Xenon Handling in the Liver: Red Cell Capacity Effect

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Xenon, despite its lack of chemical reactivity, associates preferentially with red cells in blood. To characterize the effect of this and the nature of xenon-tissue interaction in the liver, multiple indicator dilution studies were performed in the anesthetized normal dog through portal vein injection and hepatic vein collection of anaerobic blood samples. Two experimental runs were carried out in each animal, one at the prevailing hematocrit and the other at reduced hematocrit after bleeding and replacement with dextran. For comparison, the injection mixtures contained labeled red blood cells (a vascular reference), sucrose (an interstitial space reference), and labeled water (which freely enters liver cells), as well as labeled xenon. At the higher hematocrit, the labeled xenon curves generally rose earlier, peaked higher, and decayed more quickly than the labeled water curve; at the lower hematocrit, the xenon curve was delayed and diminished in magnitude in relation to the labeled water curves. Analysis of the curve shapes indicated that xenon, like labeled sucrose and water, underwent delayed wave flow-limited distribution. With knowledge of the red cell plasma partition coefficient (2.89 ml/ml), it was possible to both account for the change in form of the xenon curves with hematocrit and to use the data to estimate the liver cell tissue plasma xenon partition coefficient. Values averaged 1.93 ml/ml liver space, or 1.79 ml/g, and did not change significantly from first to second runs. Theoretical analysis indicated that flow cannot be estimated from xenon downslopes. (Circulation Research 1988;63:767–778)

Xenon is a noble gas that, despite its lack of chemical reactivity, associates with hemoglobin. A partition of xenon between red cells and plasma results, in which the concentration of xenon in the red cell is substantially above that in plasma. The distribution between red cells and plasma in blood then depends not only on the hematocrit but also on the association with hemoglobin. As a result, one would expect the behavior of xenon during transit through an organ to change with change in the hematocrit of the blood perfusing the organ.

In the present study, the tracer transient multiple indicator dilution technique was used to explore the behavior of xenon in the liver of the normal anesthetized dog. The change in the behavior of xenon with change in hematocrit was examined, and a theoretical description of this was developed that incorporates the red cell association and accounts for the variation. The red cell association makes the problem one of general interest. Oxygen, for instance, is expected to be predominantly associated with the red cells in blood (because of its binding to hemoglobin). The study of the behavior of xenon provides, by analogy, a way of developing a set of expectations for how tracer oxygen would be expected to behave in the liver in the absence of metabolic sequestration.

Materials and Methods

To explore whether binding of xenon to hemoglobin resulted in a hematocrit effect in its transit through the liver, multiple indicator dilution studies were carried out in anesthetized dogs at their normal or prevailing hematocrit and, in the same animals, after the hematocrit had been reduced by bleeding and replacement with dextran in a fashion that maintained hemodynamic stability. The indicator dilution studies were performed in dogs anesthetized with pentobarbital sodium (25 mg/kg), with supplemental doses as required. Catheters were placed in the portal vein and the left main hepatic venous reservoir, and in the femoral artery. Approximately
0.5 hours after closure of the abdominal incision, the multiple indicator dilution studies were begun.

In each case, the hematocrit of the injection mixture was matched to that in the animal, and its temperature was raised to body temperature. The bleeding and dextran infusion, in preparation for the second run, had reduced the hematocrit on average to 0.62 of the value originally present in the animal. The injection mixture contained the following reference substances: $^{51}$Cr-labeled red cells, as a vascular reference substance; $^{14}$C-labeled sucrose, as a plasma substance that undergoes flow-limited distribution into the interstitial or Disse’s space but does not enter liver cells; and $^3$H-labeled water, as a reference substance distributed within red cells and plasma, which undergoes flow-limited distribution into both interstitial and hepatic cellular spaces. In addition, the mixture contained $^{133}$Xe, a radioactive xenon species, whose transient behavior was to be defined. The mixture was combined anaerobically with no gas phase, in a glass vial capped with a rubber septum and stored above a mercury seal; aliquots for injection and for the preparation of standards were withdrawn as needed and replaced with mercury. The hematocrit of the second injection mixture was lower, matching that in the animal after bleeding and dextran infusion.

Two milliliters of the mixture were injected into the portal vein as rapidly as possible (over a period of <0.5 seconds) and, simultaneously, the collection of serial hepatic venous samples was begun. Samples were obtained by pumping hepatic venous blood at a rate of approximately 65 ml/min into serial sample wells in an anaerobic mercury trough collector at a rate of about one per 1.5 seconds over approximately 50 seconds. Anaerobic samples, collected by syringes mounted on the sample collector, and anaerobic aliquots from the injection mixture were injected into evacuated tubes capped with rubber septa and containing mercury to provide a seal on inversion. The mercury was necessary to prevent loss of $^{133}$Xe from the sample. The samples were analyzed by use of a gamma ray spectrometer set for the photopeaks of the gamma-emitting isotopes, $^{51}$Cr and $^{133}$Xe. The remaining parts of the samples and standards were processed for assay of the $^{14}$C and $^3$H activity. After protein precipitation with trichloroacetic acid, an aliquot of supernatant was added to scintillation fluid; assay for the beta-emitting isotopes was carried out in a three-channel liquid scintillation spectrometer at appropriate settings. Before assay, the contained $^{133}$Xe activity was lost from this part of the sample during its exposure to air. The proportional contribution of $^{31}$Cr to the recorded scintillation counts, established by use of suitable standards, was small; appropriate corrections were used.

