Uptake of lactate by the liver: effect of red blood cell carriage

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LACTATE PLAYS A KEY ROLE in intermediate metabolism and serves as a shuttle for oxidizable substrate between organs (4). In the liver, it is both a substrate for gluconeogenesis and a product of glycolysis. In many tissues, lactate is generated and metabolized simultaneously. The overall rate of lactate turnover in the whole body is, however, difficult to evaluate because formation and metabolism can occur simultaneously inside the same cell (27). Quantitative assessment of these processes and of their regulation thus depends on an appraisal of the involvement of membrane transport. It has been inferred that the lactate-to-

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in a dropwise manner. After 15 min, 50 µl of 1 M hydrochloric acid were added. The resultant solution of [1,1-3H]ethanol was stored frozen. The L-[2-3H]lactate was prepared enzymatically from this solution and pyruvate, as described by Vind and Grunnet (51). One-half milliliter of the frozen solution of [1,1-3H]ethanol was neutralized with 20 µl of 1 N sodium hydroxide and 100 µl of Tris, to obtain a pH of 7.5. Then 50 µl of 1 M pyruvate in water, lactate dehydrogenase (rabbit muscle, Boehringer Mannheim), alcohol dehydrogenase (yeast, Boehringer Mannheim), and 70 µl (50 µmol) of NAD (grade II, Boehringer Mannheim) were added. Lactate was separated from the resulting solution by anion-exchange chromatography (Dowex 2 × 5, chloride form) using a linear elution gradient of 0–0.1 M hydrochloric acid.

Multiple indicator dilution experiments. Mongrel dogs were anesthetized with pentobarbital (25 mg/kg) with supplemental doses as necessary, intubated, and allowed to breathe room air naturally with occasional assistance with a resuscitator bag (Ambu International, Glostrup, Denmark). The abdomen was opened, and after administration of 4 mg/kg heparin, a catheter was placed in the portal vein for injection of tracers and a sampling catheter in the left main hepatic venous reservoir in such a fashion that no outflow obstruction resulted (16). The abdomen was closed to allow its contents to return to a normal temperature. At the end of each experiment an overdose of pentobarbital followed by a saturated magnesium sulfate solution (~1 ml/kg) was used for euthanasia. The injection mixture contained three tracers: 51Cr-labeled erythrocytes, a vascular indicator that does not leave the microcirculation and that was used as a reference; labeled sucrose, a second reference indicator that enters the interstitial space (the space of Disse) freely but does not enter erythrocytes or the liver cells; and labeled L-lactate, which enters both liver cells and erythrocytes. Either of two combinations of labeled sucrose and labeled lactate was used, [3H]sucrose and L-[1-14C]lactate or [14C]sucrose and L-[2-3H]lactate. The approximate amounts of activities injected were [51Cr]-labeled erythrocytes, 20 µCi; [1-14C]-labeled activity, 15 µCi; and [3H]-labeled activity, 60 µCi. The injection mixture, constituted with a hematocrit matching that of the peripheral blood, was incubated for 2 min at 37°C. This time period proved adequate for equilibration of the lactate between erythrocytes and plasma, as judged from in vitro experiments (11, 27, 37, 47). The mixture was then placed in a syringe and introduced as rapidly as possible into the portal vein to produce cross-sectional mixing. Part of the hepatic venous outflow was pumped through the sampling catheter at a rate of ~75 ml/min. Samples were collected anaerobically with a mercury-through-syringe-type anaerobic fraction collector (13). In the experiments with L-[1-14C]lactate, the anaerobic fraction collector was cooled with ice to prevent loss of L-[2-3H]lactate and formation of [3H]HO in the samples between collection and analysis.

Previous experiments have shown that the products, labeled bicarbonate and CO2, ordinarily distribute rapidly into liver cells and that their space of distribution is smaller than that available to labeled water (43). In the experiments with L-[1-14C]lactate, a second run was therefore carried out using an injection mixture containing NaH[14CO3], [51Cr]-labeled erythrocytes, and [3H]HO, to determine the space of distribution available to the labeled bicarbonate and CO2.

Standards were prepared from each injection solution by the addition, in serial dilution, of blood obtained from the hepatic venous catheter before the collection of samples. Both the injection solution and the diluent blood were cooled to 0°C.

Analysis of samples. Total radioactivity contained in the samples, excluding H[14CO3], was assessed as follows. A 0.1-ml aliquot of each sample or diluted standard was pipetted into 1.5 ml of saline, and 0.2 ml of 25% trichloroacetic acid was added to precipitate proteins. This treatment also leads to conversion of H[14CO3] to [14CO2], which is then lost to the atmosphere. The samples were assayed in a Cobra well-type scintillation crystal or both a home ray spectrometer (Nuclear Chicago, Des Plaines, IL) or a three-channel Cobra gamma ray spectrometer (Packard Instrument, Meriden, CT) for gamma radiation originating from the [51Cr]-labeled erythrocytes. Of the supernatant from these samples, 0.4 ml were transferred to scintillation vials containing 7 ml of Scinti Verse II (Fisher Scientific, Fairlawn, NJ) or Ready Safe (Beckman Instruments, Fullerton, CA) and assayed for beta radiation in a Beckman LS5801 multi-channel liquid scintillation spectrometer. Appropriate counting standards and a set of simultaneous linear equations were used to determine the activity due to each species.

