Diffusion of oxygen from arterial to venous segments of renal capillaries

LEVY, MATTHEW N. AND GERARDO SAUCEDA. Diffusion of oxygen from arterial to venous segments of renal capillaries. Am. J. Physiol. 196(6): 1336–1339. 1959.—Injections of three types of blood preparations were made into the renal arteries of dogs, namely, a) blood equilibrated with 95% O₂, 5% CO₂, b) arterial blood containing some methemoglobin-labeled erythrocytes and c) blood containing methemoglobinemic cells, but equilibrated with 95% O₂, 5% CO₂. The initial appearance time in the renal vein was 1.25 ± 0.97 second earlier for oxygen than for the methemoglobinemic red cells. When preparation c was introduced into the renal artery, a diphasic curve was consistently registered from the renal venous blood. The initial deflection was uniformly upright, indicating a preponderant effect due to increased oxygen saturation. This was followed by an inverted deflection, resulting from the predominant effect of methemoglobin. These findings are interpreted to indicate diffusion of some of the oxygen from arterial to venous limbs of capillary loops, probably the vasa recta located in the renal medulla.

DESPITE THE HIGH OXYGEN CONTENT of the renal venous blood, a moderate reduction of renal blood flow elicits a proportionate reduction of oxygen consumption. In this respect, the kidney behaves like a flow-limited tissue, such as the myocardium, in which the major portion of the oxygen is extracted at normal rates of flow. Pappenheimer and Kintner (1) have postulated that the majority of red cells, and hence most of the oxygen passes through shunt pathways within the kidney. Thus, only a small fraction of the oxygen delivered to the kidney would actually come in contact with the parenchymal cells.

In a previous study (2), certain critical objections were raised in connection with the cell-separation theory as it pertains to oxygen utilization. It was found that, even when additional quantities of oxygen were provided in physically dissolved form, the kidney still did not extract oxygen more completely when blood flow was moderately reduced. Furthermore, 2,4-dinitrophenol increased oxygen extraction and utilization at normal renal blood flows, revealing that significantly more oxygen must be present in the peritubular capillary blood than is ordinarily extracted.

An alternative possibility, which could account for the flow-limited behavior of the kidney but which would not be contradicted by these findings, is that oxygen may diffuse between arterial and venous limbs of renal capillary loops. A considerable amount of evidence has accrued recently which indicates that certain solutes diffuse between adjacent segments of the loops of Henle located in the renal medulla (3). The first indication that diffusion may also occur between neighboring regions of the vascular system has been proffered by Longley, Lassen and Lilienfield (4). They have demonstrated that krypton ⁸⁵ attains equilibrium very slowly between the circulatory system and the renal medulla. If these findings imply that krypton ⁸⁵ diffuses from arterial to venous limbs of renal medullary capillaries, it may be predicted that oxygen would also diffuse across this path. The present study was designed to determine whether detectable quantities of oxygen do diffuse between the arterial and venous segments of renal capillaries by comparing the venous time-concentration curve of oxygen with that of labeled red blood cells after injection into the renal artery.

METHODS

Eleven experiments were performed upon mongrel dogs anesthetized with intravenous sodium pentobarbital, 30 mg/kg. Heparin (supplied through the courtesy of Dr. W. R. Kirtley, The Lilly Research Laboratories) was employed to prevent blood coagulation. Mean arterial pressure was registered on a Sanborn four-channel recorder by means of a pressure transducer. The renal vein was cannulated to permit registration of renal blood flow and optical density of the renal venous blood. The latter was measured by means of a Colson densitometer. The renal blood flow was measured by two techniques. In approximately half of the experiments, a differential...
RENAL OXYGEN DIFFUSION

FIG. 1. Changes in optical density \((D)\) of venous blood engendered by intra-arterial injection of highly oxygenated blood \((O_2)\), arterial blood containing some methemoglobin-labeled erythrocytes \((\text{met.})\), and a mixture of the two \((O_2 + \text{met.})\). Upper tracing shows mean arterial pressure \((P)\), in mm Hg. Renal blood flow \((F)\), in ml/min., is actually derived by differentiating electronically the increase in blood volume \((V)\) temporarily collected from the renal vein in a vertical cylinder. Time is indicated in seconds.

A transducer was employed to record the pressure-drop across a resistance interposed into the line leading from the renal vein cannula, as previously described (2). In the remaining experiments, the renal venous outflow was temporarily collected in a vertical cylinder. The hydrostatic pressure at the bottom of this cylinder was recorded as a function of time by means of a sensitive pressure transducer. The amplified output of this transducer was then differentiated electronically, thus permitting flow to be recorded directly.