To provide a comparison, the outflowing activity for each labeled species in each sample was normalized by dividing its measured activity by the total injected activity. The normalized outflow dilution curves, expressed in terms of its outflow fraction per milliliter, were plotted in logarithmic fashion against time and corrected for recirculation by linear extrapolation of the downslope on the semi-logarithmic plot. Liver blood flow ($F_b$) was calculated from the reciprocal of the area under the $^{51}$Cr-labeled red cell curve (i.e., from the reciprocal of its activity-time integral). The liver vascular space was calculated as the product of blood flow and the mean transit time (the time integral of the product of time and activity divided by the area under the curve) for the labeled red cells. The transit time for labeled red cells and the other indicators was corrected for the time spent in input and output catheters (their mean transit times were subtracted). The sucrose-accessible interstitial space was calculated as the product of plasma flow and the difference between the labeled sucrose and red cell mean transit times. The extravascular water space was calculated as the product of the water flow and the difference between the labeled water and red cell mean transit times. The flow of blood water ($F_{bw}$) was calculated as

$$F_{bw} = 0.70 F_b Hct + 0.93 F_b (1 - Hct)$$

where $F_b$ is the flow of blood, $Hct$ is the hematocrit, and 0.70 and 0.93 represent the proportions (ml/ml) of the red cell and plasma phases composed of water. The first term on the right corresponds to the flow of red cell water ($F_{rw}$) and the second, to the flow of plasma water ($F_{pw}$). The hepatic cellular water space is then equal to the accessible extravascular water space minus 0.93 (sucrose interstitial space). For each of the indicators, mean transit times were corrected by subtracting the mean transit times of the catheters.

The collecting system produces delay in and distortion of the recorded outflow curves. With the collecting system used, the distortion varies with the rate of change of the outflow profile; hence, effects are larger with the most rapidly changing curve and smaller for the least rapidly changing curve. The distortion does not affect estimates of extravascular volumes of distribution (which depend on transit time differences), but it does affect variables related to curve shape. Therefore, the catheter effect was removed by numerical deconvolution of the known analytical transfer function of the collecting system, and experimental variables were estimated from the recovered catheter input functions.

The indicator dilution protocols were performed in eight animals before and after bleeding. At the conclusion of the experiment, the liver was excised and weighed after it had been passively drained of blood and the gallbladder had been emptied.

### Results

#### Indicator Dilution Data

Values for flow and for transit times of the various indicators are presented in Table 1. In Figure 1, characteristic sets of dilution curves from
an animal at normal hematocrit and reduced hemato-
crit are presented. The outflow profiles are dis-
played in semilogarithmic format in the upper pan-
els and in rectilinear format in the lower panels.

Consider first the forms of the outflow curves for
the labeled reference substances. These are similar
in both runs. The labeled red cells emerge first, their
outflow fraction per milliliter reaches the highest
and earliest peak, and then decays most rapidly.
The labeled sucrose emerges next, and its outflow
fraction per milliliter reaches the later and lower peak
and decays less rapidly. The labeled water curve,
the last of the reference group, has an even more
delayed upstroke, a much reduced and much delayed
downslope. Each outflow curve is corrected for recircu-
lation by extrapolating the downslope linearly on a semilogarithmic
plot. The curves for the two diffusible substances,
sucrose and water, have previously been shown to
be related to that for the labeled red cells in a fashion
that corresponds to delayed wave flow-limited dis-
tribution of these labels into their respective extra-
vascular spaces. 38 The labeled sucrose is expected
to penetrate the Disse's or interstitial space during a
single passage and to reach the surfaces of the
hepatocytes but not enter them; the labeled water is
expected to penetrate both the Disse's space and the
water space in the cells of the hepatic parenchyma
adjacent to the perfused sinusoids.

Now consider the labeled xenon curves. These
each have a form that fits into the systematic
evolution outlined above, in which upslopes become
more delayed, peaks become lower and more
delayed, and downslopes become even more delayed
as the space of distribution available for labeling
progressively increases. Despite this, there is a
striking difference between the experimental run at
normal hematocrit and at reduced hematocrit. At
the normal hematocrit (the left-hand set of panels),
the upslope for the labeled xenon curve is earlier and
rises more steeply, its peak is earlier and slightly
higher, and its downslope is earlier and steeper than
for labeled water. When the hematocrit was reduced
(right-hand set of panels), the labeled xenon curve
followed rather than preceded the labeled water curve. Its upslope was later and less steep, its
peak was slightly lower and later, and its downslope
decayed less steeply than the labeled water curve.

The systematic rightward shift in the xenon curve
with reduction in the hematocrit was found in all of
the experiments. In Figure 2, the hematocrit is
related to the difference between mean transit times
for labeled xenon and labeled water for the whole
set of experiments. A systematic change is evident
in the two runs, even though flow values differed.
As the hematocrit decreased, the transit time differ-
eence shifted to the right in each case. For most
experiments, this involved a change from the situ-
uation in which the xenon curve preceded the labeled
water curve at higher hematocrit to one in which the
xenon curve followed the labeled water curve at
lower hematocrit (i.e., the sign of the transit time
difference changed). In two experiments, the xenon
curve in the initial instance was later than the
labeled water curve at normal hematocrit. During
the second run at reduced hematocrit, the delay in
relation to the labeled water curve became substan-
tially larger in each experiment. The shift to the
right was evident in these experiments as well.
Average values (± SD) for vascular, sucrose interstitial, and cellular water spaces expressed in terms of wet liver weight were 0.230±0.054 ml blood/g, 0.102±0.023 ml plasma equivalent/g, and 0.511±0.080 ml water/g. The values are slightly higher than those reported previously.9 The estimated liver water content corresponds on average to an estimated cell water content of 0.78 ml water/g liver cell. The value approaches the average for water content of 0.74±0.03 ml/g for liver biopsies and 0.80±0.024 ml/g for blood determined from wet and dry weights at the end of the experiment.

