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The Oxygen Supply of the Rat Kidney: Measurements of Intrarenal pO_2

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Summary. Using a newly developed platinum-O_2-microeletrode [30] based on the design of Silver [37] the construction and properties of which are described, pO_2-measurements in the parenchyma of the blood-perfused and the cell-free perfused rat kidney were carried out.

By continuous recording of the pO_2 during slow ($150\,\mu \times min^{-1}$) insertion of the O_2-electrode into the respiring tissue two regions of distinctly different mean pO_2-values were found. In the outer region which extends from the renal surface to a depth of about 3—4 mm (corresponding anatomically with the renal cortex) large pO_2-differences exist close to each other. In the blood-perfused kidney the maximum cortical pO_2-values lie in the range of arterial pO_2 the lowest values at about 10 Torr. In the cortex of the cell-free perfused kidney the maximum pO_2-values lie considerably below the arterial pO_2.

In both the blood perfused and in the cell-free perfused kidney at centripetal movement of the O_2-electrode the cortical region of high and fluctuating pO_2 is followed by a narrow zone ($\approx 200\,\mu$ radial extension) of a steep decrease of the mean pO_2. At further insertion in both preparations the pO_2 remains at low pO_2-values of ca. 10 Torr. Anatomically, this latter region of low and constant pO_2 corresponds to renal medulla and pelvis.

By recording the decrease of parenchymal pO_2 after sudden stop of the perfusion attempts were made at measuring the critical local O_2-supply pressure. In the cortex of the cell-free perfused kidney critical local O_2-supply pressures between 6 and 28 Torr with a maximum abundance at 8 Torr were found.

The qualitative and quantitative implications of the presented data on the conditions of parenchymal O_2-supply are discussed. The results are interpreted as an indication for the arteriovenous shunt (bypass)-diffusion of considerable amounts of oxygen, especially under the conditions of the cell-free perfusion. Furthermore, it follows from the data presented that even at high venous O_2-pressures and high mean pO_2-values in the parenchyma regions of local anoxia may exist.

Key-Words: O_2-Microelectrodes — Measuring of Local pO_2 — Shunt-Diffusion of O_2 — Critical O_2-Supply Pressure — Oxygen Tension in the Kidney.

Schlüsselwörter: Sauerstoff-Mikroelektroden — Lokale pO_2-Messung — O_2-Diffusions-Shunt — Kritischer Sauerstoffdruck — Sauerstoffdrucke in der Niere.

The oxygen content of the blood in the renal vein is comparatively high. The renal arterio-venous (a-v) oxygen difference amounts to only 1—1.5 vol-%, while many other organs extract 4—5 vol-% of the

oxygen supplied. The low renal oxygen extraction indicates special conditions regarding the oxygen supply and/or the oxygen demand of the respiring cells. If in the kidney—like in all other organs studied—oxygen moves from the capillaries to the cells by diffusion only, measurements of local O_2-pressures in the respiring tissue should provide information on the special conditions regarding the oxygen supply of this organ. O_2-pressures in renal tissue have so far been measured with O_2-electrodes of rather large diameter ($\leq 15\ \mu$) [1—5]. Within the cortex an almost homogenous field of high O_2-pressure (≈ 80 Torr), and within medulla and pelvis a similarly homogenous O_2-pressure between 20 and 40 Torr was found [1, 2, 5]. However, in a tissue with a high rate of respiration, like the renal cortex, one would expect relatively large O_2-pressure differences in close proximity. Thus we suspected that the low power of spacial resolution of the O_2-electrodes used had obscured O_2-pressure gradients existing across small distances. Even at high mean O_2-tissue pressures and at high O_2-pressures in the blood of the renal vein, in the parenchyma small hypoxic areas may exist. For an evaluation of the oxygen supply it is, therefore, not sufficient to know merely the mean O_2-pressure averaged over relatively large spaces.

With the aim of obtaining data which would allow a more detailed assessment of the oxygen supply of the mammalian kidney we have measured local O_2-pressures in the rat kidney in situ, and in the isolated cell-free perfused organ. A new type of platinum O_2-microelectrode, based on the design of SILVER [37], possessing high time and spacial resolution, has been developed. Since the validity of the implications drawn from the O_2-pressure data depends largely on the technique of measurement, the construction and the properties of the electrode are described.

As was to be expected from measurements with electrodes of higher spacial resolution, in the renal cortex considerable O_2-pressure gradients within small distances were found. The lowest cortical O_2-pressures lay far below the O_2-pressure in the renal vein. In the outer medullary region the pO_2 decreased to very low values, suggesting shunt diffusion of oxygen.

Material and Methods

Albino rats from the Hannover-Wistar strain of 250—300 g body weight were used throughout the experiments. The animals were narcotized by i.m. injections of 150 mg $\times$ kg^{-1} Inaktin (Promonta). The kidneys were exposed by abdominal midsection. During the in-situ experiments artificial respiration through a tracheal cannula with air from a Starling pump was carried out. In order to prevent movements of the kidneys due to heart beat and respiration the organs were held in a tight fitting perspex cup attached to an adjustable tripod arm. The renal capsule was removed over the site selected for insertion of the O_2-electrode. A small perspex

plate with an oval hole in the centre was put under slight pressure on the renal surface in a position which placed the oval hole over the site to be punctured. The O_2-electrodes were held in a micromanipulator (Leitz). An adjustable continuous movement of the electrode through the tissue was achieved by a synchronous motor belt-driving the fine movement screw of the manipulator, thus providing a smooth movement of sufficient uniformity. All data from experiments with continuously moving electrodes were obtained at a velocity of 150 $\mu \times min^{-1}$. For the isolation and artificial perfusion of rat kidneys a modified method of WEISS *et al.* [45] which has been previously described in this journal (LEICHTWEISS *et al.* [26]) was used. The isolated kidneys were fixed, decapsulated and punctured with the O_2-electrodes in the manner described above for the in-situ preparation. The isolated organs were kept at 38°C in a thermostatically controlled bath of physiological saline. The ureter was sectioned at the excision of the organ. During the experiments uretal peristalsis and urine flow were maintained. If not otherwise stated for the perfusion an osmotically and colloidosmotically isotonic solution of 3,5 g Haemaccel/litre (R) (Behringwerke) of the following composition was used: (in mMol/l) NaCl 145.0; KCl 5.1; $CaCl_2$ 3.2; glucose 4.0; bovine albumin, puriss (Behringwerke) 1.0 g/litre; pH 7.35. The medium was saturated at 38° C with pure oxygen under atmospheric pressure. Perfusion flow was measured in the venous outflow by an electromagnetic flow meter. Statham elements were used for the measurement of perfusion pressure in an arterial side arm. The perfusion pressure was obtained from an oxygen pressure bottle and adjusted by a pressure-reducing valve. For the measurement of arterial and venous O_2-pressures the platinum—"micro"-electrode of Messr. Beckman Inc., for the measurement of tissue O_2-pressures a newly elaborated platinum O_2-electrode (LÜBBERS *et al.* [30]) based on the design of SILVER [37] was used.