A T-cannula was inserted into the renal artery to enable three types of blood preparations to be injected directly into the arterial blood stream: \(a\) highly oxygenated blood, \(b\) blood containing labeled erythrocytes, and \(c\) highly oxygenated blood containing labeled erythrocytes. The high degree of oxygenation was accomplished by equilibrating a sample of the animal's arterial blood with humidified 95% O\(_2\), 5% CO\(_2\), in a rotating glass cylinder. Labeling of the red cells was accomplished by treatment with 1% sodium nitrite, followed by three washings with isotonic saline. Microscopic examination of these methemoglobinemic cells revealed no detectable alteration in size or shape. These cells were diluted with equal quantities of plasma or saline, and then with approximately five volumes of arterial blood.

Equal quantities of each preparation were injected sequentially at a constant rate of 1 ml/sec. into the renal arterial blood stream by means of a motor-driven syringe. Usually, either 3 or 4 ml of each were injected, while arterial pressure, renal blood flow, and the optical density of the renal venous blood were being registered.

RESULTS

The results of a typical experiment are shown in figure 1. The first downward spike on the densitometer tracing \((D)\) represents the beginning of infusion into the renal artery of 3 ml of blood equilibrated with 95% O\(_2\), 5% CO\(_2\). The second spike indicates the end of this infusion. An upward deflection of the densitometer tracing is evident 6.4 seconds after the beginning of infusion, indicating the increased light transmission of the renal venous blood due to increased oxygen saturation. Since the dead space time in the venous withdrawal tubing was 4.5 seconds, the initial appearance time for oxygen was 1.9 second.

The second section of figure 1 (labeled "met.") was registered just 3 minutes after the previous record was made. The blood pressure \((P)\) during this interval was slightly lower than during the previous injection. However, the blood flow \((F)\) at the beginning of this tracing was virtually identical with the preceding value. The two downward spikes on the densitometer tracing again represent the beginning and end of the intra-arterial infusion. In this case, the injection consisted of arterial blood withdrawn from the animal, and mixed with some methemoglobinemic blood. The downward deflection in this record indicates the reduced light transmission attributable to these altered erythrocytes. The initial appearance (corrected for dead space) for these labeled cells was 3.5 seconds, as contrasted with 1.9 second for oxygen.

This difference in initial appearance time for oxygen and methemoglobin was characteristic for most of the experiments of this series. The highly oxygenated blood appeared in the renal vein earlier than methemoglobinemic blood in 9 of the 11 experiments in this series. In the remaining two experiments, no difference in initial deflection time could be detected. The mean difference in initial appearance times was 1.25 ± 0.97 sec. (mean ± S.D.), which was highly significant \((P < 0.001)\). Since the measurement of the initial appearance time was
somewhat arbitrary in certain experiments because of the gradual development of the beginning deflection, the times for half of the maximum amplitude were also compared. The mean difference was $0.83 \pm 0.86$ second earlier for oxygenated than for methemoglobinemic blood, which also is significant ($P = 0.005$).

It may be noted in figure 1 that, following the injection of both the oxygenated and the methemoglobinemic blood, there was a brief but definite reduction in renal blood flow. In this experiment, the diminution was very similar both in magnitude and duration for both types of blood injected. Although many factors may have contributed to this decrease in flow, the principal cause was probably a temperature effect. When the blood was warmed to body temperature just prior to administration, this reduction of flow was minimized appreciably or abolished. However, since some degree of alteration of flow was usually observed in response to the intra-arterial injection of a blood preparation, the time-concentration curves in the renal vein for oxygenated and methemoglobinemic blood were affected accordingly.

In order to rule out conclusively the possibility that the earlier appearance time of oxygen relative to cells was a spurious result of altered flow, mixtures of methemoglobinemic cells in arterial blood were prepared and subsequently equilibrated with $95\% O_2, 5\% CO_2$ in seven of these experiments. The results of the intrarterial injection of this mixture are evident in the last segment of figure 1, labeled 'O$_2$ + met'. In the densitometer tracing, following the injection artifacts, a diphasic curve is evident. The first upward portion of this deflection reveals a reduction in the optical density of the renal venous blood, characteristic of increased oxygenation. This is followed by a downward deflection, characteristic of the presence of methemoglobinemic erythrocytes. Similar diphasic curves were obtained in each of the seven experiments in which this mixture was injected into the renal artery. However, considerable variations in the relative amplitudes of the upward and downward deflections were seen in individual experiments, and in the same experiment when the relative concentrations of the two variable ingredients were altered.