Labeled metabolic products were different depending on which variety of labeled lactate was injected. In L-[2-3H]lactate experiments, the potential metabolic products containing [3H] label are [3H]HO and [3H]glucose. Because these products were not analyzed separately, it was not possible to include them in the modeling of lactate metabolism. In L-[1-14C]lactate experiments, metabolism is expected to yield [14CO3] through oxidation of pyruvate by pyruvate dehydrogenase and in the citric acid cycle (41). At physiological pH, [14CO3] is converted to H[14CO3] by carbonate dehydratase (carbonic anhydrase) within erythrocytes and hepatocyte mitochondria and at the surface of endothelial cells to attain [14CO3]/H[14CO3] equilibrium (43, 50).

To determine combined [14C] activity of bicarbonate and CO2 in the blood sample, 0.5-ml aliquots were withdrawn and immediately injected into closed 25-ml conical flasks containing 1 ml of 1.5 M perchloric acid, equipped with a center well (Kontes Glass, Vineland, NJ) containing 0.1 ml of phenylethylamine. The flasks were opened the next day, and the radioactivity contained in the center wells was assayed by liquid scintillation spectrometry.

Anion chromatography was used to separate nonionic and ionic space products for both the [14C]- and [3H]-labeled lactate experiments. For the [14C] experiments, this allowed the assessment of nonionic labeled products such as glucose. For the [3H] experiments, this provided for the separation of the activity in lactate from that in metabolic products. However, the chromatographic system used did not allow separation of [3H]HO from other tritiated metabolites such as carbohydrates. For the [14C] experiments, the contents of the open flasks used for the determination of [14C] activity in bicarbonate and CO2 were neutralized with 50 µl of 1 M Tris base and 40 µl of 5 M KOH, resulting in a pH of ~5. For the [3H] experiments, 0.5 ml of blood was precipitated with 1.0 ml of 1.5 M perchloric acid, and the sample was neutralized as above. In either instance, the solids were removed by centrifugation and chromatographic separations were carried out on the supernatant.

Dowex 2 × 5 (200 mesh; Sigma) was washed in 1 M formic acid, suspended in 1 M sodium formate (to obtain the formate form), stirred overnight, and then washed three times with distilled water. Columns were set up in glass pipettes with 5-mm inner diameter, which were plugged at the tip with glass wool and then filled with the formate form of the anion exchange resin to a height of 6 cm. Flow in the columns was maintained by gravity.

An aliquot of 0.5 ml from the neutralized sample was applied to the column. The column was then flushed with 2.5
ml of water, of which two aliquots of 1 ml each were collected into vials containing 6 ml of scintillation fluid. Water and the nonionic species (sucrose and glucose) were found in this solution. To elute lactate, each column was flushed with 3.5 ml of 1 M formic acid, and the last 2 ml were collected in equal aliquots into vials containing 6 ml of scintillation fluid (Ready Safe, Beckman Instruments) and analyzed for beta radiation. To elute pyruvate, the column was flushed with 3.5 ml of 1 M hydrochloric acid and the last 2 ml were collected and analyzed as above.

Hemoglobin content, oxygen saturation, PO2, and PCO2 were measured in samples of venous blood collected through the portal and hepatic vein catheters and samples of arterial blood collected from a cannula inserted into the femoral artery. Unlabeled lactate was measured in plasma obtained from these samples by centrifugation, using a standard spectrophotometric technique (Ref. 21; test combination L-Lactic Acid, Boehringer Mannheim).

In vitro kinetics in erythrocytes. Lactate dehydrogenase present in erythrocytes catalyzes rapid interconversion between lactate and pyruvate. The 14C label undergoes exchange between lactate and pyruvate, whereas the 3H label appears as 3HOH. To ascertain the rate of the exchange reaction and the composition of the equilibration mixture, L-[14C]lactate or [14C]pyruvate was mixed with dog blood at 37°C. Alternatively, L-[2-3H]lactate was added to a suspension of dog erythrocytes (45%) in Krebs-Henseleit buffer solution (pH = 7.4) at 37°C. At varying intervals after mixing, the reaction was stopped with perchloric acid. The labeled lactate and pyruvate or lactate and water were then separated chromatographically, as outlined above.

Model analysis and parameter identification. The data needed to carry out the model analysis are the outflow dilution curves for labeled erythrocytes, labeled sucrose, and labeled lactate. In the case of [1-14C]lactate experiments, the H[14C]O2 curve was also used to parameterize a full precursor-product model. The outflow dilution curves were normalized in relation to the amount of tracer injected. Equivalent areas under the curves therefore signify equivalent outflow recoveries. For a substance completely recovered at the outflow (labeled erythrocytes or labeled sucrose), hepatic blood flow rate is equal to the reciprocal of the area under the curve (16). The liver vascular space was calculated as the product of blood flow rate and the mean transit time (the time integral of the product of time and concentration divided by the area under the curve). The procedure does not take into account the unlabeled input through the hepatic artery. The error introduced by this simplification was considered minor because in experiments with perfused rat livers, injection of indicators into the hepatic artery and the portal vein yielded similar distribution volumes for vascular indicators (5, 33).

The model analysis was carried out in two steps. In the first step, the relation between the labeled erythrocyte and sucrose curves was analyzed. In the second step, the relation among the labeled erythrocyte, labeled sucrose, and labeled lactate curves was analyzed.

