Effect of perfusate hematocrit on urea permeability-surface area in isolated dog lung

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PARKER, RICHARD E., ROBERT J. ROSELLI, FREDRICK R. HASELTON, AND THOMAS R. HARRIS. *Effect of perfusate hematocrit on urea permeability-surface area in isolated dog lung.* J. Appl. Physiol. 61(4): 1383–1387, 1986.—Seven dog lower left lung lobes were statically inflated and perfused at a constant rate for each lobe with a perfusate in which the hematocrit was altered over a wide range. The permeability-surface area of urea was calculated from multiple indicator dilution curves using two separate injectates for each hematocrit level. One injectate contained only ^{125}I-albumin as the vascular reference tracer and the other contained both ^{51}Cr-erythrocytes and ^{125}I-albumin as the vascular reference tracers; both contained [^{14}C]urea as the permeating tracer. The results strongly indicate that the phenomenon of "erythrocyte trapping" of urea does not affect the calculation of urea permeability-surface area product provided the appropriate albumin-erythrocyte composite reference tracer is utilized in its calculation.

erythrocyte trapping of urea; multiple indicator-dilution curves; isolated lung; pulmonary circulation

ANALYSIS OF MULTIPLE indicator-dilution data has been shown to be an effective technique in the quantitation of lung microvascular permeability-surface area product (*PS*) for both clinical (1, 3, 4, 10, 11) and laboratory (2, 12–15, 22) investigations. In the most simplistic terms, this technique is based on the comparison of normalized concentrations of an intravascular tracer (i.e., a reference tracer) and a barrier-permeable tracer as they appear after a single pass through an organ following a bolus injection of the tracers at the organ inflow.

The choice of a permeating tracer is predicated on its being barrier-limited (i.e., the tracer flux is limited by the resistance of the walls of the exchange vessels) and not flow-limited (i.e., the tracer flux is not limited by the flow rate through the organ) for the organ studied. The tracer should also have a permeability that is great enough to produce a measurable extraction and should be sensitive to alterations in the permeability and/or surface area of the organ. For the measurement of microvascular *PS*, the permeating tracer should be metabolically inactive (i.e., it should not be metabolized nor be dependent on metabolic processes for its transport).

One such tracer, urea, is commonly used in our laboratory in the measurement of lung *PS*. A major criticism of the use of urea as a permeating tracer is the possible "erythrocyte trapping" of urea (5, 8). The erythrocyte trapping of urea can affect *PS* measurement if the concentration of urea within the erythrocyte does not come to equilibrium with the plasma phase during the time the erythrocyte traverses the pulmonary microvasculature (18). Garrick and Chinard (7) found the half time of urea equilibration with dog erythrocyte was ~0.03 s at 37°C. They speculated that such rapid equilibration supported the use of urea as a lung interstitial marker, but the validity of using urea *PS* (PS_U) as a measure of endothelial permeability was questioned. This latter point was addressed in a series of theoretical papers (16–18), which showed preequilibration of blood with urea prior to injection tended to minimize any error in PS_U caused by erythrocyte exchange. This was verified by Roselli et al. (19) in a series of experiments in sheep in which the hematocrit of the urea injectate was varied. However, in this study Roselli et al. found that PS_U measured with injectates of high hematocrits was $>PS_U$ measured with normal injectate hematocrits.

Based on the PS_U results of Roselli et al. (19) with high hematocrit injectates, it is possible that substances (e.g., endotoxin and phorbol myristate acetate infusions; unpublished observations) which elevate circulating hematocrits in intact animals during the pulmonary hypertension phase of the challenge may affect measured PS_U. Therefore, this investigation was conducted to determine the importance of varying circulating hematocrit on erythrocyte trapping of urea and its influence on the measurement of PS_U in the lung.

METHODS

Animal preparation. Seven healthy mongrel dogs of either sex were anesthetized with pentobarbital sodium (30 mg/kg iv), intubated, and ventilated with room air using a Harvard respirator. The right femoral artery was cannulated with a large-bore polyethylene catheter and ~15 ml of blood drawn into heparinized syringes for labeling with radioisotopes (see below). Heparin (20,000 IU) was given and allowed to circulate for 20 min. The dogs were exsanguinated, and a thoracotomy was made at the left fifth intercostal space. After the heart and lungs were excised, the pulmonary artery and vein of the left lower lung lobe were cannulated, with great care being taken to avoid introducing air bubbles into the pulmonary vasculature. The lung lobe was connected to the ventilation-perfusion system and the remaining lung

lobes removed.