Functions of the Isolated Kidney Preparation

At flow rates between 3 and 15 $ml \times g^{-1} \times min^{-1}$ which are obtained at perfusion pressures between 50 and 180 mm Hg the glomerular filtration rate (GFR) lies between 0 and 0.8 $ml \times g^{-1} \times min^{-1}$. Autoregulation of flow, i.e. the ability to keep relatively constant the perfusion flow in spite of variations of perfusion pressure, is maintained. The U/P inulin ratio reaches 11. The urine is usually slightly hypotonic ($U/P_{osmol} = 0.8—1.0$). The urine-pH lies at 6.4—6.8. 97—99% of the filtered glucose are resorbed. The percentage of sodium resorbed from the filtered load decreases from 97% at small loads (GFR 0.2 $ml \times g^{-1} \times min^{-1}$) to 65% at the highest GFR (0.8 $ml \times g^{-1} \times min^{-1}$). U/P Na-values range between 0.4 and 0.95, U/P K-values between 1.5 and 10. High U/P K-ratios are observed with low U/P Na-ratios, low U/P K-ratios go together with high U/P Na-ratios. Within a range of flow rates from 0.3—0.8 $ml \times g^{-1} \times min^{-1}$ the O_2- consumption is linearly related to the perfusion flow. At constant flow the O_2-consumption remains practically constant for up to 120 min. At flow rates of 3 $ml \times g^{-1} \times min^{-1}$ the mean oxygen consumption lies at 0.025 ml $O_2 \times g^{-1} \times min^{-1}$, at the highest flow rates (16 $ml \times g^{-1} \times min^{-1}$), the mean O_2-consumption lies at 0.12 $ml \times g^{-1} \times min^{-1}$. The mean ATP (nucleosidtriphosphate) content of 12 kidneys perfused for 10 and 30 min resp. was found at 1.1 μ mol $\times g^{-1}$. A complete and uniform perfusion of all sections of the isolatedorgans could be demonstrated radiographically by the injection of radio-opaque material. The reactivity of the vasculature to arterenol, 5-hydroxytryptamine and their antagonists priscoline and lysergic acid diaethylamide remains unaltered during the perfusion.

Construction and Properties of the O_2 Platinum Microelectrode

A method first described by SILVER [37] has been modified: Pieces of platinum wire of 90—100 mm length (99.999% Pt) and 0.2 mm diameter are clamped in a vertical position. 10—20 mm of the free end are submerged in a solution of KCN (8.5 moles/l) contained in a cylindrical hole drilled into a block of solid graphite. Upon connection of the graphite block and the platinum wire to a source of ca. 3 volt a.c. a current begins to flow which, initially, should reach a current density at the surface of the platinum wire of 60—100 mA × $(mm^2)^{-1}$. Under these conditions the tip diameter of the platinum wire will be reduced to about 0.3—0.25 μ if—at constant voltage—the current has dropped to ca. 40% of its initial value. Subsequently the wires are cleaned in concentrated HCl, rinsed with destilled water and kept in pure ethanol. Glas tubes (Normalthermometerglas, 16 III) of 100 mm length and 3—4 mm outer diameter are cleaned by supersonic waves in a bath of equal parts of 10% RBS-solution (Fa. Roth, Karlsruhe) and methanol. The rinsed and dried tubes are drawn to capillaries. With the aid of a micromanipulator the etched platinum wires are inserted into the capillaries. With an electrically heated loop of chromium-nickel-wire the platinum wire is melted into the capillaries. During the process of melting the capillary tube with the inserted platinum wire is held in a vertical position. At the lower end of the tube a weight of 4 g is attached. By the weight the softened glas is drawn in a thin layer over the tip of the wire. Finally the film of glas usually ruptures in such a way as to leave free the conical tip of the platinum wire. Subsequently the electrodes are checked for electrical conduction. The dry electrodes are dipped several times 20—30 mm deep into a solution of 1.5% Polystyrol[1] in carbon-tetrachloride. The submerged surface of the electrodes has to be moistened completely and bubble-free. During 5 hours in a drying oven at 50°C the solvent evaporates and the electrodes are covered with a thin transparent layer of Polystyrol. After keeping them for 24 hours in destilled water they are ready for O_2-measurements. The outside diameter of the measuring tip of such electrodes lies between 1—3 μ.

For calibration solutions of physiological saline equilibrated at 37°C under atmospheric pressure with mixtures of O_2 and N_2 in different proportions were used. The gas mixtures were analysed after SCHOLANDER. Since the calibration curves for O_2 are linear between 0 and 760 Torr, 3 different gas mixtures only were used for calibration. At a pO_2 of 100 Torr the electrode current lies between 0.1 and 0.5 nA. 90% of the steady state deflection are attained in ≈ 2 sec (overall time for the complete measuring setup i.e. electrode and low impedance nanoammeter). The response time and the difference between the deflection at measuring in a stirred and at measuring in an unstirred medium depends on the thickness of the Polystyrol membrane. With membranes of average thickness the difference amounts to ≈ 5%. For 60% of the electrodes a calibration check after 1 hour revealed a practically unaltered sensitivity. The sensitivity of the other electrodes showed an average decrease of about 10%.

The measuring circuit consists of the Pt-electrode, the medium, a separate Ag-AgCl-reference electrode—which is placed on the humid surface of the organ—a shielded constant voltage supply providing the polarising tension of 700 mV, and a nA-amplifying meter (Fa. Knick, Berlin, type N 13) of a full scale sensitivity of 10^{-11} A. The d.c. resistance of the O_2-electrodes in the polarised state lies at 10^{12} ohms.

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[1] Our thanks are due to Fa. BASF, Ludwigshafen, for samples of Polystyrol III 003.

Results

1. Continuous Measurements of Local O_2-Pressures in the Blood-Perfused Rat Kidney in Situ

In Fig. 1 the local pO_2 is plotted on the ordinate against the depth of insertion of the O_2-electrode into the renal tissue on the abscissa. Zero on the abscissa corresponds to pO_2-values at the renal surface. Due to the exposition to open air the latter values are not representing the surface pO_2 under physiological conditions. From Fig. 1 it can be

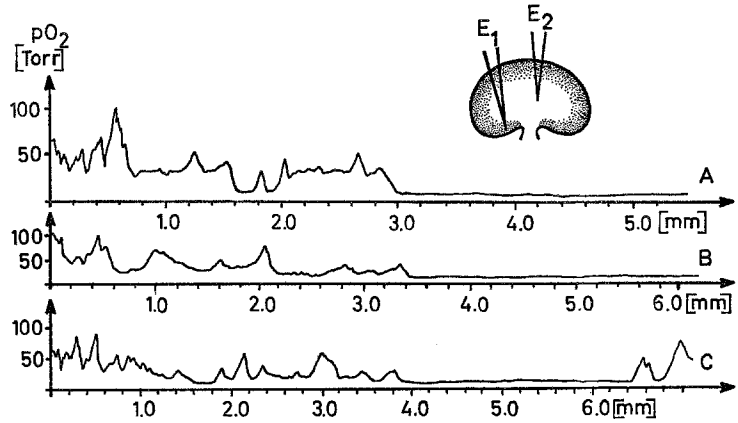

Fig. 1 A—C. Tissue pO_2-values of 3 blood-perfused rat kidneys in situ. Measurements were taken with pO_2-microelectrodes during continuous insertion of the electrodes at constant speed (150 $\mu \times min^{-1}$). In the experiments of Fig. 1 A and B the electrodes had the general direction of electrode E2, in Fig. 1 C that of electrode E1 in the schematic drawing (for further explanation see text). Ordinates: pO_2; Abscissae: Depth of insertion of electrodes

seen that within the cortex (0 to 3—4 mm depth) large differences of pO_2 exist close to each other. The highest pO_2-values correspond roughly with those in the arterial blood. The lowest values lie significantly below the pO_2-values in the renal vein as quoted in the literature. Within a layer of about 200 μ thickness situated at a depth of ca. 3 to 4 mm the pO_2 drops steeply to values of about 10 Torr. Subsequently, during further insertion of the O_2-electrode into the kidney for a considerable distance the pO_2 remains practically constant at this level. In the experiment represented by Fig. 1 C at a depth of 6.5 mm the pO_2 increases steeply and reaches values corresponding to those measured within the first 3—4 mm of insertion. The extension of the region of low and rather constant pO_2 depends on the direction of the electrode

movement relative to the kidney. Preliminary gauge measurements in kidneys which were sectioned along the intrarenal path of the electrode showed that the regions of low and almost constant pO_2 and the zones of high and fluctuating pO_2 correspond with the length of the intrarenal electrode paths in pelvis and medulla and in the cortex respectively.