Succrose is distributed into the interstitial space (the space of Disse) in a flow-limited fashion but does not enter the liver cells. It enters the interstitial space as rapidly as it is presented because, with the fenestrae perforating the sinusoidal lining cells, there is no resistance to exchange between the sinusoidal plasma and the very shallow interstitial space. As a consequence, the labeled sucrose impulse propagates along the sinusoid in both the sinusoidal plasma and interstitial spaces; it travels less rapidly than the labeled erythrocytes, which are carried along the sinusoids by flow, so that the labeled sucrose impulse emerges later (16, 19). Labeled sucrose thus marks out the interstitial space, which would be available to labeled lactate if it did not enter hepatocytes or erythrocytes. The distribution of transit times in the large vessels and sinusoids is such that virtually all of the heterogeneity occurs in the sinusoidal bed (this is the predominant part of the contained blood, in the liver), and the transit times of the nonsinusoidal ("large") vessels were assumed to be uniform. In a similar way, labeled water, bicarbonate, and CO2 enter the interstitial space as well as the erythrocyte and hepatocyte space in a flow-limited fashion (16, 43). Simultaneous analysis of the outflow profiles of labeled erythrocytes and sucrose according to the delayed-wave model (16) allowed estimation of the ratios of extravascular to vascular space of sucrose (γv) and of the common nonsinusoidal transit time, t0 (APPENDIX A). Similarly, simultaneous analysis of outflow profiles obtained for second runs was used for estimating t0 and the ratios of extravascular (combined interstitial and parenchymal) to vascular (combined plasma and erythrocyte) space ratio for bicarbonate/CO2 (γm) and water (γw).

The description of lactate tracer disposition in mathematical form is developed in APPENDIX B and is presented in schematic form in Fig. 1. Because the data demonstrate a limiting erythrocyte exchange of label, plasma and erythrocytes are represented by two compartments interconnected by the exchange of tracer between plasma and erythrocytes. Lactate in erythrocytes travels along the sinusoid with the same velocity as [3H]labeled erythrocytes, whereas lactate in plasma travels with the lower velocity of [12C]sucrose. Unlabeled lactate is assumed to be in a Donnan equilibrium
Table 1. Physiological parameters of animals

<table>
<thead>
<tr>
<th>Blood Sample</th>
<th>pH</th>
<th>PCO₂, Torr</th>
<th>PO₂, Torr</th>
<th>Hemoglobin Concentration in Blood, gl</th>
<th>O₂ Saturation of Hemoglobin, %</th>
<th>Unlabeled Plasma Lactate Concentration, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>7.34 ± 0.05</td>
<td>34.7 ± 7.5</td>
<td>93.8 ± 10.9</td>
<td>124 ± 29</td>
<td>95.3 ± 4.1</td>
<td>2.60 ± 1.94</td>
</tr>
<tr>
<td>Portal venous</td>
<td>7.33 ± 0.08</td>
<td>42.1 ± 8.4</td>
<td>60.2 ± 13.4</td>
<td>123 ± 27</td>
<td>75.9 ± 10.7</td>
<td>2.43 ± 1.88</td>
</tr>
<tr>
<td>Hepatic venous</td>
<td>7.30 ± 0.06</td>
<td>44.8 ± 8.2</td>
<td>48.3 ± 14.3</td>
<td>124 ± 31</td>
<td>62.4 ± 14.7</td>
<td>2.93 ± 2.02</td>
</tr>
</tbody>
</table>

Values shown are means ± SD obtained from 10-19 animals.
Parameters from the fit of superimposed erythrocyte and sucrose profiles to lactate and bicarbonate/CO₂ profiles are shown in Table 4. For the [1-14C]lactate experiments that had no second-run data, the value of γₘ used to fit first-run precursor and product profiles was the average value obtained by the fit of second-run profiles in the other experiments. In a first attempt, only the lactate profile was fitted to data, and the product bicarbonate/CO₂ data were not used. However, the resulting standard deviations of k₉ₗ (estimated using the matrix of sensitivities) were too large to allow for a reliable k₉ₗ estimate. Therefore, the outflow profiles for lactate and its metabolite, bicarbonate/CO₂, were fitted simultaneously to lactate and bicarbonate/CO₂ data. This provided for adequate reliability of the k₉ₗ estimates. Because [2-3H]lactate experiments had no product bicarbonate or CO₂ identified, the k₉ₗ value used was the average value obtained from the analysis of [1-14C]lactate experiments.

Fits to first-run labeled lactate and bicarbonate/CO₂ data of the representative experiments are shown in Fig. 3. Also shown in the illustration are the following components of the total predicted outflow response: tracer present in erythrocytes at the time of injection and that passes through the microcirculation without being released in the plasma (erythrocyte throughput), tracer in erythrocytes and plasma that never enters the liver (total throughput component, which includes erythrocyte throughput), and tracer that returns from hepatocytes to the vascular space and then exits the liver (returning component). The components show that, although the magnitude of erythrocyte throughput is significant, the lactate early peak is due to plasma as well as erythrocyte throughput.

Reliability of the fitted parameters was further established by assessing the effect of changes in parameter values on calculated outflow profiles (Fig. 4). For one parameter at a time, its value was set to a fixed value equal to either one-half or double the optimal value. With highly correlated parameters, changing several parameters simultaneously can have a smaller effect on the calculated outflow profile than changing each parameter separately. Therefore, the remaining parameters were optimized by refitting the calculated outflow profiles. In all four cases examined, the parameter values had a definite effect on the shapes of the calculated outflow profiles, confirming their pertinence. It is noted that the permeability of red blood cell membranes influences only the peak of the lactate