Ventilation-perfusion system. The ventilation-perfusion system is depicted schematically in Fig. 1. Briefly, the perfusion system was designed as follows: the perfusate was pumped by a variable speed roller pump (Masterflex model 7545) from a venous reservoir through a heat exchanger (to maintain the inflow perfusate temperature at 37°C), and through an injection system. The injection system consisted of bifurcating tubing in which one segment contained an injection port and a mixing chamber and a second segment which was set at an equal resistance to flow with screw clamps. The perfusate then passed through an electromagnetic flow probe (connected to a Carolina square-wave electromagnetic flowmeter) and into the lung lobe. The venous perfusate passed through a venous mixing chamber, by a sample collection port, and into a second venous reservoir which was connected to the first venous reservoir. The arterial and venous mixing chambers were used to mimic normal mixing that occurs during passage of a substance through the right and left heart chambers. Static inflation of the lung lobe was accomplished by a compressed gas mixture of 95% O_2-5% CO_2 that was connected to a water column that allowed airway pressure to be set at any desired level.

The perfusate for five experiments was initially a plasma-dextran solution that consisted of half Dextran 40 (Rheomacrodex, Pharmacia), which was diluted with saline to the same oncotic pressure of plasma, with the other half being autologous plasma. The pH of the plasma-dextran solution was adjusted to 7.4 with sodium bicarbonate. Hematocrit was increased by the addition of packed erythrocytes (see below).

Radiolabeling procedure. Two radiolabeled injectate solutions were made. The first injectate (Inj-1) consisted of the plasma-dextran solution to which ^{125}I-albumin and [^{14}C]urea were added. The second injectate (Inj-2) contained radiolabeled erythrocytes. In brief, the procedure for labeling the erythrocytes of Inj-2 was that the blood obtained prior to exsanguination was incubated for 45 min with sodium ^{51}Cr-chromate. The blood was centrifuged and the plasma and leukocytes were discarded. After erythrocytes were washed three times with 0.9% saline the cells were resuspended with saline to its original hematocrit (i.e., the same hematocrit prior to exsanguination). ^{125}I-albumin and [^{14}C]urea were added to the resuspended erythrocytes to obtain equal per unit volume concentrations as Inj-1. Both injectates were gently mixed on a rocker for a minimum of 30 min prior to their use.

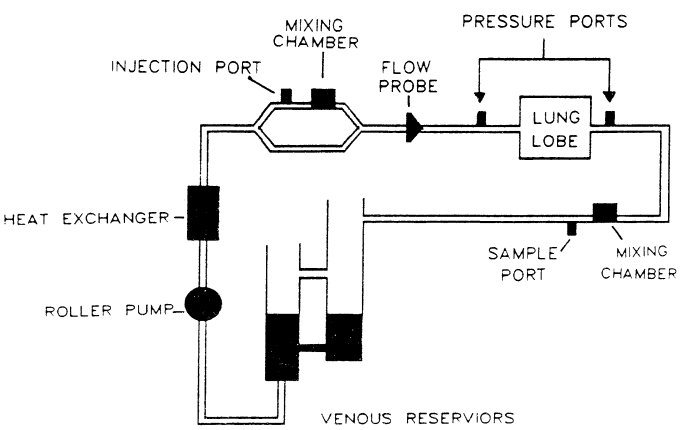

FIG. 1. Schematic of perfusion system.

Experimental protocol. In five of the seven experiments the lung lobes were connected to the perfusion-ventilation system and residual blood flushed from the lobes by perfusing at a rate of 700 ml/min for 20 s, and the resulting effluent being discarded. Perfusion was stopped, the lobes hyperinflated three times to reverse any observable atelectasis, and static airway pressure reset to 4 cmH_2O. Perfusion was then slowly increased until the rate was ~50% higher than that to be used in the experimental protocol. This elevated perfusion rate was maintained for 30 s while the venous outflow pressure (Ppv) was held at 15–20 cmH_2O so as to overcome any artifactual increase in vascular resistance that may have resulted from cessation of perfusion during the isolation procedures. The perfusion rate was adjusted to the rate utilized in the experiment (ranging from 360 to 580 ml/min; mean = 492 ± 20 ml/min). Ppv was set at 5 cmH_2O by raising or lowering the height of the venous blood reservoirs. During the experimental procedures pulmonary arterial pressure (Ppa), Ppv, airway pressure, and perfusion rate were measured. All pressures were referenced to the upper most portion of the lung lobe, thereby maintaining the lung in zone 3 conditions as described by West et al. (21).