Modeling the Outflow of Label

To gain insight into the outflow patterns for xenon, we extended the flow-limited10 modeling previously used to describe the behavior of labeled red cells, sucrose, and water in the liver.5,8 In this modeling, flow is assumed to be confined to the vascular compartment. Diffusion equilibration is assumed to take place so rapidly in the small extravascular space that the concentration at each point along the length is equal to the concentration in the vascular space, and the mechanism transporting material along the length is assumed to be flow (diffusional transport along the length is assumed negligible in comparison with the rate at which material is transported along the length by flow). Flow in the sinusoids is bolus flow (the red cells, which are slightly larger than the sinusoidal lumen, travel single file and segment and mix the plasma). With these assumptions, a simple physical model can be developed.

Consider a sinusoid of length (L) in which blood flows with a velocity (W). Let the concentration of tracer in the red cells be \( y(x,t) \), in plasma \( u(x,t) \), in the Disse’s space \( v(x,t) \), and in the hepatic cellular space \( z(x,t) \), and the relative space ratios for red cells, Disse’s space, and liver cells with respect to the sinusoidal plasma space be \( \beta \), \( \gamma \), and \( \theta \). A common equation of conservation,5 including the behavior of the three references, is then found to be

\[
\frac{\partial u}{\partial t} + W \frac{\partial u}{\partial x} + \frac{\partial v}{\partial t} + \beta \frac{\partial z}{\partial t} + \frac{\partial y}{\partial t} + \beta \frac{\partial v}{\partial x} = 0 \quad (2)
\]

The red cell \(^{51}\)Cr-tracer is found only in the red cells. Its conservation expression is therefore
FIGURE 2. Relation between hematocrit and the difference between the mean transit times of labeled xenon and labeled water. When the value for the transit time difference was negative, the labeled xenon curve precessed that for labeled water; and when it was positive, the labeled xenon curve lagged that for labeled water.

described by the expression in brackets on the left-hand side of the equation. The outflow solution for this, in response to an impulse function or Dirac delta function \( q_0 \delta(x) \) introduced at the origin (where \( q_0 \) is the amount of activity introduced), is

\[
y(L,t) = \frac{q_0}{F_r} \delta(t - \frac{L}{W})
\]

where \( F_r \) is the flow rate of red cells. Thus, the labeled red cells propagate along the sinusoid with flow at the velocity \( W \).

Labeled sucrose does not penetrate the red cells or the liver cells but does instantaneously equilibrate into the Disse's space, so that concentrations in plasma and accessible Disse's space are equal at each point along the length. With this, the outflow profile for labeled sucrose is

\[
u(L,t) = \frac{q_0}{F_p} \delta(t - [1 + \gamma \frac{L}{W}])
\]

where \( F_p \) is the sinusoidal plasma flow. The tracer sucrose travels as a delayed wave, with the velocity \( W/[1 + \gamma] \).

For tracer water, instantaneous concentration equilibration is assumed at each point along the length for red cells, plasma, Disse's space, and liver cells and, with this, the outflow response becomes

\[
u(L,t) = \frac{q_0}{F_{bw}} \delta(t - \frac{1 + \beta + \gamma + \theta}{1 + \beta} \frac{L}{W})
\]

where \( F_{bw} \) is the flow of blood water. The labeled water impulse function again travels as a delayed wave, now with the velocity \( W/(1 + \beta)(1 + \beta + \gamma + \theta) \). Its outflow transit time is \( (1 + \beta + \gamma + \theta)/(1 + \beta)(L/W) \). The ratio \( (1 + \beta + \gamma + \theta)/(1 + \beta) \) corresponds at the level of the microvasculature to the ratio of the total water space to the vascular water space.

Now consider the behavior of labeled xenon. Its description must be formulated in a fashion compatible with the information available on xenon solubility in dog blood.\(^\text{2,3}\) This has been measured on a volumetric basis (ml/ml) and has been formulated in terms of the volumetric solubilities in the two phases, plasma and red cells. For the present description, we will use volumetric space ratios. The parameter \( \beta' \) is defined as the sinusoidal (red cells/plasma) volume ratio; this corresponds to the ratio \( \text{hematocrit}/(1 - \text{hematocrit}) \). At equilibrium, the xenon concentration in the red cells is substantially higher than that in plasma. Hence, to provide an estimate of relative contents in red cells and plasma, the space ratio \( \beta' \) must be multiplied by the relative partition coefficient \( \lambda_{wp} \), describing the ratio of xenon concentrations, red cells/plasma, at equilibrium. For dog blood at 37°C, the \( \lambda_{wp} \) value is 2.89.\(^\text{2,3}\) The partition of xenon between hepatocytes and plasma will similarly be described by a volumetric space ratio, \( \theta' \), and a partition coefficient, \( \lambda_{hp} \). The latter varies with the lipid content of the liver, increasing greatly when it is large because of the high relative solubility of xenon in lipid.\(^\text{2,11}\) Liver retention of \(^{133}\)Xe after inhalation thus becomes very long, for instance, in the presence of hepatic steatosis.\(^\text{12}\) In the present study, we estimate the value of the parameters \( \lambda_{wp} \theta'/\theta \) and \( \lambda_{hp} \) for the normal dog liver. For the two runs, at high and low hematocrits, identical values are expected.