### Table 2. Parameters obtained from analysis of experimental profiles

<table>
<thead>
<tr>
<th>Lactate Label</th>
<th>n</th>
<th>Dog, kg</th>
<th>Liver, g</th>
<th>Hematocrit</th>
<th>Mean Transit Times, s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Flow Rate, ml·s⁻¹·g⁻¹</td>
</tr>
<tr>
<td>³H</td>
<td>4</td>
<td>22 ± 7</td>
<td>432 ± 130</td>
<td>0.43 ± 0.04</td>
<td>0.023 ± 0.016</td>
</tr>
<tr>
<td>¹⁴C</td>
<td>16</td>
<td>23 ± 8</td>
<td>667 ± 199</td>
<td>0.34 ± 0.10</td>
<td>0.025 ± 0.011</td>
</tr>
<tr>
<td>All</td>
<td>20</td>
<td>23 ± 7</td>
<td>620 ± 203</td>
<td>0.36 ± 0.09</td>
<td>0.024 ± 0.012</td>
</tr>
</tbody>
</table>

Values are means ± SD for n experiments. In the case of [1-14C]lactate, second-run data were available in only 13 of 16 experiments.
profile, whereas that of the hepatocyte membrane has an impact on the whole profile. These parameters have only a small effect on the product profile, especially considering that the scatter of product data is larger than that of precursor data. In contrast, $k_{seq}$ has no effect on the lactate profile and thus cannot be determined from lactate data alone (see above); it has, however, a strong effect on the product profile: the magnitude of the latter is roughly proportional to the value of $k_{seq}$.

The change in the vascular volume with flow rate is illustrated in Fig. 5A. The best fit from linear regression is $V_{\text{vasc}} = (6.9 \pm 0.9)F + (0.05 \pm 0.02)$, where $V_{\text{vasc}}$ is the vascular volume in milliliters per gram of liver and $F$ is flow rate in milliliters per second per gram of liver. The values for the slope and the ordinate intercept are approximately one standard deviation away from the values obtained in a previous study for normal hematocrit data (20).

### DISCUSSION

Lactate carriage by erythrocytes. Modeling analysis of the results suggests that the sinusoidal membrane of hepatocytes and the membrane of erythrocytes constitute barriers for lactate transport. Lactate is thus partially trapped within erythrocytes during transit through the sinusoidal bed, and this part is not avail-

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Table 3. Parameters obtained from fit of flow-limited substance profiles

<table>
<thead>
<tr>
<th>Lactate label</th>
<th>First Run (erythrocytes/sucrose curve fit)</th>
<th>Second Run (erythrocytes/water/HCO$_3^-$ curve fit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t_0, s$</td>
<td>$\gamma$</td>
</tr>
<tr>
<td>$^3$H</td>
<td>4</td>
<td>3.4 1.1</td>
</tr>
<tr>
<td>$^{14}$C</td>
<td>16</td>
<td>3.4 1.0</td>
</tr>
<tr>
<td>All</td>
<td>20</td>
<td>3.4 1.0</td>
</tr>
</tbody>
</table>

$n$, No. of experiments. In the case of $[1-^{14}$C]lactate, second-run data were available in only 13 of 16 experiments. $t_0$, Common nonsinusoidal transit time; $\gamma$, ratio of interstitial to plasma space; $\gamma_w$ and $\gamma_m$, ratios of extravascular to vascular space of water and bicarbonate/CO$_2$, respectively; $\pm$ SD$_A$, mean of individual SD of fitted values, determined from fitting procedure; $\pm$ SD$_B$, SD of parameters from different experiments, representing interindividual variability.

The variation in the lactate influx permeability-surface area product with flow rate is shown in Fig. 5B. The equation for the fitted line is $P_{in}S = (3.1 \pm 0.5)F + (0.021 \pm 0.014)$, where $P_{in}S$ is the influx permeability-surface area product in milliliters per second per gram of liver and $F$ is flow rate in milliliters per second per gram of liver.

There was no significant correlation between plasma lactate concentration and $P_{in}S$ for lactate or the sequestration constant.

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Table 4. Parameters obtained from fit of lactate and CO$_2$ profiles

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>$k_{r_p}$, s$^{-1}$ Value $\pm$ SD</th>
<th>$k_{p_h}$, s$^{-1}$ Value $\pm$ SD</th>
<th>$k_{h_p}$, s$^{-1}$ Value $\pm$ SD</th>
<th>$k_{seq}$, s$^{-1}$ Value $\pm$ SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.75 0.12</td>
<td>0.59 0.05</td>
<td>0.264 0.026</td>
<td>0.0532 0.00033</td>
</tr>
<tr>
<td>2</td>
<td>1.12 0.11</td>
<td>0.71 0.04</td>
<td>0.126 0.009</td>
<td>0.0038 0.00020</td>
</tr>
<tr>
<td>3</td>
<td>0.25 0.03</td>
<td>0.36 0.04</td>
<td>0.082 0.014</td>
<td>0.0029 0.00016</td>
</tr>
<tr>
<td>4</td>
<td>0.88 0.14</td>
<td>0.22 0.01</td>
<td>0.060 0.006</td>
<td>0.0009 0.00014</td>
</tr>
</tbody>
</table>