The preparation was allowed to reach a steady state for a minimum of 15 min relative to pressures and perfusion rate. During this time we estimated the delay time from the injection port to the sampling port by injecting a precooled bolus of the perfusate and measuring its appearance time at the sampling port with a thermistor tipped probe.

Indicator dilution data were collected by clamping the segment of the injection system that contained the injection port and loading a 1.5-ml aliquot of Inj-1 into this segment. The injection segment was unclamped while concomitantly clamping the other segment of the bifurcation, thereby allowing the injectate bolus to enter the circulating perfusate. After an appropriate delay time we collected venous perfusate from the collection port into a rotating collection wheel at a rate of 2 ml/s and a sampling interval of 0.5 s. The sample collection interval was increased to 1.17 s/sample when the estimated downslope of the indicators began, thereby increasing the overall collection period for maximal tracer recovery measurement. To prevent tracer recirculation during the sampling period, we clamped the lower connection between the two venous reservoirs when we began the sample collection. This procedure was repeated with Inj-2 after allowing a minimum of 5 min for the circulating isotopes to equilibrate throughout the perfusate. The perfusate hematocrit was then increased by the addition of packed autologous erythrocytes which were filtered to remove any large aggregates. The multiple indicator dilution curves were repeated as described above for several perfusate hematocrit levels. To compensate for increased levels of background isotope activity after each set of

curves, we increased the injectate volume in 0.5-ml increments. To minimize edema formation due to the relatively high vascular pressures resulting from the perfusion rate used in the experiments, we decreased the perfusate flow rate to approximately one-third while allowing the added erythrocytes to circulate in the system (usually 12–15 min). However, we always increased the perfusion rate to 50% greater than that of the experimental protocol for 20 s 2 min prior to the next set of multiple indicator dilution curves.

In two of the experiments we altered the experimental protocol in that we began with a high hematocrit level and proceeded to decrease the hematocrit level after each set of indicator dilution curves. This we accomplished by the addition of buffered dextran-saline solution to the venous reservoirs.

The collected blood samples from the multiple indicator dilution curves were then processed as described previously (12) and the radioactivity of ^{125}I-albumin and ^{51}Cr-erythrocytes measured with a gamma spectrometer. After ethanol precipitation, the activity of [^{14}C]urea was measured in a liquid scintillation spectrometer. Both gamma and beta activities were corrected for overlap of the other radioactive tracers.

Theoretical methods. The isolated lung lobe PS_U was computed from the multiple indicator-dilution data using integral extraction techniques as described by Crone (6) and Harris et al. (13). This technique assumes that all capillaries have equal capillary transit times (length/velocity); moreover, the permeating tracer is assumed to be rapidly diluted in the extravascular space, and does not diffuse back into the vasculature during the course of the appropriate segment of the indicator curve as described below. This technique can be expressed by the equation

$$PS_U = -F_W \cdot \ln \int_0^{t_p} (C_D dt) \Big/ \int_0^{t_p} (C_R dt) \qquad (1)$$

where F_W is the intravascular water flow rate for Inj-2 and is plasma water flow for Inj-1, t_p is the time when the intravascular reference tracer concentration (C_R) is maximal, and C_D is the permeating tracer concentration; with both C_R and C_D being normalized to their respective injectate concentrations.

The reference tracer concentration was calculated by

$$C_R = C_{Alb}/C_{Alb0} \qquad (2)$$

for Inj-1 and

$$C_R = \frac{0.7Hct(C_{RBC}/C_{RBC0}) + 0.92(1 - Hct)C_{Alb}/C_{Alb0}}{0.7Hct + 0.92(1 - Hct)} \qquad (3)$$

where Hct is hematocrit and for Inj-2; C_{Alb0} and C_{RBC0} refer to the injectates' concentrations of ^{125}I-albumin and ^{51}Cr-erythrocytes, respectively. *Equation 3* was proposed by Goresky et al. (9) and applies to a tracer for which erythrocytes and plasma phases achieve rapid equilibration, whereas *Eq. 2* applies to a tracer which is unable to enter erythrocytes. The hematocrit value used in *Eq. 3* was assumed to be that of the perfusate since the volume of the bolus was relatively small and passed through a mixing chamber prior to entering the exchange vessels of the lung.