With this background, the conservation equation for xenon is

\[
\frac{\partial u}{\partial t} + W \frac{\partial u}{\partial x} + \gamma \frac{\partial v}{\partial t} + \theta' \frac{\partial v}{\partial x} + \beta'(\gamma + \theta) + \phi = 0
\]

If xenon undergoes instantaneous equilibrium partition between red cells, plasma, and hepatocytes at each point along the length, rather than slow exchange,\(^\text{6,13}\) and if the relative solubility of xenon in the plasma and Disse's spaces is identical, the description becomes

\[
\frac{1 + \lambda_{wp} \beta' + \gamma + \lambda_{hp} \theta'}{1 + \lambda_{wp} \beta'} \frac{\partial u}{\partial t} + W \frac{\partial u}{\partial x} = 0
\]

From this, with an impulse input, the expected output response is

\[
u(L,t) = \frac{q_0}{F_b} \delta(t - \frac{1 + \lambda_{wp} \beta' + \gamma + \lambda_{hp} \theta'}{1 + \lambda_{wp} \beta'} \frac{L}{W})
\]

The labeled xenon impulse travels as a delayed wave, with the velocity \( W/((1 + \lambda_{wp} \beta' + \gamma + \lambda_{hp} \theta')/1 + \lambda_{wp} \beta') \). Its outflow transit time is \( [1 + \lambda_{wp} \beta' + \gamma + \lambda_{hp} \theta']/(1 + \lambda_{wp} \beta') \). With the magnitude of the parameter \( \lambda_{wp} \), the ratio \( [(1 + \lambda_{wp} \beta' + \gamma + \lambda_{hp} \theta')/1 + \lambda_{wp} \beta'] \) will change faster than the ratio \( [(1 + \beta + \gamma + \theta)/(1 + \beta)] \) with change in hematocrit.

The relation between the outflow curve from the whole liver for labeled red cells and the curves for each of the diffusible substances depends on the relation between the distributions of transit times in the large vessels and sinusoids. Goresky\(^3\) has shown
previously that the appropriate description for the liver approaches that extreme in the relations between these distributions in which the large vessel transit times are uniform and the distribution of outflow times is dominated by the distribution of sinusoidal transit times. The proposition was based on the finding that in the analysis of a set of normalized multiple-indicator outflow dilution curves, after a common initial time, the curves for labeled albumin, a group of extracellular reference substances (inulin, sucrose, and sodium), and labeled water could be made to superimpose on the labeled red cell curve by decreasing the subsequent times along each curve by a single factor and compensatorily increasing its magnitude by the inverse of the factor. The phenomenon appears rooted in the substantial proportion of the liver which is vascular, and the extensive proportion of this which is sinusoidal.

The finding can be modeled in the following fashion. The liver has an innate symmetry in which acinar pathways exhibit only a small variation in sinusoidal lengths (L). The distribution of sinusoidal transit times will result chiefly from variations in sinusoidal velocity (W) across the various pathways. F is defined as the total flow through the liver; n(L/W)d(L/W) as the proportion of blood flow through sinusoids with sinusoidal transit times from (L/W) to (L/W) + d(L/W), arising from the distribution of velocities in the sinusoids; C0RBC as the outflow fraction per milliliter outflowing blood for labeled red cells; h(t)RBC as the transport function or probability density function of transit times in the system for labeled red cells; t0 as the common large vessel transit time; and t' = t - t0. Then, we find

\[
F(t)_{RBC} = h(t'_{RBC}) = \int_{t_0}^{t'} n(t) \, dt 
\]

where \( t \) is the dummy variable of integration and (L/W)min is the minimum sinusoidal transit time.

Now consider the behavior of a flow-limited diffusible substance binding to hemoglobin in much greater proportion (oxygen, for instance), the hematocrit effect is larger for xenon (for which \( \lambda_{hp} \) is substantially larger than one) than for water. For a substance binding to hemoglobin in much greater proportion (oxygen, for instance), the hematocrit effect will be expected to be much larger.