$[2-^{3}$H]lactate experiments

| 5         | 0.21 0.01                         | 0.48 0.03                       | 0.174 0.013                     | 0.00532 0.00033                  |
| 6         | 0.70 0.09                         | 0.34 0.02                       | 0.084 0.008                     | 0.0038 0.00020                  |
| 7         | 0.80 0.14                         | 0.36 0.02                       | 0.126 0.007                     | 0.00129 0.00020                  |
| 8         | 0.36 0.32                         | 0.41 0.03                       | 0.192 0.017                     | 0.00442 0.00018                  |
| 9         | 0.48 0.05                         | 0.20 0.01                       | 0.042 0.005                     | 0.00335 0.00070                  |
| 10        | 0.73 0.09                         | 0.48 0.04                       | 0.132 0.014                     | 0.00200 0.00026                  |
| 11        | 0.75 0.08                         | 0.53 0.03                       | 0.269 0.017                     | 0.00335 0.00024                  |
| 12        | 0.83 0.07                         | 0.47 0.02                       | 0.081 0.004                     | 0.00057 0.00014                  |
| 13        | 0.19 0.01                         | 0.63 0.07                       | 0.237 0.031                     | 0.00399 0.00046                  |
| 14        | 0.76 0.06                         | 0.52 0.01                       | 0.168 0.006                     | 0.00061 0.00009                  |
| 15        | 0.35 0.03                         | 0.43 0.02                       | 0.147 0.008                     | 0.00146 0.00027                  |
| 16        | 0.58 0.07                         | 0.52 0.02                       | 0.106 0.005                     | 0.00097 0.00020                  |
| 17        | 0.48 0.07                         | 0.27 0.02                       | 0.076 0.007                     | 0.00073 0.00029                  |
| 18        | 0.61 0.05                         | 0.40 0.01                       | 0.097 0.004                     | 0.00076 0.00014                  |
| 19        | 0.53 0.04                         | 0.46 0.02                       | 0.140 0.006                     | 0.00094 0.00014                  |
| 20        | 0.77 0.10                         | 0.44 0.02                       | 0.119 0.007                     | 0.00064 0.00016                  |
| Mean      | 0.62 0.24                         | 0.44 0.13                       | 0.136 0.065                     | 0.00167 0.00015                  |

$k_{r_p}$, $k_{p_h}$, $k_{h_p}$, and $k_{seq}$, rate constants for erythrocyte efflux, hepatocyte influx, hepatocyte efflux, and metabolism, respectively.
able for exchange with plasma and uptake by hepatocytes. This results in an early peak portion in the outflow profiles for lactate. In some experiments, the peak is diminished to a shoulder (curves not displayed).

Depending on the rate of tracer exchange between erythrocytes and plasma, three different cases generally must be considered. If the permeability of the erythrocyte is high such that the inverse of the efflux rate constant is very large compared with the sinusoidal transit time of the erythrocytes through the acinus, erythrocytes can be viewed as an extension of the plasma compartment, resulting in an erythrocyte capacity effect. This case has been previously investigated theoretically and experimentally for water, urea, chloride, and bicarbonate/CO₂ (6, 16, 18, 43). If the efflux rate constant is very small compared with the inverse of the transit time of erythrocytes through the acinus, a substance trapped within erythrocytes will not be removed to a significant extent during one passage through the sinusoidal bed. In this case, the kinetics of erythrocyte exchange between plasma and erythrocytes cannot be studied using the multiple-indicator dilution method. For example, the efflux rate coefficient for the immunosuppressant tacrolimus from human erythrocytes was <1 min⁻¹ and thus much lower than the inverse of the sinusoidal transit time. Hepatic uptake of this drug was investigated with recirculating perfusion of rabbit liver. These experiments were evaluated using compartmental analysis, with consideration of tracer exchange between plasma and erythrocytes (7, 34). However, this evaluation does not contribute to elucidation of the mechanism of hepatic uptake, because most of the exchange takes place outside the liver within the reservoir of the perfusion apparatus.

In the present case, the efflux rate constant is of the same order of magnitude as the inverse of transit time of erythrocytes through the acinus, resulting in “red blood cell carriage,” i.e., partial trapping of the indicator within erythrocytes. This phenomenon has originally been observed with thiourea in the dog kidney (6) and analyzed quantitatively in the dog liver, where thiourea enters hepatocytes rapidly (18). A similar carriage phenomenon was described more recently with acetaminophen in rat liver perfused with human erythrocytes (32). In this study, lactate was found to undergo barrier-limited uptake into hepatocytes where it is metabolized, a situation similar to that previously described for acetaminophen (32). Analytical solutions
have been found for the pertinent differential equations, and they have been used for modeling. A system of six differential equations was used to describe hepatic acetaminophen disposition. Numerical solutions were sought to calculate outflow profiles of the precipor, acetaminophen, and the metabolic product, acetaminophen sulfate. Investigation of the present case led to a system of four differential equations, which are very similar to the case described for acetaminophen. An analytical solution could be formulated for the outflow profile for the precipor, lactate, but not of the products, bicarbonate/CO₂, water, or glucose (Appendix C).

Lactate has previously been found to be transported across canine erythrocyte membranes primarily via a monocarboxylate carrier, with minor contributions of capnophorin (band 3 protein) and nonionic diffusion of lactic acid (11, 47). Because unlabeled lactate is near Donnan equilibrium between plasma and erythrocyte matrix, the rate coefficients reported here represent equilibrium exchange. Direct measurements of equilibrium exchange rates at physiological temperature have not been reported, because this process seems to be very fast and difficult to measure. Deuticke et al. (11) measured equilibrium exchange rates at 10°C and found a rate constant for efflux of 0.004 s⁻¹. If this value is extrapolated to 37°C, using data for human erythrocytes and assuming that the ratio of the activities at the two temperatures are the same for the two species, an estimated rate constant of 0.04 s⁻¹ at 37°C is obtained, which is one order of magnitude slower than the fitted efflux coefficients obtained from the present experiments. However, attempts to fit to the data a model with a fixed value of 0.04 s⁻¹ for the coefficient of efflux from erythrocytes, kᵣₑ, did not result in acceptable fits. This discrepancy between the fitted and the in vivo equilibrium exchange values does not have an obvious explanation. Possibly, dog plasma contains unknown factors that enhance lactate transport into erythrocytes.