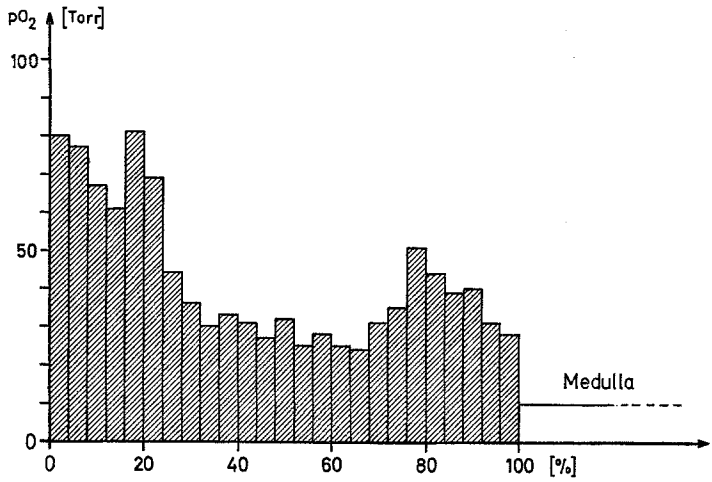

Fig. 2. Cortical mean pO_2-values (averaged over distances of 100 μ) of the kidney of Fig. 1 A. Ordinate: pO_2, Abscissa: Percentage distance from the renal surface to the cortico-medullary boundary. For further explanations see text

The size of the outer region of high and fluctuating pO_2 varies considerably from one kidney to another. In order to facilitate comparison of data obtained from different kidneys we have standardized the distance between the renal surface and the 200 μ wide region of steep change of pO_2 by calling this distance 100%. Thus Fig. 2 contains the mean pO_2-values (averaged over distances of 100 μ) measured in the experiment of Fig. 1 A plotted in percentage of the distance from the renal surface to the cortico-medullary boundary. In the outer 30% of the distance (corresponding to ca. 1 mm depth) regularly a mean pO_2 above that of the deeper cortical regions is found. In the deeper cortical regions pO_2-values above 50 Torr rarely occur. In Fig. 3 the frequency distribution of pO_2-values in the cortex is shown. Almost 50% of all values lie in the range between 20—40 Torr. Only 7 values fall in the range of arterial O_2-pressures. 85% of all pressures measured lie below 70 Torr—the pO_2 in the renal vein. It is noteworthy that even in the cortex some pO_2-values below 10 Torr were found.

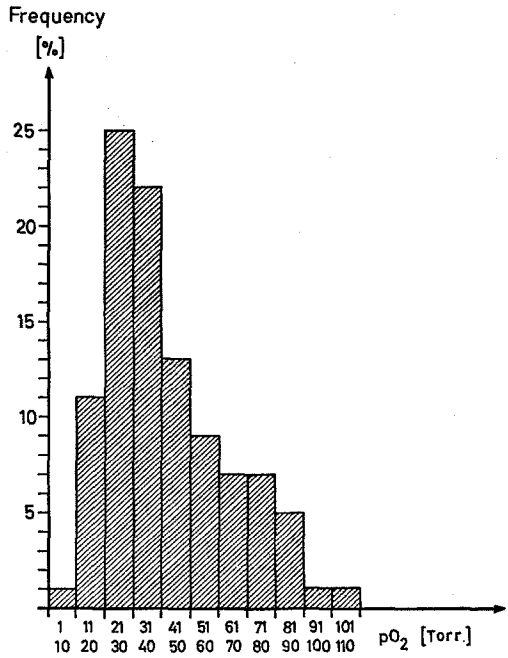

Fig. 3. Frequency-distribution (abundance) of pO_2-values (averaged over distances of 100 μ) measured in the cortex of the blood-perfused rat kidney in situ. No. of measurements (n): 102 (3 kidneys). Ordinate: Percentage of pO_2-values; Abscissa: Ranges of pO_2

2. *Continuous pO_2-Measurements in the Isolated Cell-Free Perfused Rat Kidney*

The data in the Table refer to the isolated kidney. The table contains data of the arterial pO_2, the venous pO_2, the perfusion flow ($ml \times g^{-1} \times min^{-1}$) and the oxygen consumption ($ml\ O_2 \times g^{-1} \times min^{-1}$) of the kidneys in which local pO_2-measurements in the tissue were carried out. The arterial pO_2 ranges between 640 and 690 Torr, the venous pO_2 lies above 250 Torr. At a mean perfusion flow of 9.2 $ml \times g^{-1} \times min^{-1}$ the mean O_2-consumption amounted to 0.10 $ml\ O_2 \times g^{-1} \times min^{-1}$. It can be seen in Fig. 4 that similar to the in-situ experiments a variety of pO_2-profiles is obtained. In contrast to the in-situ preparations, however, in the cell-free perfused kidneys even the highest pO_2-values measured lie far below the arterial pO_2.

The 200 μ wide region of steep change of pO_2, which is found in the in situ organs is also present in the isolated kidneys where the region has practically the same width ($\approx$ 200 μ), and lies in a depth of 3—4 mm. Again, like in the in situ preparation, beyond this zone

Table

Experiment	art. pO_2 Torr	ven. pO_2 Torr	AVD (pO_2) Torr	Perfusion flow $ml \times g^{-1} \times min^{-1}$	O_2-consumption $ml \times g^{-1} \times min^{-1}$
Group No. I. Medium equilibrated with pure O_2, full perfusion flow					
a	640	250	390	11.6	0.145
b	677	410	267	11.0	0.095
c	635	315	320	7.5	0.078
d	665	300	365	8.7	0.103
e	640	270	370	8.9	0.105
f	660	310	350	10.0	0.115
g	690	360	330	8.2	0.087
h	690	300	390	9.3	0.115
i	687	340	347	7.1	0.081
j	690	410	280	10.2	0.092
Mean	667	326	341	9.2	0.102
Group No. II Medium equilibrated with pure O_2 at 50% reduction of the perfusion flow					
k	675	265	410	4.2	0.056
l	690	280	410	5.1	0.067
m	675	265	410	4.1	0.056
n	680	270	410	4.6	0.061
Mean	680	270	410	4.5	0.060
Group No. III. Medium equilibrated with 50% O_2, 50% N_2 at full perfusion flow					
o	360	100	260	8.8	0.073
p	360	100	260	9.5	0.079
q	360	90	270	8.2	0.070
r	300	40	260	10.0	0.083
s	300	20	280	8.6	0.076
Mean	340	70	270	9.0	0.076
A	B	C	D	E	F

a region of relatively constant and low pO_2 ($\approx$ 12 Torr) exists. According to the method used in Fig. 2 the distribution of mean pO_2-values (averaging the pO_2 over 100 μ wide steps) is depicted in Fig. 5. Clearly, the highest pO_2-values occur in the superficial region. Even within the cortex high pO_2-values get significantly rarer with increasing depth. The mean pO_2 of the 100 μ steps decreases almost continuously from the surface towards the cortico-medullary boundary. Similar to Fig. 3 in Fig. 6A the frequency distribution of pO_2-values is depicted for the isolated organ. As was to be expected in view of the higher arterial pO_2-values in these experiments, the distribution of pO_2-values covers a comparatively wider range. The maximum of abundance lies at