**Values Obtained by Fitting the Outflow Curves**

For tracer undergoing delayed wave flow-limited distribution in the fashion expected in the liver,\(^5\) Equation 10 indicates that, after a common large vessel transit time, each point along each diffusible label curve (compared with the reference labeled red cell curve) will have its transit time increased by the factor \( 1 + \gamma \). Because the areas underlying all of the normalized curves are the same, the magnitude of the diffusible label curves will be decreased by the factor \( 1/(1 + \gamma) \). The common to and the respective \( \gamma \) parameters were optimized by reversing these expectations, so that each of the diffusible label curves was superimposed on the labeled red cell curve; a least-squares minimization approach was used to guide the transformation. The optimization was carried out on both the raw experimental curves and the curves corrected for catheter distortion. Average coefficients of variation for the optimized fits for labeled sucrose, water, and xenon for the raw experimental data were 0.067, 0.047, and 0.056, and for the corrected curves, 0.088, 0.066, and 0.075. Values for the respective \( \gamma \) values are given in Table 2. For labeled water, the ratio \( 1 + \gamma \) equals \( (1 + \beta + \gamma + \theta)/(1 + \beta) \). The value for \( \beta \) can be calculated, and the value for \( \gamma \) is known from the sucrose curve; hence, the value for \( \theta \) can be calculated. For labeled xenon, the ratio \( 1 + \gamma \) equals \( (1 + \lambda_{hp} + \lambda_{hp}/(1 + \lambda_{hp} + \beta)) \). In this expression, the parameter \( \beta \) can be calculated and \( \lambda_{hp} \) is 2.89; hence, \( \lambda_{hp} \) is known. The value for \( \gamma \) is known, and, hence, the value for \( \lambda_{hp} \) can be calculated. From this, the value for the combined parameter \( \lambda_{hp} \theta/\theta \) can be determined. Calculation in this fashion provides an estimate of \( \lambda_{hp} \), multiplied by \( \theta/\theta \), where the latter is the quotient of (cellular volume/plasma volume) to (cellular water space/plasma water space). The parameter \( \lambda_{hp} \) itself will be expected to have an average normal value but to be sensitive to the cellular lipid content, to increase greatly when the cellular lipid content is increased.

**Values for \( \gamma \) are given in Table 2.** The derived parameter is \( \lambda_{hp} \theta/\theta \). This parameter reflects the partition of xenon into the liver tissue and will be expected to be independent of hematocrit; it should be the same in both first and second runs in a particular experiment. The values for this parameter, derived from the curves corrected for catheter distortion, are displayed in Figure 3 (values derived from curves for an experiment before and after removal of catheter distortion were also essentially...
TABLE 2. Values for $\lambda_0 \theta' / \theta$, from first and second runs. Lines join the corresponding pairs of values.

<table>
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<tr>
<th>Exp No.</th>
<th>$\beta$</th>
<th>$\beta'$</th>
<th>Parameters from Raw Curves $\gamma_{\text{Xenon}}$</th>
<th>$t_0$ (sec)</th>
<th>Parameters from Corrected Curves $\gamma_{\text{Xenon}}$</th>
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Discussion

Experimental Findings

There are three salient findings that emerge from the present study. The first is that for xenon, the hematocrit has an effect on the shape of the outflow dilution curve. This is a consequence of the higher relative concentration of xenon in red cells than in plasma. The effect can be accounted for by considering xenon red cell and plasma distribution separately. The finding and the approach to it lay a base for future reasoning concerning the distribution of oxygen within the liver. The second finding is that, with the hematocrit effect considered, the labeled xenon outflow curve corresponds to a delayed wave flow-limited distribution within the liver. The labeled xenon outflow curve is symmetrically delayed and diminished in magnitude in relation to the labeled red cells; the flow-limited transformation applies to the xenon curve equally as well as to the diffusable compartment labels, labeled sucrose, and water. The third finding is that a value for the liver cell/plasma xenon partition coefficient arises naturally, as part of

the value for $\theta'$ is (liver cell volume)/(sinusoidal plasma volume). Therefore, $\theta'/\theta=1.11$. Hence, from the average $\lambda_0 \theta'/\theta$ value, $\lambda_0=1.93$. The value indicates that at equilibrium, the association of xenon with a milliliter of liver cells is almost twice that with a milliliter of plasma. This is, of course, why the labeled xenon curve consistently lags that for labeled water at low hematocrits. The partition for xenon may also be expressed in terms of liver weight; its value for liver cells in relation to plasma is then, 1.93/1.08 or 1.79 mL/g. This value is somewhat higher than that found for the kidney at 37° C, also expressed in relation to plasma, 1.50 mL/g, but the lipid content of the normal liver is also higher.

The approximate value for $\lambda_0$ can be deduced as follows. If the specific gravity of liver is ordinarily approximately 1.08 g/ml, if its water content is 0.78 mL/g, and if the water content of plasma is 0.93 mL/ml, the value for $\theta$ is approximately (0.78 x 1.08 x liver cell volume)/(0.93 x sinusoidal plasma volume), and the average value of $\lambda_0 \theta'/\theta$ in the first run was 2.11 and in the second, 2.07. When the values for the two runs were compared by a paired $t$ test, the null hypothesis could not be rejected at $p=0.05$. Our expectation that this parameter would not change systematically, from the first to the second run, was confirmed. For the whole data set, the average value ($\pm$ SD) of the parameter $\lambda_0 \theta'/\theta$ was 2.089 ±0.184, and the average value for the ratio of second run to first run values was 0.991 ±0.074.

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flow fraction per milliliter \((t - t_0)\). Thus, the description of the data. When the xenon partition coefficient is formulated in this fashion, in relation to plasma, its value is found not to change as a function of change in the hematocrit of the blood perfusing the organ, as would have been expected.

**Past Use of Downslopes of Xenon Outflow Curves to Estimate Organ Blood Flow**

In 1951, Kety\(^1\) formulated a set of principles then thought to govern the exchange of an inert gas between blood and tissue. He utilized the ideas that 1) uptake by tissue would be reflected by the product of flow and the arteriovenous difference; 2) the effective concentration of inert gas in tissue, in relation to that in blood, could be expressed by the local tissue concentration divided by the partition coefficient tissue/blood for that gas; 3) if a single tissue concentration value is assumed, exchange between blood and tissue could be described by an ordinary differential equation; and 4) for an inert gas, blood and equivalent tissue concentrations would virtually instantaneously become the same. The solution to this description indicated that for the special case in which the inert gas is cleared from the tissue by flow in the presence of negligible arterial concentrations, the outflow was an exponential washout, with a rate constant equal to flow divided by the product of the partition coefficient and the weight of the organ.