Skelton et al. (47) measured zero-trans influx into erythrocytes at 37°C and found, at the lowest lactate concentration of 1.6 mM, influx rates of 1.7 ± 0.14 µmol·min⁻¹·ml cellular volume⁻¹. Because at Donnan equilibrium, the intracellular concentration is about one-half the extracellular concentration, zero-trans influx can therefore be expressed as 1.7/0.8 = 2.13 µmol·min⁻¹·µmol intracellular lactate⁻¹ or 0.035 µmol·s⁻¹·µmol intracellular lactate⁻¹. According to membrane carrier kinetics, equilibrium exchange is generally expected to be faster than zero-trans uptake (10, 12, 49). The rate constant for equilibrium exchange of 0.04 s⁻¹ could thus be considered a minimal value.

Rates of lactate transport into erythrocytes have been shown to vary widely between species (11, 47). In “athletic” species (dogs and horses) with high maximal oxygen uptake, lactate transport is up to 160 times faster than in “nonathletic” species (cattle and goats). Presumably, rapid partition of lactate into erythrocytes increases the capacity of blood to remove excess lactate from the musculature during heavy exercise. At normal hematocrit (0.42 for dog) and Donnan equilibrium, 27% of blood lactate is present in erythrocytes, so the capacity would increase by ~36%. The rates reported in the literature, however, are not sufficiently high to ensure that this mechanism is operative, because the characteristic times for filling the erythrocyte lactate pool exceed typical transit times through the capillary beds of exercising skeletal or cardiac muscles, which are on the order of a few seconds (1, 22, 38). The present analysis yielded much higher activities of lactate transport across the erythrocyte membranes, and it provides more direct evidence that, in the liver, lactate within the erythrocytes can partly exchange with lactate in plasma and in hepatocytes during the hepatic transit time of blood.

Lactate transport into hepatocytes. Hepatic lactate transport has previously been studied only in the rat. Near-equilibrium exchange rates were measured in the isolated, perfused rat liver using the multiple-indicator dilution method in the absence of erythrocytes (2, 44). In agreement with our results in dogs, saturation was not observed with plasma concentrations up to 8 mM. A carrier-mediated transport mechanism was nevertheless postulated on the basis of inhibition by α-cyanocinnamate. At 8 mM extracellular lactate, the transport rate was 240 µmol·min⁻¹·ml cell⁻¹. With a cellular volume of 0.8 ml/g, this value yields an influx permeability-surface area product of 0.4 ml·s⁻¹·g⁻¹, which is about twice the maximal value obtained in the dog liver. In experiments with isolated hepatocytes, zero-trans influx of L-lactate was found to be carrier mediated (12, 14, 25). The most reliable values are those by Jackson and Halesstrap (25) using intracellular pH indicators. At 25°C, the Kₘ value was 4.5 mM and the maximal influx rate was 20 µmol·min⁻¹·ml cell⁻¹. Extrapolation to 37°C yields a maximal influx rate of 46 µmol·min⁻¹·ml cell⁻¹. This is slower than equilibrium exchange rates, as also observed for monocarboxylate transport in erythrocytes (10, 12), and as expected for exchange transport systems (49). Lactate transport across the basolateral membranes of canine hepatocytes is fast (values of P_inS are, on average, 4 times those of hepatocyte blood flow rate) and carrier mediated. A large proportion of intracellular lactate returns into the plasma space, and a much smaller proportion is metabolized, as judged from comparison of kₜ₀₉ to kₑₛₑ; thus the intracellular lactate is maintained near equilibrium with plasma lactate, and the available intracellular lactate does not limit overall lactate metabolism. Also, this means that rates of disappearance of labeled lactate from plasma provide acceptable estimates for cellular lactate metabolism (27).

The observed correlation of the influx permeability-surface area product for lactate with hepatic blood flow rate is analogous to that previously described for rubidium (20). This phenomenon is tentatively attributed to a portion of the sinuses that are intermittently stagnant at low flows such that they are not accessible to the tracers; at higher flows, the proportion of sinuses available for tracer exchange at any time would increase.
Hepatic lactate metabolism. The initial step in hepatic lactate metabolism is oxidation to pyruvate by the action of lactate dehydrogenase. This reaction is reversible, and is probably near equilibrium under physiological conditions (45, 51, 54). The final products are mainly [1H]2O for [2-3H]lactate and mainly [14C]bicarbonate and [14C]O2 for [1-14C]lactate. The only other known pathway of lactate metabolism is peroxysomal oxidation by glycolate oxidase, which is negligible (29).

The role of lactate dehydrogenase in sequestration of labeled lactate depends on the kind of labeling. In the case of [1-14C]lactate, the label is retained in pyruvate formed by oxidation (46). This pathway is a dead end in erythrocytes, which lack mitochondria and therefore the ability to further oxidize pyruvate. Label contained in pyruvate within erythrocytes and plasma will not exceed a few percentage points, as shown by the reported in vitro experiments with dog blood. In the majority of the animals, net release of unlabeled lactate by the liver was observed despite sequestration of labeled lactate. Labeled lactate is thus at least partly replaced by unlabeled lactate produced from other intrahepatic metabolites. This can be attributed to production of lactate by glycolysis and its simultaneous consumption by oxidation and gluconeogenesis (53).