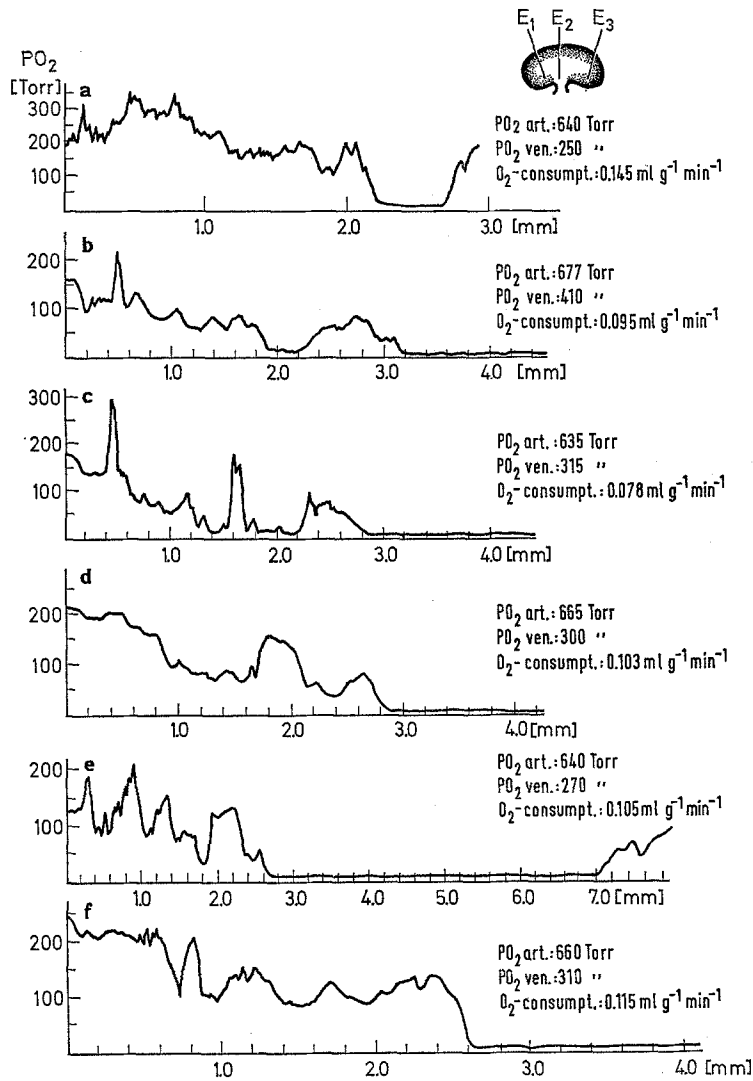

Fig. 4a—f. Tissue pO_2-values of 6 isolated cell-free perfused rat kidneys. Measurements were taken with pO_2-microelectrodes inserted at constant speed (150 $\mu \times min^{-1}$). The direction of insertion of the electrode in a is diagrammatically represented by electrode E3, that of b, c, d, and f by electrode E2, and that of e by electrode E1 in the schematic drawing. Ordinates: pO_2; Abscissae: Depth of insertion of electrodes

pO_2-values between 121 and 140 Torr. 50% of all values range below 140 Torr. Remarkably, only 2% lie above the pO_2 in the renal vein. Also in the isolated kidney pO_2-values below 10 Torr were measured.

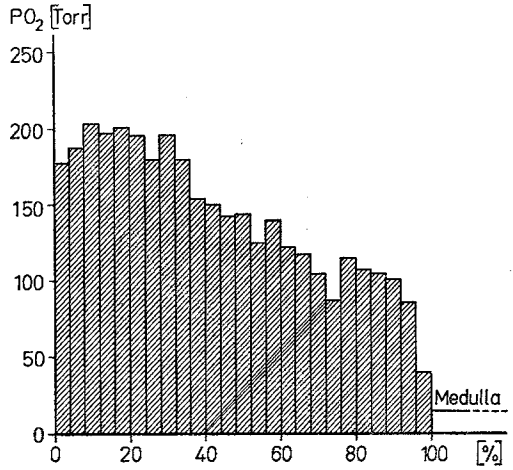

Fig. 5. Cortical mean pO_2-values (averaged over distances of 100 μ) in an isolated cell-free perfused rat kidney. Ordinate: pO_2; Abscissa: Percentage distance from the renal surface to the cortico-medullary boundary

3. pO_2-Distribution in the Isolated Kidney at 50% Reduction of the O_2-Supply

A 50% reduction of the oxygen supply was achieved in two different ways. Firstly by a 50% reduction of the perfusion flow due to lowering of the perfusion pressure (Fig. 6B), and secondly, at full perfusion flow, by saturation of the medium not with pure oxygen but instead with a mixture of 50% O_2 and 50% N_2 (Fig. 6C).

In Fig. 6B a distinct maximum of pO_2-values occurs at 71—80 Torr. All values lie far below the O_2-pressure in the renal vein. Compared to Fig. 6A and Fig. 3 an increase of the number of values below 10 Torr is apparent ($\approx$ 5% of all measurements). The increase of the number of low pO_2-values becomes even more pronounced at perfusion with the medium equilibrated with 50% O_2 and 50% N_2 (12% of all values $<$ 10 Torr) (Fig. 6C). The venous pO_2 in the experiment of Fig. 6C is much lower than that in the experiment of Fig. 6B.

Compared to the experiments with a 50% reduction of the flow rate (Fig. 6B) where all measured parenchymal pO_2-values range below the pO_2 in the renal vein, at perfusion with a medium of reduced pO_2 12% of all tissue pO_2-values lie above the venous pO_2.

Plotting the pO_2-distribution (Fig. 7A) according to the method used in Fig. 2 one sees that the mean pO_2 of the superficial cortical regions—unlike that in the blood-perfused kidney—no longer lies much

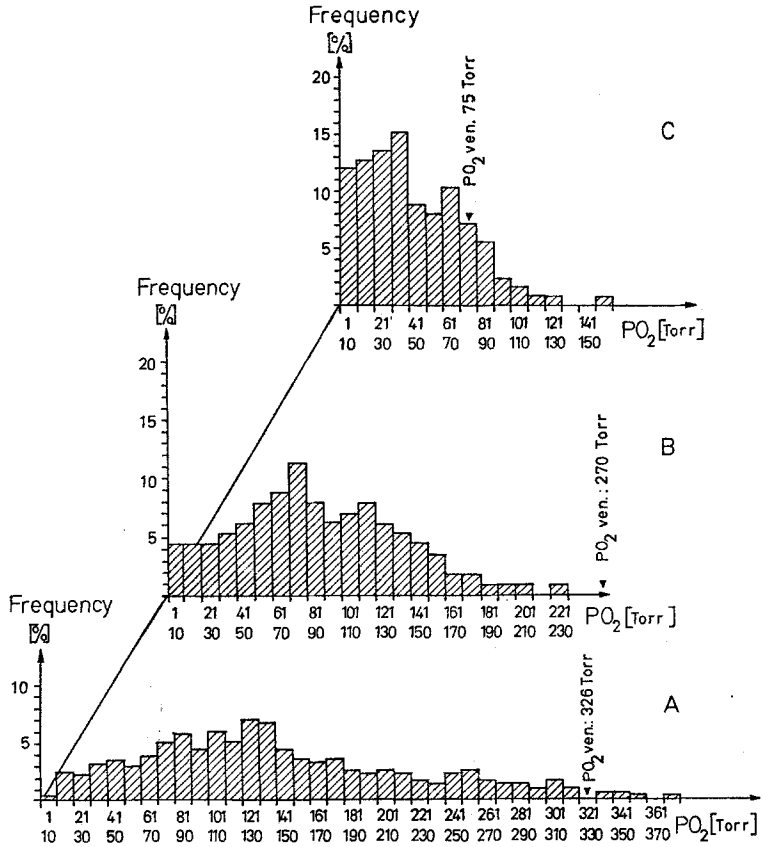

Fig. 6. A: Frequency-distribution (abundance) of pO_2-values (averaged over distances of 100 μ) measured in the cortex of isolated rat kidneys perfused with standard medium saturated with pure O_2 under atmospheric pressure. No. of measurements (n): 314 (11 kidneys). B: Frequency-distribution of cortical pO_2-values (averaged over distances of 100 μ) in isolated rat kidneys perfused at 50% reduced flow rate with standard medium saturated with pure O_2 under atmospheric pressure. No. of measurements (n): 124 (15 kidneys). C: Frequency-distribution of cortical pO_2-values (averaged over distances of 100 μ) in isolated rat kidneys perfused at full flow rate with standard medium saturated under atmospheric pressure with a gas mixture of 50% O_2 and 50% N_2. No. of measurements (n) 125 (4 kidneys). Ordinates: Percentages of pO_2-values; Abscissae: Ranges of pO_2

above the pO_2-values in the deeper cortical regions. This levelling tendency is still more apparent at perfusion with the medium of 50% O_2-saturation (Fig. 7B). Here the scatter of the pO_2-values is somewhat less, and the overall mean pO_2 lower.