### DISCUSSION

Injection of highly oxygenated blood and of blood containing methemoglobin labeled red cells into the renal artery reveals that the oxygen appears approximately one second earlier than the labeled cells in the renal vein. This is confirmed by the finding that when a mixture of highly oxygenated blood containing some methemoglobinemic cells is introduced into the renal artery, the optical density of the renal venous blood is always initially reduced, and subsequently increased. Such a diphasic densitometer curve does not imply, however, that all of the excess oxygen appears first, and is then followed by the methemoglobin-labeled cells. The situation can be explained on the basis of the time-concentration curves shown in figure 2. The solid curve in the upper portion of this figure (labeled 'O$_2$') is a redrawing of the corresponding densitometer tracing in figure 1. The curve is plotted as deflection (in mm) as a function of time (in sec.), corrected for dead-space with withdrawal time. The dotted curve is a redrawing of the densitometer tracing for methemoglobinemic blood shown in figure 1, plotted on the same time axis, but inverted. Furthermore, the amplitude of the curve (in mm) was multiplied by a constant factor (of 1.65) in an effort to reproduce graphically the ratios of optical density changes attributable to increased oxygenation and methemoglobin which must have existed in the mixture prior to injection. In recording the actual curves, the change in optical density produced by the methemoglobinemic blood was considerably greater than that produced by the highly oxygenated blood; accordingly, a higher degree of amplification was employed in registering the curve for oxygenated blood. In preparing the mixture of highly oxygenated, methemoglobinemic blood, thrcorfec, more arterial blood was added to the methemoglobinemic blood preparation prior to equilibration with $95\% O_2$ in an attempt to balance more closely the optical effects.

In passing through the cuvette, the optical effects of methemoglobin are subtracted from those of oxygenated hemoglobin. If the oxygen and cells travelled through the same pathways in the kidney at the same velocities, then the renal venous time-concentration curves should be exact mirror images of each other, except for possible differences in amplitude. Under such circumstances, a mixture of methemoglobinemic cells with highly oxygenated normal cells should produce either no deflec-
tion, a monophasic upright deflection, or a monophasic inverted deflection when the renal venous blood passes through the cuvette, depending upon whether the optical effects due to increased oxygenation are equal to, greater than, or less than those due to methemoglobin in any particular mixture.

The fact that oxygenated blood appears earlier in the renal vein than methemoglobin-labeled cells accounts for the observed diphasic curve obtained when the mixture is injected into the renal artery. It is evident that subtraction of the curve for methemoglobinemic blood from that for highly oxygenated blood will result in a diphasic curve, initially upright. The curve representing the differences between the two redrawn curves is presented in the lower half of figure 2. This curve is quite similar in configuration to the actual densitometer tracing reproduced in the right-hand segment of figure 1.

The finding of dissimilar time-concentration curves for oxygen and labeled cells for the renal circulation indicates that some of the oxygen which passes through the kidney must traverse a shorter pathway than do the red cells. It has already been demonstrated conclusively that the red cells pass through the renal circulation more rapidly than does the plasma (5-7). Since the erythrocytes represent that component of the blood which passes through the vasculature most rapidly, it may be concluded that some of the oxygen must diffuse from the vascular system and re-enter at some point downstream.

Two distinct types of postglomerular capillary networks are present within the kidney (8). The peritubular capillaries supplying the cortical nephrons are arranged in the form of a reticular network. On the other hand, the capillaries located within the renal medulla have a unique anatomical configuration. These vessels, known as the vasa recta, arise from the efferent arterioles of the juxtamedullary glomeruli. They traverse the renal medulla in a radial direction, and form hairpin loops in which the arterial and venous limbs lie in close proximity to each other. Furthermore, in the outer zone of the medulla, they form compact vascular bundles, where numerous arterial and venous segments are interspersed.

On the basis of their anatomical arrangement, it is probable that the diffusion of oxygen indicated by the present series of experiments occurs between the arterial and venous limbs of the vasa recta. This is consistent with the observations of Longley and his collaborators (4), which revealed that krypton\(^{85}\) equilibrated very slowly between the vascular system and the renal medulla. In a system where the arterial and venous limbs of capillary loops lie in such proximity, one would anticipate that a reduction in blood flow would permit more complete equilibration of diffusible constituents, such as oxygen. It is conceivable, therefore, that such a phenomenon may account, at least in part, for the failure of the kidney as a whole to extract additional quantities of oxygen from each unit of blood during periods of reduced renal blood flow. Before the exact role of oxygen diffusion in the apparent flow-limited behavior of the kidney can be evaluated, however, quantitative measurements of the actual rates of diffusion must be obtained under conditions of normal and reduced blood flow.

REFERENCES