The Kety development was succeeded by a more detailed kind of capillary modeling, one in which formulations were of a distributed in space nature and were applied to concrete biological problems. The formulations of Renkin\(^3\) and Crone\(^3\) focused on the permeation of materials leaving capillaries poorly, and approximated permeability-surface area values by assuming tracer lost from the vasculature could never return to it. At the same time, Goresky\(^5\) developed the delayed wave flow-limited approach, the large permeability extreme used here,\(^3\) and subsequently showed that when return of tracer to the capillary was allowed and when longitudinal (or axial) diffusion was considered negligible relative to transport (as it would ordinarily be, in view of the magnitude of diffusion coefficients, the lengths of capillaries, and capillary transit times\(^1\)), the analytical solution to the distributed in space description encompassed both the Renkin-Crone situation and, in the large permeability asymptote, the delayed wave flow-limited case.\(^1\)

The true nature of the Kety formulation for the description of the distribution of inert gas became clear when Perl and Chinard\(^19\) developed a distributed in space formulation with instantaneous equilibration between blood and tissue, and inserted into this an axial or longitudinal diffusion coefficient which could be increased from zero to intermediate values to the case in which axial diffusion was essentially infinite. In the absence of longitudinal diffusion, tracer underwent delayed wave flow-limited propagation. With infinite longitudinal diffusion, diffusible tracer introduced into the capillary-tissue unit at the entrance instantaneously equilibrated from input to output, arriving at the output before a nondiffusible vascular reference (labeled red cells, for instance, which are carried along by flow), and then subsequently left the capillary-tissue unit as a single exponential washout, with a rate constant corresponding to that described by Kety. With intermediate but substantial values for the longitudinal diffusion coefficient, the outflow profiles assumed intermediate forms.

Perl and Chinard\(^19\) developed normalized plots for this single capillary model, in which time was divided by the mean capillary transit time for the indicator, and normalized magnitude (that is, outflow fraction per milliliter) was multiplied by the product of flow and the mean transit time (see Figure 4). The normalized profiles for various longitudinal diffusion coefficients vary from that for the delayed wave impulse function (of unit area), now centered at unit normalized time, to the monoexponential outflow profile, beginning at zero time, with intermediate profiles rising to intermediate peaks and decaying more rapidly. The various profiles illustrated depend on a characteristic parameter \(Pe\), the ratio of a
diffusive effect of the structural elements.

perse the input to produce the observed transit
diffusible and nondiffusible labels is identical leads
that the apparent axial dispersion coefficient for the
times. The value \( D_N \) for the data set illustrated
(0.25) thus appears to characterize only the axial
disperse of transit times in the system, rather
longitudinal diffusion. The findings reemphasize the
ideas that the delayed wave asymptote of this spec-
trum is the realistic biological case for the liver and
that dispersion of transit times in the system, rather
than longitudinal diffusional displacement, is domi-
nant. For the liver, the labeled xenon curve also
superimposes on the labeled red cell curve, and the
mechanism underlying the kinetics for xenon corre-
spond to those underlying the kinetics for labeled
sucrose and labeled water.

The lack of displacement between the trans-
formed diffusible label and red cell curves produces
some additional insight into those models in which
axial dispersion is regarded as kinetically equivalent
to a longitudinal dispersion process. The results
of this modeling have been characterized in terms of
an axial dispersion coefficient \( D_N \), which is the
inverse of the apparent Peclet number. From this
point of view, the results illustrated above can be
reinterpreted. The plot of data for both the nondif-
susible and diffusible labels on the family of normal-
ized profiles would be expected to indicate whether
there is measurable longitudinal dispersion in addi-
tion to the structure-dependent dispersion present
in the data for the nondiffusible label. The finding
that the apparent axial dispersion coefficient for the
diffusible and nondiffusible labels is identical leads
one to infer that longitudinal dispersion has produced
no additional dispersion for the diffusible labels; the
dispersion is, in each case, due only to the struc-
tural elements, to the manner in which they dis-
perse the input to produce the observed transit
times. The value \( D_N \) for the data set illustrated
(0.25) thus appears to characterize only the axial
dispersive effect of the structural elements.

Both the above graphical analysis and the under-
lying theoretical development indicate that there is
no evidence that the Kety downslope hypothesis can
validly be applied to the present set of labeled xenon
data to derive values for flow, even though residue
curves corresponding to the present data set and
external detection studies in which the liver is
shielded from other organs indicate that labeled
xenon leaves the organ with a terminal single expo-
nential downslope characterizing the data. Even if
the hematocrit effect were accounted for, the present
data indicate that, at equivalent flows, xenon down-
slpoe would be more delayed at lower hematocrits.

The findings also bring up the more general ques-
tion of whether the Kety downslope hypothesis is
ever valid in a biological system. The question comes
particularly into focus with the demonstration of
Ceretelli et al that, although microsphere estimates
of flow in dog gastrocnemius muscle at rest and
during exercise correspond precisely to volumetric
venous outflow rates, values estimated from xenon
clearance by use of the Kety hypothesis substantially
underestimate blood flow rates. It appears that as
originally applied, downslope estimates for flow
should be expected to be incorrect since the under-
lying theoretical formulation is inappropriate.