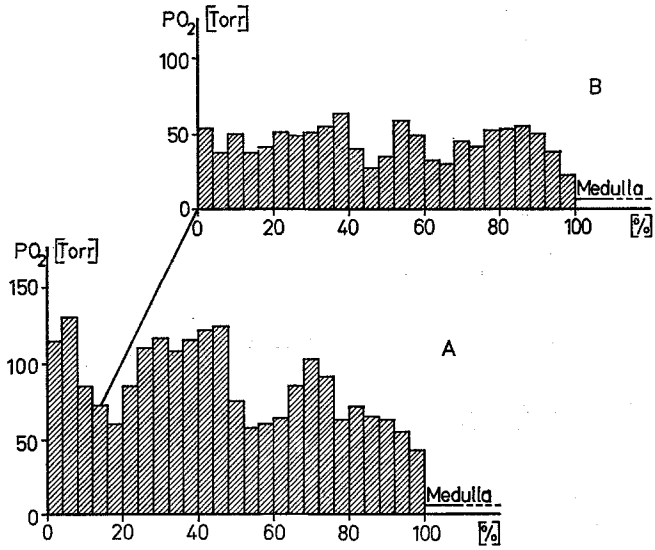

Fig. 7. A: Cortical mean pO_2-values (averaged over distances of 100 μ) of an isolated rat kidney perfused at 50% reduced flow rate with standard medium saturated with pure O_2 under atmospheric pressure. B: Cortical mean pO_2-values (averaged over distances of 100 μ) of an isolated rat kidney perfused at full flow rate with standard medium equilibrated under atmospheric pressure with a gas mixture of 50% O_2 and 50% N_2, Ordinates: pO_2; Abscissae: Percentage distance from the renal surface to the cortico-medullary boundary (for explanation see text of Fig. 2)

4. Attempts at Measuring the Critical O_2-Supply-Pressure in the Isolated Kidney

In Fig. 8 the time course of the pO_2-decrease in cortex and medulla after sudden stop of the perfusion (i.e. the O_2-supply) is demonstrated. In the cortex the pO_2 decreases much steeper than in the medulla. The pO_2-decrease in the cortex proceeds with two different slopes before it reaches its final bent part. The point at which the curve begins to bend away from the straight line into the final portion is taken to indicate the parenchymal critical O_2-supply-pressure, i.e. the minimum pO_2-value above which the O_2-consumption becomes independent of the pO_2. With this method critical parenchymal O_2-supply-pressures were determined by 49 measurements in 7 isolated kidneys. As is demonstrated in Fig. 9 A the critical pO_2-values lie between 6 and 28 Torr. A significant accumulation is found around 8 Torr. Fig. 9 B contains critical local O_2-supply pressures from 30 measurements in 2 kidneys which were perfused with a medium containing the decoupling agent 2,4-dinitrophenol (DNP) in a concentration which, under our experimental condi-

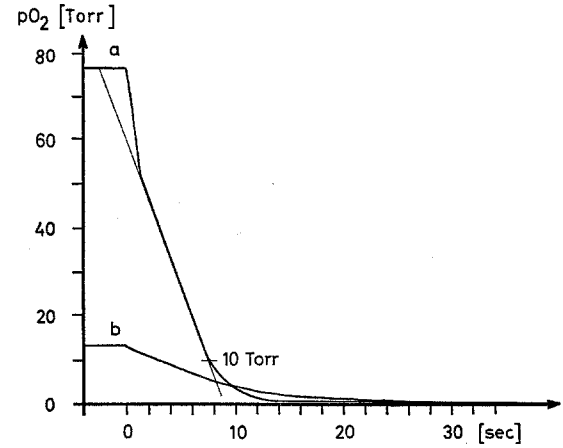

Fig. 8. The time course of the pO_2-decrease in renal cortex (*a*) and medulla (*b*) after sudden stop (t_0) of the perfusion (the O_2-supply). Ordinate: pO_2, Abscissa: Time in seconds

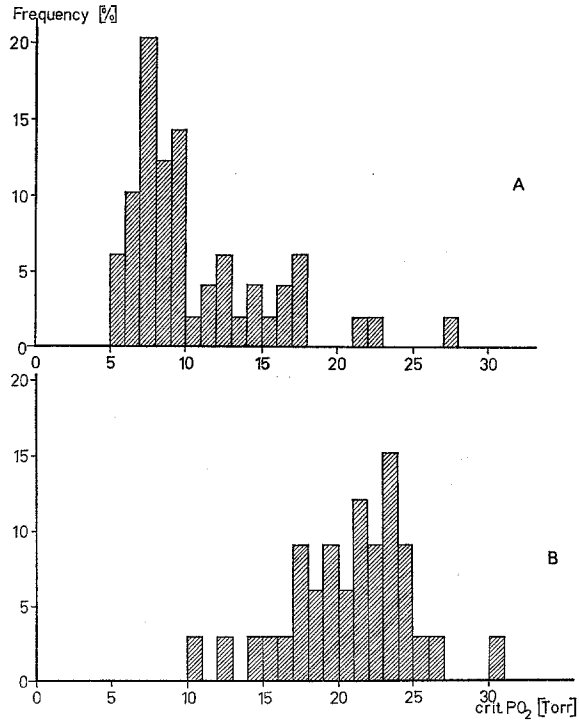

Fig. 9. A: Frequency-distribution of local critical O_2-supply pressures in the cortex of isolated cell-free perfused kidneys. Number of measurements (*n*): 49 (7 kidneys). B: Frequency-distribution of local critical O_2-supply pressures in the cortex of isolated kidneys perfused with standard medium containing 2,4-dinitrophenol (for further explanations see text). Number of measurements (*n*): 33 (3 kidneys). Ordinates: Frequency of pO_2-values in percent, Abscissae: Critical pO_2-values

tions, transiently increases the O_2-consumption. The measurements were carried out during the period of increased O_2-consumption. The mean of the critical local O_2-supply pressures in these experiments is considerably higher than in the experiments of Fig. 9 A. The maximum abundance of critical pO_2 values lies between 21 and 24 Torr.

Discussion

1. The Cortical Oxygen Supply of the Kidney in Situ and of the Cell-Free Perfused Isolated Kidney

The patterns of parenchymal O_2-pressure, represented in Fig. 1 and Fig. 4 from the cortex of the blood-perfused and of the cell-free perfused kidney respectively, are similar to those which have been described by KESSLER [15] for the liver, by KUNZE [22, 23] for skeletal muscle, by SCHUCHHARDT and LÜBBERS [36] for heart muscle and by LÜBBERS [36] for the brain of cat and guinea-pig. In all organs large differences of O_2-pressure occur in close proximity. This is well demonstrated in Fig. 10 (perfused kidney).

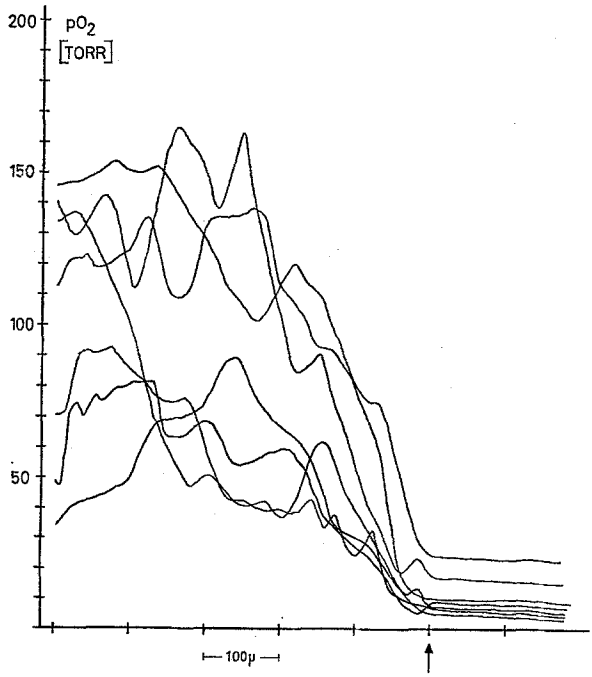

Fig. 10. Curves of the pO_2 of the cortico-medullary region obtained from 7 isolated perfused kidneys during insertion of the O_2-microelectrodes at constant velocity ($150\,\mu \times min^{-1}$). Ordinate: pO_2, Abscissa: Distance in the direction of electrode progress. Abscissa: Distance, taking the low value of the inner zone as beginning (arrow)

The highest measured pO_2-values in the blood-perfused organs lie in the range of the arterial pO_2 between 90 and 100 Torr. The lowest pO_2-values lie at 10 Torr, far below the O_2-pressure in the renal vein.