Statistics. Data are expressed as means ± SE where appropriate. Two-tailed paired *t* tests were used to compare the calculated PS_U at any given perfusate hematocrit between the two injectates (20). Dunnett's method of analysis of variance was used to test for significance among the calculated PS_U values for a given injectate over the various perfusate hematocrit ranges.

RESULTS

The perfusate hematocrits used in this investigation ranged from essentially 0–0.66; whereas, the hematocrit of Inj-2 was equal to the original circulating hematocrit of each dog prior to being exsanguinated and ranged from 0.36 to 0.46 (mean = 0.41 ± 0.02). The effect of altering the perfusate hematocrit on PS_U for the two injectates as calculated by their respective reference tracers is depicted by Fig. 2. As can be seen from this figure only PS_U values of Inj-1 were affected by alterations of perfusate hematocrit. This phenomenon was consistent for all seven experiments; regardless of whether the hematocrit was being increased or decreased. Since the hematocrit of the collected samples for Inj-2 was essentially zero when zero hematocrit perfusate was being used, both curves for the zero hematocrit perfusate conditions utilized ^{125}I-albumin as the reference tracer.

A typical experiment for one dog lung lobe is illustrated by Figs. 3 and 4. Figure 3 depicts the extraction patterns and normalized indicator dilution curves for zero hematocrit perfusate conditions for both injectates. Figure 4 is the same as Fig. 3 except that the perfusate hematocrit was increased to 0.34. The PS_U's for Inj-1 and Inj-2 in Fig. 3 were 1.25 and 1.12 ml/s, respectively. However, in Fig. 4 the PS_U for Inj-1 decreased to 0.58; whereas, for Inj-2 PS_U was 1.19 ml/s. The recoveries of urea were within 5% of the recoveries of albumin in most instances.

The effects of hematocrit on the extraction pattern for Inj-1 are even more pronounced at higher perfusate hematocrit levels, as shown by another experimental preparation on Fig. 5. Here the extraction pattern for

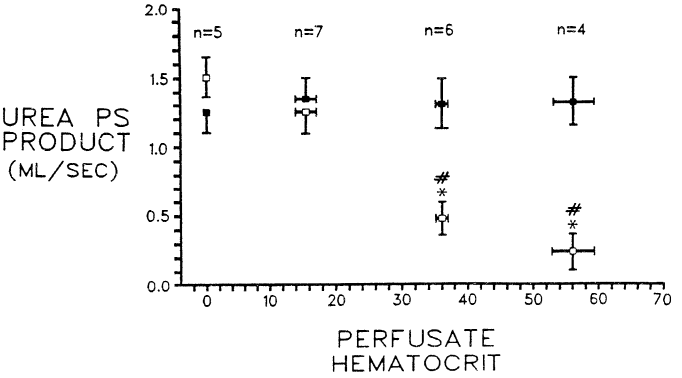

FIG. 2. Effects of perfusate hematocrit on urea permeability-surface area (*PS*) product. First injectate (Inj-1) represented by *open squares* and second injectate (Inj-2) by *solid squares*. *Significant difference for values compared with those obtained for zero perfusate hematocrit; #, significant difference for values obtained for Inj-1 compared with corresponding value of Inj-2.