Determination of Mean Transit Time and Blood
Flow From the Area Under the Residue Curve

The logic of indicator methodology, as developed
by Zierler, indicates that the mean transit time (\( I \))
of an indicator is equal to the area under the residue
curve; that is,

\[
I = \int_0^\infty R(t) \, dt = \int_0^\infty [1 - H(t)] \, dt \tag{11}
\]

where \( R(t) \) is the residue function obtained by
dividing the quantity of tracer externally detected at
time, \( t \), by the initial quantity, which is the maximal
quantity detected shortly after injection, and \( H(t) \) is
the cumulative residence time distribution function
defined by

\[
H(t) = \int_0^t h(\tau) \, d\tau \tag{12}
\]

The mean transit time for each indicator is equal to
the ratio of the volume of distribution, \( V \), now
expressed in equivalent milliliters of blood to blood
flow, \( F_b \); that is,

\[
I = \frac{V}{F_b} \tag{13}
\]

From this, blood flow per unit organ weight, \( F_b/wt \),
has usually been obtained by use of the intermedi-
ate relation

\[
V = \lambda_{ob} \left( \frac{wt}{p} \right) \tag{14}
\]

where \( \lambda_{ob} \) is an organ/blood partition coefficient and
\( p \) is the density of the organ. From this
The idea is that, given a known value for $\lambda_{ob}$ and $\overline{t}$ for a material like xenon, one should be able to estimate flow per unit liver weight. This is the manner in which the expression has commonly been used.26-27

This expression can be used with the present data in a converse fashion. With values obtained for $F/R$ and $\overline{t}$ from the dilution curves and liver weight, $\lambda_{ob}$ can be estimated. When determined in this fashion, $\lambda_{ob}$ is the ratio of the amount of indicator contained in 1 ml of the organ (including blood in small and large vessels) divided by the amount of indicator contained in 1 ml of blood when both are in equilibrium. The average value derived from the present data was $0.87 \pm 0.18$. The average was $0.81 \pm 0.19$ ml/ml for first run values and $0.93 \pm 0.16$ ml/ml for second run values. An increase in the $\lambda_{ob}$ value with decrease in hematocrit is evident. The change appears systematic rather than random; the value $\lambda_{ob}$ as derived in this fashion is thus not constant and is substantially smaller than that for $\lambda_{ob}$.

The value $\lambda_{ob}$, the organ/blood partition coefficient, can also be expressed in terms of the parameters developed to describe the present data set. The blood content of the organ needs to be included to carry this out properly. For the liver, this is especially important since the blood content is so much larger than that of most other organs. This can be illustrated by comparing it with another organ, the heart. In the heart, the capillary blood volume is, anatomically, approximately 0.03 ml/g28 (but not all of this is used at low cardiac outputs), and the large vessel volume is approximately the same or slightly larger.29 In the present studies, the total vascular volume in the liver averaged 0.23 ml/g, and the apparent sinusoidal volume, calculated by use of $t$ values, was 0.15 ml/g. The hepatic microvascular volume is therefore about eight times as large as that in the heart. To include the vascular volume in the large vessels in the calculation of $\lambda_{ob}$, we made use of the ratio $V_b/V_s$, where $V_b$ is the total blood volume and $V_s$ is the sinusoidal blood volume, and

$$\frac{V_b}{V_s} = \frac{t_{RBC}}{t_{RBC} - t_0} \quad (16)$$

The resultant expression for $\lambda_{ob}$, the ratio of the xenon content in the whole liver at equilibrium, including the blood space, divided by that in the blood space, each relative to the appropriate volume, is then

$$\lambda_{ob} = \frac{\left(V_b/V_s\right)[1 + \lambda_{ob}b''] + \gamma + \lambda_{ob}\theta']}{\left(V_b/V_s\right)[1 + \beta'] + \gamma + \theta'}$$

And since

$$\frac{\left(V_b/V_s\right)[1 + \beta']}{\left(V_b/V_s\right)[1 + \beta'] + \gamma + \theta'} \frac{1}{\rho} = \frac{V_b}{wt}$$

where $V_b/wt$ is the liver blood volume per unit liver weight, the organ/blood partition coefficient for xenon can be expressed as

$$\lambda_{ob} = \left[1 + \left(\gamma_{ob}/x_{ob}\right)\lambda_{ob}\right] \times \frac{V_b}{wt/\rho} \quad (19)$$

For the present data set, the average value for $\lambda_{ob}$ recalculated in this fashion was $0.95 \pm 0.20$. The average was $0.89 \pm 0.20$ for the first run and $1.02 \pm 0.20$ for the second. The values are, on average, 1.09 times larger than those estimated by use of $F/v/(wt/\rho)$ and xenon transit times. On comparison of the values calculated in the two ways with a paired $t$ test, it was not possible to reject the null hypothesis. The values would have been expected to be identical with ideal data in any case.