It seems reasonable to presume that the high pO_2-values occur near arterial vessels, the low pO_2-values in regions which lie far away from capillaries. However, moving the electrode through the tissue we were unable to relate single pO_2-values to topographically defined positions. Therefore, the pO_2-values measured are presented in the form of frequency-distributions (Figs. 3 and 6) which allow a quantitative assessment of the conditions of oxygen-supply [31].

Comparing the cortical pO_2-distribution of the blood-perfused and the cell-free perfused kidney, one sees that pO_2-values in the range of arterial O_2-pressures are absent in the cortex of the cell-free perfused organ. pO_2-values above the pO_2 in the renal vein are much less abundant in the cell-free perfused than in the blood-perfused kidney (see Fig. 1, Fig. 3, Fig. 4 and Fig. 6 A).

Part of the oxygen supplied seems to bypass the respiring parenchyma. Arterio-venous anastomoses in a number and capacity which would explain the apparent shunt effect for O_2 could not be demonstrated so far. The recently elaborated concept of O_2 shunt-diffusion [28, 29, 46] offers a reasonable explanation for the lack of arterial O_2-pressures in the cortex of the cell-free perfused kidney if one assumes that oxygen diffuses in significant amounts from preglomerular and/or precortical arteries into venous vessels. Under conditions of equal oxygen consumption of both preparations—due to the low transport capacity of the cell-free medium and its high arterial pO_2—in the isolated kidney the pO_2-difference between arterial and venous vessels, and thus the effect of O_2 shunt-diffusion should be much larger than in the blood-perfused organ. DUME *et al.* [9] after studying the relationship between the O_2-consumption and the arterial and venous pO_2 arrived at principally similar conclusions.

A 50% reduction of the O_2-supply either by a reduced rate of perfusion (Fig. 7 A) or by perfusion at full flow rate with a medium equilibrated with a mixture of 50% O_2 and 50% N_2 (Fig. 7 B), results in a more uniform distribution and a general reduction of cortical pO_2-values (Fig. 2). In the experiments with a medium saturated with 50% O_2 and 50% N_2, low pO_2-values ($\approx$ 20 Torr) are more abundant than under condition of a 50% reduction of the flow rate (Fig. 6 B, C). Obviously, in spite of equal amounts of oxygen supplied per unit time and unit tissue weight, the relation between oxygen-need and -supply is worse in the first case than in the latter. The Table provides the explanation for this finding: Though the venous pO_2 of the kidneys perfused at reduced flow rate is higher, their O_2-consumption is lower

(0.06 ml $\times$ g^{-1} $\times$ min^{-1}) than that of the organs perfused at full flow rate but half the arterial pO_2 (0.075 ml $O_2 \times g^{-1} \times min^{-1}$).

This finding fits into the concept according to which the renal O_2-requirement (above a certain basal need) is limited by and linearly related to the filtered tubular load. The experimentally well documented assumption of a causal relationship between active tubular transport (especially that of Na^+) and the suprabasal O_2-consumption explains the observed difference of the O_2-consumption between the organ perfused at full flow rate and that perfused at reduced flow rate (Table).

Within the range of experimentally attainable flow rates a linear relationship between flow rate and rate of glomerular filtration (GFR) (i.e. the tubular work load) exists. Therefore, a 50% reduction of the rate of perfusion leads to a 50% reduction of the GFR (the tubular load), thus decreasing the O_2-requirement accordingly. On the other hand, in the organ perfused at the full flow rate but with a medium of a 50% reduced pO_2 the GFR, the tubular load and thus the O_2-requirement remain unchanged. The O_2-supply, however, is cut down by 50%. The Table shows that under these conditions the pO_2 in the venous outflow falls considerably below the value measured at perfusion with a fully O_2-saturated medium. In spite of the increased O_2-extraction, however, only about 75% of the O_2-requirement is satisfied, as can be seen by a comparison of the mean O_2-consumption of the kidneys in the two groups of experiments (Table, group I, column F, and group III, column F).

2. The Oxygen Supply of Renal Medulla and Pelvis

Preliminary coarse gauge measurements of the thickness of the regions of different mean O_2-pressures in the kidney have revealed that the low, relatively constant O_2-values of about 10 Torr (maximal deviations of only 2—3 Torr) occur in the renal medulla and pelvis (urine). This is true for the blood-perfused, as well as for the cell-free perfused organ.

The mean pO_2 in medulla and pelvis lies significantly below the pO_2 in the venous blood or the outflowing medium. This complies with results of renal pO_2-measurements with O_2-electrodes of comparatively large diameter (Ulfendahl [43]; Aukland and Krogh [1]; Deetjen *et al.* [5]). Low medullary pO_2-values which lie below the pO_2 in the renal vein, fit well into the concept of O_2 shunt-diffusion mentioned above. After injection into the renal artery of fully oxygenated blood containing labelled red cells, Levy and Sauceda [28] observed a rise of O_2-pressure in the renal vein before labelled erythrocytes had reached the site of venous pO_2-measurement. With regard to the specific topomorphological arrangement of the vasa recta in the renal medulla

where—at the open end of the hair-pin-like vasa recta loops-arterial inflow and venous outflow lie close to each other (and run parallel over the entire length of the vessel) the authors have assumed O_2-shunt diffusion to occur in the upper region of this vascular system. LONGLEY *et al.* [29] who observed a delayed equilibration of the medullary parenchyma with the swiftly diffusing Krypton 85 supplied supporting evidence for this hypothesis.

3. Critical Oxygen Supply and Local pO_2

In order to ascertain independence of the rate of O_2-consumption from the local pO_2 even of those respiring structures which lie farthest away from the capillaries, a defined minimum local pO_2 has to be maintained. The value of this critical local O_2-supply pressure is determined firstly by the critical pO_2 of the O_2-consuming structures, the mitochondria, and secondly by the minimum pO_2-difference which is necessary to drive per diffusionem a sufficient amount of oxygen from the place of actual pO_2-measurement to the place of O_2-consumption. Hence the latter distance significantly influences the values of the measured critical local O_2-supply pressure. Since in our experiments this distance varies statistically, a considerable scatter of the values of the measured critical local O_2-supply pressures has to be expected. Accordingly, the critical local O_2-supply pressures which we determined in 7 kidneys range from 6—28 Torr with a maximum abundance at 8 Torr.

All local critical O_2-supply pressures measured lie above the critical pO_2 of liver mitochondria of about 1—3 Torr, as reported by KESSLER and LÜBBERS [16]. Supposing, a critical pO_2 within a similar range would apply for renal mitochondria, at the lowest measured local critical pO_2 of 6 Torr a mean pressure difference of 3 Torr would remain for the diffusional transport of O_2 from the place of measurement to the place of consumption. In all probability the tip of the O_2-electrode should eventually get in direct contact with the surface of mitochondria. If the latter assumption is correct it could indicate that the lowest measured local critical pO_2-values of 6 Torr represent the critical pO_2 of renal mitochondria. However, considering the average size of mitochondria (≈ 0.5—$1.2\ \mu$) and the extension of the sphere around the O_2-electrode the pO_2 of which significantly (to 93%) influences the measurement (—at O_2-electrodes of a tip diameter of $2\ \mu$ a sphere of $4\ \mu$ diameter—) the above conclusion is obviously of no value.