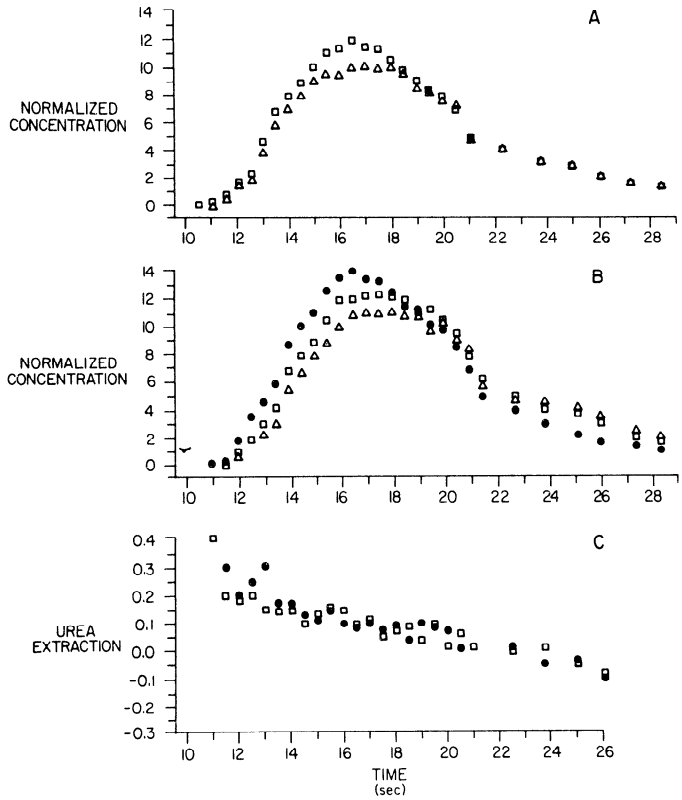

FIG. 3. Normalized indicator dilution curves for first injectate (Inj-1, *A*) and second injectate (Inj-2, *B*) at zero perfusate hematocrit (erythrocytes, albumin, and urea are represented by *solid circles, open squares,* and *open triangles,* respectively); urea extraction pattern (*C*) for Inj-1 is depicted by *open squares* and for Inj-2 by *solid circles.*

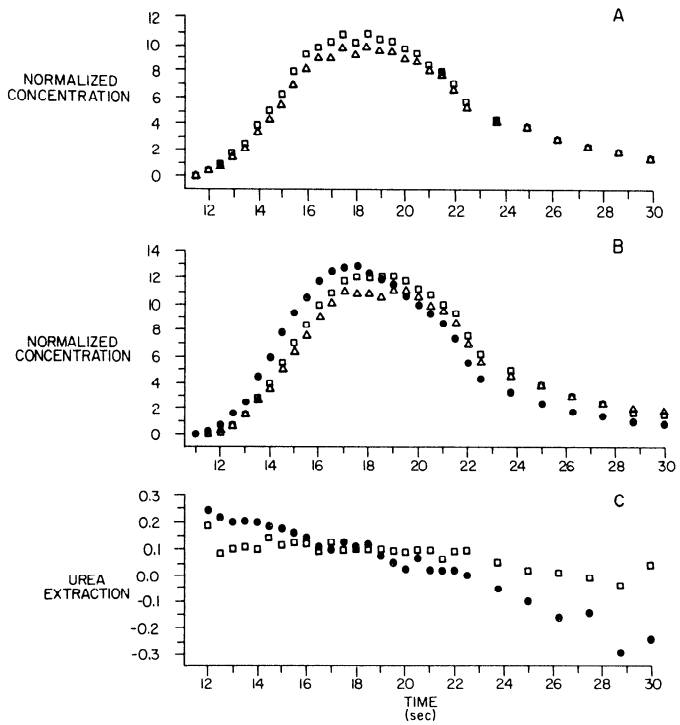

FIG. 4. Normalized indicator dilution curves for first injectate (Inj-1, *A*) and second injectate (Inj-2, *B*) at 0.34 perfusate hematocrit (*symbols* are same as Fig. 3); urea extraction pattern (*C*) for Inj-1 is depicted by *open squares* and for Inj-2 by *solid circles.*

zero hematocrit perfusate is compared with that of a perfusate hematocrit of 0.66.

DISCUSSION

The use of [^{14}C]urea as a permeating tracer for multiple indicator-dilution studies of the lung microvessels offers several advantages over many other commonly used tracers. These include the fact that urea is electrostatically neutral, hydrophilic, has a relatively high permeability, and should not be affected by possible metabolic alterations of the endothelial cells following pathological challenges such as endotoxemia and hypoxia. However, urea enters erythrocytes and thus the erythrocyte trapping phenomenon could affect PS_U calculations. Recently, Roselli et al. (19) reported that PS_U computed with C_R based on *Eq. 3,* in which erythrocytes of normal hematocrit were preequilibrated with urea, was not significantly influenced by this trapping phenomenon in the intact sheep. However, they did find that using a packed erythrocyte bolus preequilibrated with urea gave a PS_U only 60% that of normal hematocrit injectate. Although the authors attributed this to the exchange time of urea into and out of the erythrocyte (7), it is possible that the packed erythrocyte per se may have influenced the measured PS_U (e.g., erythrocyte aggregates being trapped by the lung).

