% N₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine flow, ml/g.min</td>
<td>0.33 ± 0.02</td>
<td>0.39 ± 0.02*</td>
<td>0.23 ± 0.03*</td>
<td>0.3 ± 0.02*</td>
<td>0.22 ± 0.02*</td>
</tr>
<tr>
<td>Perfusion flow, ml/g.min</td>
<td>20.5 ± 0.17</td>
<td>16.5 ± 0.14*</td>
<td>24.0 ± 0.25*</td>
<td>26.3 ± 0.28*</td>
<td>25.6 ± 0.18*</td>
</tr>
<tr>
<td>U/T₀</td>
<td>2.19 ± 0.02</td>
<td>1.55 ± 0.02*</td>
<td>1.94 ± 0.01*</td>
<td>1.26 ± 0.06*</td>
<td>2.21 ± 0.07*</td>
</tr>
<tr>
<td>Glomerular filtration rate, ml/g.min</td>
<td>0.77 ± 0.02</td>
<td>0.96 ± 0.02*</td>
<td>0.24 ± 0.03*</td>
<td>0.37 ± 0.09</td>
<td>0.27 ± 0.11*</td>
</tr>
<tr>
<td>Na reabsorption, pmol/g.min</td>
<td>67.6 ± 2.73</td>
<td>31.7 ± 1.1*</td>
<td>3.0 ± 0.57*</td>
<td>11.3 ± 0.91</td>
<td>7.74 ± 0.97*</td>
</tr>
<tr>
<td>Fractional Na reabsorption, % of tub load</td>
<td>60.1 ± 0.41</td>
<td>34.2 ± 0.26*</td>
<td>8.9 ± 0.16*</td>
<td>20.8 ± 0.73*</td>
<td>17.8 ± 0.53*</td>
</tr>
<tr>
<td>O₂ consumption, ml/g.min</td>
<td>0.150 ± 0.004</td>
<td>0.100 ± 0.009*</td>
<td>0.313 ± 0.01*</td>
<td>1.70 ± 0.03*</td>
<td>0.07 ± 0.03*</td>
</tr>
<tr>
<td>ΔPN, %†</td>
<td>No flash</td>
<td>+7.0</td>
<td>-18.0</td>
<td>+33</td>
<td>+39</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 20); perfusion pressure: 112 Torr; temperature: 37°C. *Significantly different from control values. † ΔPN, %: change in fluorescence from control to treated state. ‡ Ischemia started from this state resulted in an increase of PN fluorescence by 56% during the fast phase.
kidney cortex toward state 4 resulting in a more reduced level of the electron carriers and thus an increased PN fluorescence.

Uncoupling of oxidative phosphorylation by PCP (≈2 × 10^{-7} M) caused a decrease in PN fluorescence by about 18% which was not reversed on returning to PCP-free perfusate. In ouabain pretreated kidneys PCP infusion led to a PN oxidation of 25% (Fig. 6). Ischemia starting from the uncoupled state induced a PN reduction of 60%, compared to 33% at control conditions. Thus changes in NADH fluorescence during transitions to different metabolic states seem to be additive. The functional parameters (Table 3) reflect uncoupling by increased O_{2} consumption and decreased work. O_{2} consumption increased to 215% of the control while the GFR decreased to 32% of control values. The ability to produce an osmotically concentrated urine was completely lost (U/P_{\text{in}} - 1.0) together with the function to reabsorb Na^{+}.

DISCUSSION

The steady-state redox level of mitochondrial electron carriers in tissue slices may be determined directly by absorption spectroscopy (24). However, to obtain information in the intact perfused kidney we measured the change in redox level of an electron carrier, PN, upon a transition from the conditions of interest to a rather well-defined condition. To determine whether the oxygen supply to the tissue was adequate the effects of work, temperature, and perfusion pressure on the changes in PN fluorescence were studied. Since surface fluorometry provides only information about the first 0.5 mm of the kidney cortex we are unable to tell what happens in deeper areas under different conditions of isolated perfusion. On the other hand, micropuncture studies on the isolated rat kidney have shown that the decrease in fractional and absolute Na^{+} reabsorption during isolated perfusion is mainly due to a reduced functional ability of the proximal tubules (1) which are located directly under the surface. Furthermore, former experiments have demonstrated (14) that morphological deviations during isolated perfusion occur mainly in proximal tubular cells and that the number of histological alterations has a closed relationship to the Na^{+} reabsorption, while the tissue of the medulla showed only very little and unspecific changes. Therefore we expect a close relationship especially between the fractional and absolute Na^{+} reabsorption and the fluorescence of reduced pyridine nucleotide obtained from kidney surface.