According to studies of WEISS [44] the critical O_2-pressure of isolated tubular cells from rat kidneys lies at 16.5 Torr. This critical pO_2 is considerably higher than the most frequently observed critical O_2-supply pressure of 8 Torr, which we measured in the present experiments.

It lies, however, still within the scatter (6—28 Torr) of our measurements. Higher critical O_2-pressures at the cell surface of the isolated cells could be caused by a spacial rearrangement of mitochondria in the form of columns. Another possible explanation for the relatively high critical O_2-pressure of isolated cells from the renal cortex is furnished by the observations of KESSLER [15] according to which the critical O_2-pressure of liver mitochondria increases linearly with the rate of respiration. The mean O_2-consumption of the isolated cells in the experiments of WEISS [44] and GRUBE *et al.* [11] lay at 0.129 ml $O_2 \times g^{-1} \times min^{-1}$, the mean O_2-consumption of the kidneys in the present study (as is shown in the Table) at only 0.1 ml $O_2 \times g^{-1} \times min^{-1}$. The question remains open wether these two explanations apply. Under conditions of increased respiration, brought about by dinitrophenol, higher local critical O_2-supply pressures are measured (Fig. 9B). These increased critical O_2-supply pressures can be explained by the higher pO_2-gradients being a prerequisite for the diffusion of a larger amount of oxygen from the capillaries to the more actively respiring mitochondria. Higher supply pressures could also indicate that the critical O_2-pressure of kidney mitochondria too is related to the rate of respiration.

The decrease of the local parenchymal pO_2 after stop of the perfusion does not proceed at a uniform rate (Fig. 8). In recordings of typical experiments three curve sections can be distinguished. The initial section of the curve decreases with constant maximum slope. The slope of the second part is less but also rather constant. Presumably a number of different factors, the relative influence of which cannot yet be estimated, are responsible for the shape of the curve.

1. Immediately after stop of the perfusion a swift equalisation of local pO_2-differences has to be expected. This breakdown of parenchymal pO_2-gradients can best be observed in experiments where the O_2-electrodes lie initially in a zone of high pO_2. This can be prevented by selecting a position for the electrode prior to the stop of perfusoin in a region of relatively low pO_2.

2. After stop of the perfusion and cessation of glomerular filtration tubular transport proceeds until the tubules collapse. Therefore the O_2-consumption due to active tubular transport will go on for some time. According to HIERHOLZER and WEISS (unpubl. obs.) the time elapsing from perfusion stop to tubular collapse in the isolated kidney lies between 9 and 15 sec. This is much longer than the duration of the steep initial phase of the pO_2-decrease which lasts one or two seconds only.

3. Inevitably, after stop of the perfusion—presumably due to intravasal shifting of vascular contents from the high pressure to the low pressure sections of the system—movements of the tissue relative to the fixed O_2-electrode occur, precluding a detailed interpretation of the initial fast sloping sections of the pO_2-curve. The response of the O_2-electrode is slow (90% of the steady state deflection are reached in $\approx$2 sec) compared to the rate of pO_2-decrease during the initial phase ($\approx$ 15 Torr $\times$ sec^{-1}). Therefore the slope of the pO_2-decrease cannot be used for an evaluation of the rate of parenchymal respiration.

During the second phase of pO_2-decrease which proceeds also at constant but slightly slower rate (9—10 Torr $\times$ sec^{-1}), the levelling by diffusion of parenchymal pO_2-gradients as well as the resorption from the collapsing tubules should come to an end. The "basal O_2-consumption", i.e. the O_2-consump-

tion of the non-filtering kidney, should go on until the local O_2-supply pressure or the O_2-pressure at the mitochondria reach the critical values. With regard to the above mentioned factors which contribute in a complex manner to the O_2-consumption after stop of the perfusion it seems highly improbable that the O_2-consumption at any moment during this phase is truly constant. However, only if this condition were granted the critical pO_2-values measured in our experiments would represent characteristic limiting values for the renal O_2-supply.

4. Conclusions

In the kidney neither the vascular arrangement nor—as far as it is known—the intrarenal distribution of blood-flow (flow of medium) fit the model of KROGH's cylinder or the cone-counter-current model elaborated by DIEMER [6, 7]. In the renal cortex the vessels are arranged as a three-dimensional network. In such capillary meshworks O_2-diffusion-shunts according to the concept of WIRZ [46] and LEVY and SAUCEDA [28] as well as preferential hydrodynamic connections between arterial inflow and venous outflow as discussed by PASSOW *et al.* [33] can occur. It cannot yet be decided whether a special arrangement of capillary loops in the form of "asymmetric meshes" (GRUNEWALD and LÜBBERS [12]) is realized in the kidney. Such asymmetric meshes would provide conditions for an optimum effect of the oxygen supplied. In all models except that of KROGH, arterio-venous O_2-shunts can occur. At present, measurements of the local pO_2 in the parenchyma are the only direct method supplying quantitative information on the condition of oxygen supply in respiring tissue. By surveying fields of pO_2-differences in the parenchyma pO_2-gradients between capillaries are measured which occur as a result of intercapillary O_2-transport by diffusion. For any steady state condition of the oxygen supply a characteristic frequency-distribution of parenchymal pO_2-values can be found. From pO_2-frequency-distribution diagrams the actual situation regarding the relation between O_2-requirement and O_2-supply can be quantitatively evaluated. The pO_2-frequency-distribution diagrams presented are based on data obtained by continuous measurements with an electrode moving at constant velocity through the respiring tissue. The mean pO_2-values do not allow to recognize even large pO_2-differences which may exist across small distances. Hence such diagrams do not provide information on the conditions of O_2-supply on a micro-scale. The values of the mean pO_2 in the above mentioned diagrams represent the mean values averaged over a distance of 100 μ (Fig. 2). Accordingly, finer details of the parenchymal pO_2-profile are not resolved. The information in the diagrams of the (100 μ) mean pO_2-values correspond to a power of spacial resolution which would be obtained by using O_2-electrodes of a tip diameter of about 50 μ. Photometric studies have shown that provided the cytochromoxydases are oxidised to 97% or more, no anoxic areas

exist. Under this condition, however, local pO_2-values as low as 1—2 Torr may be found. The measurement of low local pO_2-values alone, without information on the frequency-distribution of pO_2-values, does not allow valid conclusions to be drawn on the parenchymal O_2-supply.

From those of our results which suggest that significant amounts of O_2 bypass the respiring tissue it follows that the finding of high pO_2-values in the renal vein does not exclude anoxic situations in the parenchyma. In kidneys perfused with cell-free media of high O_2-pressure and low O_2-capacity conditions are favouring the bypass diffusion of oxygen. Indeed, such experimental conditions are a means for demonstrating the phenomenon of shunt-diffusion. If significant amounts of O_2 bypass the respiring tissue raising the arterial pO_2 does not lead to a proportional increase of the amount of oxygen available to the tissue.