In hepatocytes, the proportion of tracer pyruvate is also expected to be insignificant because of equilibration with lactate with a similar lactate-to-pyruvate ratio (50:1). [14C]Pyruvate contained in the cytosol of hepatocytes can thus be combined with cytosolic [14C]lactate to form a single pool. Tracer exchange between lactate and pyruvate is expected to be faster than maximal net rates of lactate production or consumption, because it can take place without dissociation of the enzyme-coenzyme complex (46). Appearance of the 14C label in bicarbonate/CO2 is most probably due to the action of pyruvate dehydrogenase. It has been shown in the rat liver that labeled bicarbonate/CO2 is formed from [1-14C]pyruvate in two different ways that can be distinguished kinetically: the part that is formed directly from pyruvate by the action of pyruvate dehydrogenase appears in the perfusate very quickly, whereas bicarbonate/CO2 formed indirectly (e.g., via oxaloacetate or tricarboxylic acid intermediates) appears after a lag time of 2 min (3, 41). Because in our experiments, the sampling time was shorter than that lag time, it may be presumed that formation of H[14C]O3 reflects the activity of pyruvate dehydrogenase. However, no attempt was made to quantify the rate of the pyruvate dehydrogenase reaction.

When [2-3H]lactate is used as a tracer, sequestration and formation of labeled products occur in a different manner. Lactate dehydrogenase will mediate the transfer of 3H to the α-position of NADH, from where it can be transferred to other metabolic intermediates by the action of other NADH-dependent dehydrogenases (24, 30). This transfer is expected to be slower than that of the 14C label, because it requires the dissociation of the coenzyme from the enzyme. Moreover, the lactate dehydrogenase reaction shows a considerable kinetic isotope effect (31). Part of the label will eventually appear as 3HOH because of oxidation reactions and hydrogen exchange with cellular water. Another part may be incorporated into metabolites released by the liver, such as into glucose formed by gluconeogenesis (30, 52). A detailed analysis of the fate of the 3H label is beyond the scope of this work.

General conclusions. Lactate transport across the sinusoidal membranes of hepatocytes and across the erythrocyte membrane is bidirectional under in vivo conditions, resulting in an approximate equilibrium between lactate in plasma and in the cytosol of hepatocytes. Lactate carried in erythrocytes can exchange with that in plasma and is available in part for hepatic metabolism.

APPENDIX A

Linear Superposition According To Delayed Wave Model With Catheter Correction

To evaluate the experimentally obtained outflow profiles, the dispersion of the injected bolus by the injection apparatus and the inflow and outflow catheters must be considered, as previously described in detail (12, 13). For example, the experimental erythrocyte curve, C_{RBC}(t), is the convolution of the organ erythrocyte transport function (catheter-corrected outflow profile or impulse response), h_{RBC}(t), with the outflow profile obtained from the apparatus in the absence of a liver, C_{cath}(t)

$$C_{RBC}(t) = (C_{cath} * h_{RBC})(t)$$  \hspace{1cm} (A1)

where * is the convolution operator. Similarly, for sucrose

$$C_{suc}(t) = (C_{cath} * h_{suc})(t)$$  \hspace{1cm} (A2)

The experimental outflow profiles are distorted by the effect of the catheter and pump used for sample collection from the hepatic vein. The response of the latter has been shown to be adequately approximated by a monoeponential decay with delay constant, α_{cath}, combined with a simple delay, τ_{cath}. The outflow profile of the collection catheter alone was represented by the function

$$C_{cath}(t) = 1/F_{cath} e^{-\alpha_{cath}(t - \tau_{cath})}$$  \hspace{1cm} (A3)

The impulse response of the liver was then obtained by deconvolution using the following relation

$$h_{RBC}(t) = F \left[ C_{RBC}(t + \delta_{cath}) + \frac{1}{\alpha_{cath}} \frac{dC_{RBC}(t + \delta_{cath})}{dt} \right]$$  \hspace{1cm} (A4)

The erythrocyte curve, C_{RBC}, was approximated by a piecewise third-order polynomial (8), which is easily differentiated. The organ sucrose transport function, h_{suc}(t), was calculated from the organ red blood cell transport function, h_{RBC}(t), using linear superposition according to the flow-limited model of Goresky (16) with uniform large-vessel transit time

$$h_{suc}(t) = \frac{1}{1 + \gamma} h_{RBC}(t - t_0)$$  \hspace{1cm} (A5)

where γ is the interstitial to vascular distribution spaces and t is the common large-vessel transit time. The sucrose outflow profile, C_{suc}(t), was then calculated by convolution according to Eq. A1, using a numerical integration algorithm (QDAGS from Visual Numerics, Houston, TX). The result was fitted to the experimental outflow profile for sucrose by a
nonlinear least-squares procedure to find optimal values for γ and $t_0$.

**APPENDIX B**

**Matrix Approach To Multiple-Indicator Dilution Equations**

The evaluation of the multiple-indicator dilution data is similar to that previously used for experiments with acetaminophen (32). It is based on a published mathematical representation of transport and metabolism in the liver (42). Labeled material is contained in various pools, as shown in Fig. 1. Mobile pools include lactate in blood plasma, with concentration $c_p$, lactate in erythrocytes, with concentration $c_r$, and a combined bicarbonate/CO₂ pool representing bicarbonate and CO₂ contained in blood plasma, erythrocytes, and hepatocytes, with a plasma concentration $m$. The combined pool was formulated based on the observation that bicarbonate and CO₂ exchange very rapidly between these spaces (43). The only stationary pool is the intracellular lactate pool, which corresponds to the lactate content of hepatocytes, with concentration $c_i$.