With decreasing temperature respiration slows down as indicated by the increased induction time of the PN reduction accompanied by a decrease of the Na reabsorption. At 14°C the Na net transport amounted to 29.8% and the O_{2} consumption to 27.9% of the control at 37°C. These results indicate that in the isolated kidney, as in the in situ organ (11), the main part of the consumed O_{2} is used for the active transport of Na^{+}. Moreover, there is no significant difference in the regression line obtained from the ratio of Na^{+} transported per milliliter of oxygen consumed between normothermic and hypothermic conditions, although this value is in both cases much lower than under in situ conditions (28). One reason for the increased O_{2} consumption of the isolated kidney could be the change of the percentage distribution of the perfusion flow of the medulla. In the dog kidney in situ for instance the medullary blood flow could be increased by lowering the hematocrit (22). Consequently it can be expected that under the condition of a perfusion with a cell-free medium, as it was used in our preparation, the perfusion flow of the renal medulla is increased. Then the energy demand of the medulla, which is covered under in vivo conditions almost by anaerobic metabolism (17), may thus be switched over to oxidative processes. Moreover, at an increased medullary "blood flow" the rat kidney has a high rate of glucose production from lactate, which is not related to the renal transport of Na^{+} especially at high lactate concentrations as used in these experiments (own unpublished observations).

The fact that the fractional O_{2} uptake from the perfusate shows a small but significant increase at lower perfusion temperatures may be due to changes in flow distribution between cortex and medulla, since the increased U/P_{\text{in}} and the higher fractional Na reabsorption at 26°C indicate that the decreased flow rate seems to impair mainly the medullary perfusion flow. On the other hand, the oxygen tension in neither the arterial nor the venous limb of the perfusion system seems to reflect the conditions in the renal cortex. As demonstrated by studies in perfused rat kidneys (19) using O_{2} microelectrodes the highest PO_{2} pressures in the renal cortex were about 200–250 torr compared to 600–700 torr in the arterial perfusate. Thus due to the vascular countercurrent system and the steep oxygen gradients between the arterial influx and the venous efflux a main part of the oxygen probably diffuses directly into the venous limb without passing the capillary bed (Lübbers, personal communication).

The percent fluorescence increase upon ischemia at 37°C amounted to only 28%, compared to 40–48% at

![Fig. 6. Response of PN fluorescence to partial inhibition of active transport of Na\(^{+}\) by infusion of ouabain and uncoupling of oxidative phosphorylation by PCP; final ischemia provides a transition to a fully reduced state.](http://ajplegacy.physiology.org/DownloadedFromhttp://ajplegacy.physiology.org/)
lower temperatures. This indicates that tissue oxygenation is better under hypothermic conditions than at 37°C because of the slower respiration at lower temperatures. If tissue oxygenation were sufficient at 37°C and decreasing the temperature served only to reduce the work load the steady-state PN level would become more reduced, as seen during ouabain infusion, rather than more oxygenated. Consequently it must be concluded that the O₂ delivery cannot be adapted to the O₂ requirement of the renal tissue under normothermic conditions and physiological perfusion pressures, when a cell-free perfusate is used.

Effect of ouabain and PCP. The work load of the kidney could be lowered by partial inhibition of the Na-K ATPase enzyme system by the addition of ouabain to the perfusate. This resulted in a PN fluorescence increase of about 7% (Fig. 6), consistent with a shift to the resting state 4 (7). Perfusing thereon with normal medium the initial state in fluorescence activity was reached after 180 s. The Na net transport, the most important change, was reduced to 46% of the control period (Table 3). Simultaneously the O₂ consumption fell to 0.108 ml/g·min. Similar results were obtained in earlier studies on the CO₂ production from exogenously offered substrates of the isolated kidney (3). In contrast to uncoupling where the ability to transport actively Na⁺ was almost completely lost, it is not possible to inhibit the Na reabsorption in the same range by ouabain, even at fourfold higher ouabain concentrations where the Na-K ATPase enzyme system is completely inhibited (13). These results indicate that a reasonable amount of the Na.net transport is not dependent on the activity of the Na-K ATPase enzyme system.

Uncoupling by PCP led to a decrease in NADII fluorescence by 18 and 26%, respectively, under the action of ouabain. Ischemia starting from the uncoupled state 3a (Fig. 6) led to a fast PN reduction of 55% instead of 28% starting from control conditions. Kidney function of the uncoupled state was drastically reduced (Table 3), the capability to transport Na⁺ was almost completely lost. The O₂ consumption at 26°C was raised to 215% of the control. This demonstrates that this agent is effective in the same range as 2,4-dinitrophenol (DNP), which induced under comparable experimental conditions an increase of the O₂ consumption to 198% of the control (12). But in contrast PCP is effective at much lower concentrations than DNP, where concentrations of 5 × 10⁻⁴ M were required. The fact that no immediate decrease of NADH fluorescence is observed, and that the response is prolonged over a long interval and is relatively small, in spite of the measured increase of respiration, suggests that two conflicting phenomena are superimposed: the abrupt oxidation of mitochondrial PN due to the transition from the coupled (state 3) to the uncoupled state (state 3a) and the increased tissue anoxia due to the higher rate of respiration (6).

Effect of perfusion pressure. The size of the change in PN fluorescence upon anoxia was dependent on the rate at which oxygen was delivered by the perfusate. As the perfusion pressure was increased from 80 to 155 torr at 37°C, the PN fluorescence increase upon CO anoxia went from 16.0 to 26.3%. Even at 155 torr the steady-state PN redox level was still oxygen dependent.