References

1. Aukland, K., and J. Krogh: Renal oxygen tension. Nature (Lond.) **188**, 671 (1960).
2. Clara, M.: Die arterio-venösen Anastomosen. Wien: Springer 1956.
3. Deetjen, P., u. K. Kramer: Na-Rückresorption und O_2-Verbrauch der Niere. Klin. Wschr. **38**, 680 (1960).
4. — — Die Abhängigkeit des O_2-Verbrauchs der Niere von der Na-Rückresorption. Pflügers Arch. ges. Physiol. **273**, 636 (1961).
5. — D. W. Lübbers, F. Sugioka, and K. Kramer: Unveröffentlichte Befunde, zit. nach P. Deetjen: Normal and critical oxygen supply of the kidney. In: Oxygen transport in blood and tissue. Ed. Lübbers, Luft, Thews, and Witzleb. Stuttgart: Thieme 1968.
6. Diemer, K.: Über die Sauerstoffdiffusion im Gehirn, I. Mitteilung. Pflügers Arch. ges. Physiol. **285**, 99 (1965).
7. — Über die Sauerstoffdiffusion im Gehirn, II. Mitteilung. Pflügers Arch. ges. Physiol. **285**, 109 (1965).
8. Doby, T.: The number of direct arterio-venous anastomoses in the kidney. Acta med. Acad. Sci. hung. **3**, 209 (1952).
9. Dume, Th., K. M. Koch, H. H. Krause u. B. Ochwadt: Kritischer venöser Sauerstoffdruck an der erythrocytenfrei perfundierten isolierten Rattenniere. Pflügers Arch. ges. Physiol. **290**, 89 (1966).
10. Gänsslen, M.: Der feinere Gefäßaufbau gesunder und kranker menschlicher Nieren. Ergebn. inn. Med. **47**, 275 (1934).
11. Grube, G., K. Philipps u. Ch. Weiss: Die Wirkung von Änderungen der Natriumkonzentration im Suspensionsmedium und von Strophanthin auf die Sauerstoffaufnahme isolierter Nierenzellen der Ratte. Z. ges. exp. Med. **143**, 150 (1967).
12. Grunewald, W., u. D. W. Lübbers: Die Bedeutung asymmetrischer Capillarstrukturen für die Sauerstoffversorgung der Organe. Pflügers Arch. ges. Physiol. **289**, R 98 (1966).
13. Hammersen, F.: Gibt es arterio-venöse Anastomosen in der Niere? Dtsch. med. Wschr. **87**, 147 (1962).
14. Hardwick, D. F., J. Strauss, and G. A. Misrahy: Kidney oxygen availability. Amer. J. Physiol. **205**, 322 (1963).

15. Kessler, M.: Normale und kritische Sauerstoffversorgung der Leber bei Normo- und Hypothermie. Habilitationsschrift, Marburg 1967.
16. —, u. D. W. Lübbers: Bestimmung des kritischen Sauerstoffdruckes an isolierten Lebermitochondrien. Pflügers Arch. ges. Physiol. **281**, 50 (1964).
17. Kiil, F., K. Aukland, and H. E. Refsum: Renal sodium transport and oxygen consumption. Amer. J. Physiol. **201**, 511 (1961).
18. Kramer, K., and P. Deetjen: Sodium reabsorption and oxygen consumption of the kidney. Excerpta med. (Amst.) **29**, 50 (1960).
19. — — Beziehungen des O_2-Verbrauchs der Niere zu Durchblutung und Glomerulusfiltrat bei Änderung des arteriellen Druckes. Pflügers Arch. ges. Physiol. **271**, 782 (1960).
20. Kruhøffer, P.: Transport of fluid in the nephron as a whole (relationship between glomerular and tubular factors). Handb. Exp. Pharmakol., vol. 13, II. Kap. IV, p. 261. Berlin-Göttingen-Heidelberg: Springer 1960.
21. Kügelgen, v., A., W. Kuhlo u. K. J. Otto: Die Gefäßarchitektur der Niere. Stuttgart: Thieme 1959.
22. Kunze, K.: Die lokale, kontinuierliche Sauerstoffdruckmessung in der menschlichen Muskulatur. Pflügers Arch. ges. Physiol. **292**, 151 (1966).
23. — Normal and critical oxygen supply to the muscle. In: Oxygen transport in blood and tissue. Ed.: Lübbers, Luft, Thews, and Witzleb. Stuttgart: Thieme 1968.
24. Langley, J. N.: The course of the blood of the renal artery. J. Physiol. (Lond.) **60**, 411 (1925).
25. Lassen, N. A., O. Munck, and J. Hess-Thaysen: Oxygen consumption and sodium reabsorption in the kidney. Acta physiol. scand. **51**, 371 (1961).
26. Leichtweiss, H.-P., H. Schröder u. Ch. Weiss: Die Beziehung zwischen Perfusionsdruck und Perfusionsstromstärke an der mit Paraffinöl perfundierten isolierten Rattenniere. Pflügers Arch. ges. Physiol. **293**, 303 (1967).
27. Levy, M. N.: Effect of variations of blood flow on renal oxygen extraction. Amer. J. Physiol. **199**, 13 (1960).
28. —, and G. Sauceda: Diffusion of oxygen from arterial to venous segments of renal capillaries. Amer. J. Physiol. **196**, 1336 (1959).
29. Longley, J. B., N. A. Lasser, and L. S. Lilienfeld: Tracer studies on renal medullary circulation. Fed. Proc. **17**, 99 (1958).
30. Lübbers, D. W.: Methods of measuring oxygen tensions of blood and organ surfaces. In: Oxygen measurements in blood and tissues. Ed. J. P. Payne and D. W. Hill. London: Churchill 1966.
31. — Normal and critical oxygen supply of the brain. In: Oxygen transport in blood and tissue. Ed.: Lübbers, Luft, Thews, and Witzleb. Stuttgart: Thieme 1968.
32. Möllendorff, v., W.: Der Exkretionsapparat. Handbuch d. mikrosk. Anatomie d. Menschen, Bd. 7, 1. Teil, S. 1—328. Berlin: Springer 1930.
33. Passow, H., H. Schniewind u. Ch. Weiss: Die Abhängigkeit der Sauerstoffaufnahme der mit zellfreier Blutersatzlösung perfundierten Rattenniere von der Perfusionsstromstärke und der arteriellen Sauerstoffspannung. Pflügers Arch. (im Druck).
34. Piper, J., u. E. Schürmeyer: Über den Nachweis arterio-venöser Anastomosen in der Hundeniere. Pflügers Arch. ges. Physiol. **261**, 543 (1955).
35. Reid, J., and J. R. Derrick: Effect of reduced segmental blood flow on renal parenchymal oxygen tension. Surgery **58**, 390 (1965).

36. Schuchhardt, S., u. D. W. Lübbers: Sauerstoffversorgung und Mikrozirkulation der Herzmuskulatur. Wiss. Z. d. Univ. Halle-Wittenberg (im Druck).
37. Silver, J. A.: The measurement of oxygen tension in tissues. In: Oxygen measurements in blood and tissues. Ed. J. P. Payne and D. W. Hill. London: Churchill 1966.
38. Staubesand, J., u. F. Hammersen: Zur Problematik des Nachweises arteriovenöser Anastomosen im Injektionspräparat. Z. Anat. Entwickl.-Gesch. **119**, 365 (1956).
39. —, u. M. Stoeckenius: Zur Problematik des Nachweises arterio-venöser Anastomosen. II. Mitteilung. Gefäßinjektionen mit Wachskugeln bekannter Größe. Z. Anat. Entwickl.-Gesch. **120**, 115 (1957)
40. Thaysen, J. H., N. A. Lassen, and O. Munck: Sodium transport and oxygen consumption in the mammalian kidney. Nature (Lond.) **190**, 919 (1961).
41. Thurau, K.: Renal Na-reabsorption and O_2-uptake in dogs during hypoxia and hydrochlorothiazide infusion. Proc. Soc. exp. Biol. (N.Y.) **106**, 714 (1961).
42. Trueta, J. A., E. Barclay, P. M. Daniel, K. J. Franklin, and M. M. L. Prichard: Studies of the renal circulation. Oxford: Blackwell 1947.
43. Ulfendahl, H. R.: Intrarenal oxygen tension. Acta Soc. Med. upsalien. **67**, 95 (1962).
44. Weiss, Ch.: Oxygen tension and rate of respiration of isolated kidney cells. In: Oxygen transport in blood and tissue. Ed. Lübbers, Luft, Thews, and Witzleb. Stuttgart: Thieme 1968.
45. — H. Passow, and A. Rothstein: Autoregulation of flow in isolated rat kidney in the absence of red cells. Amer. J. Physiol. **196**, 1115 (1959).
46. Wirz, H.: The kidney. CIBA Foundation symposium, pp. 38—45. Boston: Little 1954.

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