This possibility led us to reinvestigate the effects of the erythrocytes on the measured PS_U in the isolated dog lung lobe. To circumvent the possible problems of packed erythrocytes on the multiple indicator dilution curves we "reversed" the experimental protocol of Roselli et al. (19) in that we altered the perfusate hematocrit instead of the injectate hematocrit. Using a constant injectate hematocrit and varying the perfusate hematocrit should be a more realistic approach for investigation; in that, in intact animal experiments it is the hematocrit of the animal that often changes in response to infusions of substances such as endotoxin. Moreover, since many of these hematocrit alterations are transient it would be impossible for an investigator to ascertain the hematocrit alteration and label erythrocytes prior to the effect subsiding.

This protocol precluded the use of intact animal preparations in that alterations in cardiac output may occur by inducing anemia and thereby alter lung microvascular surface area. Moreover, substantial increases in hematocrit above base line are difficult to attain using autologous blood, and the time required to accomplish the alterations in hematocrit and reach a reasonable steady state would be prohibitive. These and other problems associated with an intact preparation were avoided with an isolated organ preparation. In addition, with an isolated lung lobe preparation we were able to perfuse the lobe with an essentially erythrocyte free perfusate.

The results as indicated by Figs. 2–5 clearly indicate that, provided the appropriate reference tracers are used to calculate a composite reference tracer curve, perfusate hematocrit has no effect on the measured PS_U. This would indicate that the erythrocytes have a great permeability for urea and there is no real trapping of urea. These results are consistent with theoretical predictions

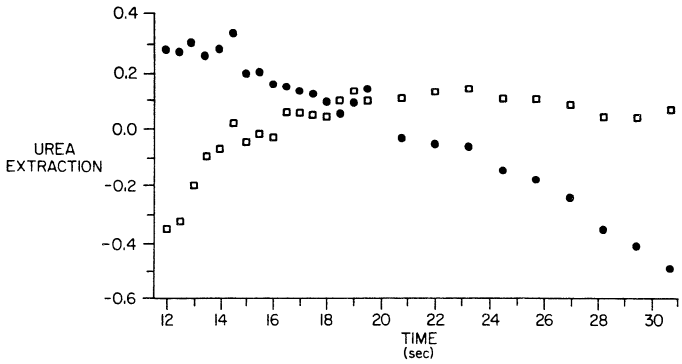

FIG. 5. Urea extraction pattern for a single dog lung lobe using first injectate at perfusate hematocrits of 0 (*closed circles*) and 0.66 (*open squares*).

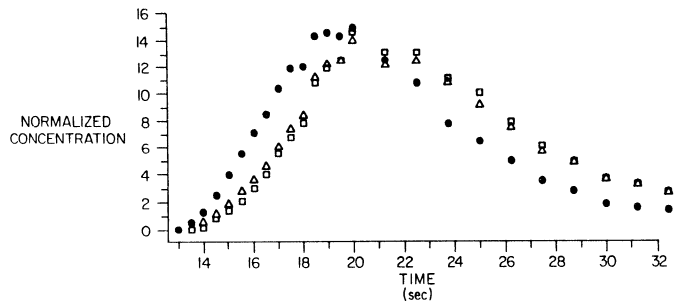

FIG. 6. Normalized indicator dilution curve using second injectate with perfusate hematocrit of 0.66; *symbols* are same as Fig. 3.

(16–18) in which variations of perfusate hematocrit values from 0 to 0.66 had very little effect on the calculation of PS_U, provided the erythrocyte injectate was preequilibrated and PS_U/F_W was >0.3.

The error introduced in the calculation of PS_U, when a plasma reference tracer (albumin) is used instead of a whole blood reference tracer is illustrated by Fig. 6. Figure 6 shows the normalized indicator dilution curves of erythrocytes, albumin and urea for one dog lung lobe in which the injectate had a hematocrit of 0.47 and perfusate a hematocrit of 0.66. It can be seen that except for the last point used in the calculation of PS_U (i.e., the peak of the reference tracers) the concentration of urea was always higher than that of albumin, thereby a negative PS_U would be calculated. This phenomenon is most likely due to the erythrocyte "streaming" ahead of the labeled plasma and the urea within the red cells diffusing out into the unlabeled plasma. Thus urea would be transported ahead of a plasma reference indicator, but not ahead of the composite reference indicators.

In conclusion, the results of this investigation indicate that the calculation of PS_U is not affected by erythrocyte trapping, provided that the proper composite reference curve is used in its calculation. Significant errors may thus occur for any tracer that can enter the erythrocyte if this property is not taken into consideration in the tracer PS calculation.

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