The photolysis of cytochrome oxidase-CO in the presence of oxygen to initiate PN oxidation makes it possible to avoid the O₂ diffusion limitations in the anaerobic aerobic transition so that the measured kinetics of a redox change represent the kinetics of the respiratory pigments. Moreover, because all energy consuming functions of the cell, for instance ATP formation, ion pumping and substrate transport, are also rapidly activated (9), this technique can be of great value for the study of overall physiological activities. A basic requirement for all flash photolysis techniques is that the mixing time of the oxidase-CO compound with O₂ must be shorter than the time for the spontaneous dissociation of the a₃-CO complex (9). While this is easier to achieve in mitochondrial preparations (6), it becomes difficult in tissue, where it depends on the rate of perfusion and the O₂ diffusion time from the capillaries to tissue mitochondria.

The rate of PN oxidation from the steep phase of the PN fluorescence immediately after the flash and the extent of this rapid oxidation depends on the perfusion pressure and consequently on the rate of perfusion. At a perfusion pressure of 155 torr the initial rate of NADH fluorescence decrease following photolysis was 40% (9), while it was only 16% at a pressure of 80 torr (Fig. 3, Table 2). The extent of the fast redox change seems to be limited by the amount of oxygen present in the tissue at the moment of the flash. Thus at high perfusion pressure the bulk of the tissue is well perfused and a high O₂ tension is present in the moment of photolysis. At lower pressures more and more areas of the tissue become insufficiently perfused resulting in a slower initial rate and smaller extent of PN oxidation after photolysis. In contrast to the extent or initial oxygen at the mitochondria at the moment of photolysis, the half time for rapid PN oxidation is independent of oxygen concentration and reflects the inherent rate of mitochondrial electron transfer.

The amount of Na⁺ reabsorbed correlates well with the extent and the speed of the flash response. This connection between flash response and Na net transport is supported by the approximately linear relationship between Na net transport and O₂ consumption (12) indicating that the main energy of the kidney metabolism is used for the active transport of Na⁺. At high pressures the bulk of the kidney cortex cells are well oxygenated and only a very small number of cells are anoxic. At lower perfusion pressures the percent distribution of anoxic cells increases and consequently the Na reabsorption is reduced as well as the extent of the fast flash response.

The biphasic change in PN fluorescence as a response on ischemia at different temperatures, the fact that the PN fluorescence increase during the first few seconds of CO-anoxia was higher at lower perfusion pressures, and the greater extent of the flash response at higher perfusion pressures suggest that the reduced functional state of the isolated mammalian kidney is not the result of a homogenous cell population with a nearly identical but reduced functional ability. The results indicate that there exist two different types of cells in the tissue: one,
which is well oxygenated with an approximately normal function, and a second type, which is nearly anoxic. Thus anoxia, as far as the mitochondria are concerned, appears to a first approximation to be an "all-or-nothing" phenomena, and only small numbers of mitochondria are to be found in border zones between normoxia and anoxia. This assumption is supported by the fact that in the kidney cortex of the dog (2) and of the rat (17) the tissue PO\textsubscript{2} gradient is very high, and that even under in situ conditions, areas are existing where the PO\textsubscript{2} is lower than 1 Torr. These findings can be explained from the architecture of the vascular bed of the renal cortex (18). Since the capillary grid surrounding the kidney tubules results in a heterogeneous flow pattern, in some areas exists a high perfusion flow while others have very low flow rates and consequently a much lower tissue PO\textsubscript{2} than the well-perfused tissue areas. Especially under the conditions of the perfusion with a cell-free medium it is evident that the increased perfusion flow cannot totally compensate the drastically reduced O\textsubscript{2} content of a hemoglobin free medium. Thus it can be expected that in those areas where the PO\textsubscript{2} is extremely low in situ the tissue becomes completely anoxic upon perfusion. Moreover, the morphological alterations occurring in perfused kidneys (12, 14) are identical with those following anoxia (5, 27). As a consequence of the markedly reduced functional state of kidneys perfused with dextran 40 solutions some restrictions have to be made if these data are interpreted with regard to organs perfused with other artificial colloids or albumin solutions. It can be assumed that anoxic tissue areas are much smaller in those kidneys where better functions are obtained (4, 12, 14, 20, 23); thus most of the described phenomena such as the biphasic response of PN fluorescence to ischemia or the slow and small decrease in photolysis activated PN oxidation will be less distinct under those conditions.

That tissue hypoxia, nevertheless, is a main reason for the reduced physiological function of artificially perfused kidneys is shown by recent experiments. Perfu-
sing rat kidneys with solutions containing 7.5 ~01% of the fluoro-carbon FC-43 as an oxygen carrier a GFR of 1.0 ml/g.min and a Na reabsorption of 139.5 mmol/g.min which correlates to a fractional Na reabsorption of 93% could be obtained (15). These values reach almost the in situ levels and are much higher than in kidneys perfused under the same conditions but without an oxygen carrier.

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