The development of the $\lambda_{ob}$ expression in this second fashion allows us to gain some insight into the factors underlying this parameter. The hepatic blood volume per unit liver weight has been found to depend on blood flow; its value increases from 0.13 to 0.27 ml/g, on average, when blood flow increases from 0.01 ml/sec/g to 0.04 ml/sec/g.9 Therefore, the sinusoidal volume could be expected to rise parallel with the flow-induced increment in total blood volume in the absence of the blood volume-reducing effect of sympathetic activation.9 The values of $\lambda_{ob}$ for the liver thus depend not only on the fat content of the liver cells and the hematocrit of the blood but also, more importantly, on the blood flow. Because the cellular volume is not affected by blood flow, the value of $\theta'$ will decrease with an increase of blood volume, and thus it will be expected that the effect on $\lambda_{ob}$ of the increase in blood volume with blood flow will be compensated, to a certain extent, by that of a concomitant decrease in $\gamma_{ob}x_{ob}$. Note that in organs where the microvascular volume is small and $\theta'$ is very high (such as the brain), $\lambda_{ob}$ will, from Equation 17, be approximated by $\lambda_{ob}/(1 + \lambda_{ob}b')/(1 + \beta')$; it will then be almost independent of blood volume.

The residue approach to the determination of the transit time and its utilization, when an appropriate $\lambda_{ob}$ value is available, has the advantage that the whole residue curve could be used, rather than only a part of the downslope of the curve. However, the contribution of extrahepatic tissues and of recirculation (even though small) to the measured residue curve20 must be removed from the curve, a task that can be quite difficult if the shape of these contributions is not exactly known. Because of the convex form of the first part of the residue curve, it is important that values for the first 10 seconds of the residue curve be accurately determined, through the use of short intervals between measurements, to provide accurate values for the height and the area.
of the curve. The mean transit time obtained by the 
area/height method will include nonexchanging large 
vessels to the extent they appear in the collimated 
window of the detector. Thus, although the area/
height method is potentially reliable for determining 
blood flow, it appears that it will not usually be 
possible to obtain more than a rough estimate for 
the in situ liver because the substantial and variable 
proportion of the organ which is blood and its 
change with flow will make it impossible to arrive at 
a unique value for the organ/blood partition coeffi-
cient for xenon. Even in an organ like the heart, 
where there are depots of adipose tissue, it becomes 
apparent that a single value for $k_{\infty}$ cannot be used 
(since values for muscle and adipose tissue are 
widely different), and without a way of treating the 
two differing parts, the flow estimates from xenon 
residue curves are not accurate.26

How Xenon Data Can Be Used To Approximate Flow: Use of a Mixed Approach30

The methodology for acquiring xenon data by 
external scanning has become well developed, so it 
is now appropriate to seek a substitute for the Kety 
downslope approach that could be used to obtain 
flow values. The Kety downslope approach, as it 
has been applied to efflux slopes, is not directly 
related to the principle of conservation of label, 
although most other approaches for flow estimation 
are. Therefore, it is appropriate to scrutinize resi-
due data (which are what is ordinarily recorded) and 
seek a conservation relation. There is one that can 
be used. Suppose that a known amount of activity, 
$m(0)$, is introduced as an impulse into the organ at 
zero time and that residual activity is recorded as a 
function of time, so that at any given time the rate of 
change of residual activity in the organ, $\frac{dm(t)}{dt}$, 
can be estimated. Then, if the mixed venous out-
flow leaves the organ by some accessible channel, 
the rate of loss of activity from the organ will be 
equal to the product of blood flow, $F_b$, and the 
concentration in the mixed venous outflow, $c_v(t)$; 
that is

$$\frac{dm(t)}{dt} = -F_b c_v(t) \quad (20)$$

The equation will be true for any label in any organ 
and will be independent of the kinetics of distribu-
tion for the label within the organ. Practically, the 
problems associated with synchrony (i.e., of mak-
ing sure that values for the rate of change of residual 
activity in the organ and for measured mixed venous 
blood concentration are simultaneous) will be least 
for an indicator like xenon, which is cleared less 
quickly from the organ.

When the rate of efflux of activity from the organ 
is monoexponential, we can write

$$\frac{dm(t)}{dt} = -k m(t) \quad (21)$$

where $k$ is the rate constant for the exponential 
decay (this corresponds to the quotient $\ln 2/t_{1/2}$, 
where $t_{1/2}$ is the half time for exponential decay); 
then,

$$-k m(t) = F c_v(t) \quad (22)$$

and

$$F = -\frac{k m(t)}{c_v(t)} \quad (23)$$

Thus, when there is monoexponential decay, flow 
can be obtained directly from the simultaneous 
observation of the rate of change of the residue of 
the dose by external detection, and of the mixed 
venous outflow concentration values.

The approach is applicable even when the rate of 
efflux is not monoexponential:

$$\frac{dm(t)}{dt} = -F c_v(t) \quad (24)$$

and the relation

$$F = -\frac{[\frac{dm(t)}{dt}]}{c_v(t)} \quad (25)$$

will provide an estimate of the flow. The major 
problem will be the correct determination of the 
derivatives, a task that is easiest when the data 
change in an apparently monoexponential fashion. 
The approach could also potentially be used to 
estimate local flow from a local depot of activity, 
provided that the concentration in the local venous 
outflow, not contaminated flow by from adjacent 
areas, could be determined.

This approach is much different from that usually 
used with xenon data in that it is model indepen-
dent. It will provide an estimate of flow that is 
independent of the intraorgan kinetics of xenon 
passage between blood and tissue and which, with a 
recorded residue function, can be obtained for 
xenon by use of single well-timed anaerobic venous 
sample rather than an anaerobically obtained out-
flow dilution curve. The usual precautions needed 
for handling the sample and for the determination of 
outflow activity need to be observed, and the quan-
titation of the dose introduced needs to be exact.

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