The behavior of tracers is described by the following system of partial differential equations:

\[
(1 + \gamma) \frac{\partial c_p}{\partial t} + \frac{\partial c_r}{\partial x} - \beta k_{rp} c_p + c_r \left( 1 + \frac{1}{\beta} k_{rp} c_p - k_{ip} c_r \right) = 0
\]  

\[
\frac{\partial c_r}{\partial t} + \frac{\partial c_i}{\partial x} - k_{ip} c_r + \left( 1 + \frac{1}{\beta} k_{rp} c_p - k_{ip} c_r \right) = 0
\]  

\[
(1 + \gamma) \frac{\partial m}{\partial t} + \frac{\partial m}{\partial x} = \theta k_{imp} c_i
\]

where $t$ is time, $x$ is the quotient of the distance along the length and the velocity of flow; $\gamma$ is the ratio of the Disse space to that in sinusoidal plasma; $\beta = \text{hematocrit}(1-\text{hematocrit})$ is the erythrocyte-to-sinusoidal plasma volume ratio; $\theta$ is the hepatocyte-to-sinusoidal plasma volume ratio; and $\gamma_m$ is the ratio of the stationary to the mobile part of the product. The latter is given by

\[
\gamma_m = 1 + \frac{\gamma + \lambda_{\text{rh}_{0}}}{1 + \lambda_{\text{rh}}}
\]

where $\lambda_{m}$ and $\lambda_{\text{rh}_{0}}$ are partition coefficients for bicarbonate/CO₂ between plasma and erythrocytes and between plasma and hepatocytes, respectively (43).

Exchange between pools is determined by transfer coefficients, with the dimension of reciprocal time, related to permeabilities and enzymatic activities in the following fashion. The transfer coefficient $k_{rp}$ is the turnover number for lactate within erythrocytes. The transfer coefficient is equal to the permeability of the erythrocyte membrane multiplied by the ratio of the surface area to the volume of the erythrocyte, $P_{rp} S_{v_{\text{rh}}}$. Because the distribution space ratio of lactate in the Disse space to that in sinusoidal plasma, $\gamma$, is the same as that for the reference indicator, sucrose, the transfer coefficient $k_{ip}$ is determined by the relation

\[
k_{ip} = \frac{\lambda_{\text{rh}}}{1 + \gamma}
\]

where $\lambda$ is the equilibrium partition coefficient between lactate concentration in erythrocytes and the unbound lactate concentration in plasma. The following equations relate the transfer coefficients for hepatocellular membrane passage of lactate to membrane permeabilities

\[
k_{ip} = \frac{P_{ip} S_{v_{cell}}}{V_{cell}(1 + \gamma)}
\]

\[
k_{ip} = \frac{P_{ip} S_{v_{cell}}}{V_{cell}(1 + \gamma)}
\]

where $P_{ip} S_{v_{cell}}$ and $P_{ip} S_{v_{cell}}$ are the permeability-surface area products for the exchange of lactate across the hepatocyte cell membranes in the inward and outward direction, respectively; $V_{cell}$ is the sinusoidal plasma volume; and $V_{cell}$ is the hepatocellular volume. The transfer coefficient $k_{seq}$ represents the irreversible hepatic biotransformation activity and is given by the ratio of the reaction rate to the amount of intracellular precursor.

The dose introduced at the origin ($x = 0$) of the initially tracer-free sinusoid had previously been equilibrated between plasma and erythrocytes. The system of differential equations, therefore, must be solved with the following initial conditions (18):

\[
\begin{align*}
&c_{cp} |_{x=0} = \frac{q_{0} (1 + \beta)}{F_{s} (1 + \lambda \beta)} \delta(t) \\
&c_{rp} |_{x=0} = \frac{q_{0} \lambda (1 + \beta)}{F_{s} (1 + \lambda \beta)} \delta(t)
\end{align*}
\]

where $q_{0}$ is the amount of tracer initially applied to the entrance of the sinusoid, $F_{s}$ is sinusoidal blood flow rate, and $\delta$ is the impulse function.

An analytical solution of the system of partial differential equations B1a–B1d with initial conditions B5a–B5b is presented in **APPENDIX C** for precursor concentrations $c_{r}$ and $c_{i}$. However, no solution was found for the product plasma concentration $m$. Moreover, the numerical evaluation of the solution detailed in **APPENDIX C** is expensive in terms of computer time. We therefore use an eigenvalue method previously developed by Schwab (42) for our calculations. For this purpose, the transfer coefficients are collected into the compartmental matrix $A$

\[
A = \begin{pmatrix}
-k_{ip} + k_{rh} & k_{rp} & k_{ip} \\
k_{pr} & -k_{ip} & 0 \\
0 & 0 & k_{seq}
\end{pmatrix}
\]

Each pool travels along the sinusoids with a relative velocity that is defined to be 1 for erythrocytes and 0 for hepatocytes. The average relative velocity of lactate in plasma contained in the combined sinusoidal and the interstitial spaces is $1/(1 + \gamma)$, and the average relative velocity of total bicarbonate/CO₂ is $1/(1 + \gamma_m)$. The relative velocities are collected in the diagonal matrix $W$

\[
W = \begin{pmatrix}
1 & 0 & 0 \\
0 & 1 + \gamma & 0 \\
0 & 0 & 1 + \gamma_m
\end{pmatrix}
\]

Concentration terms are collected in a vector, $u$, whose elements are amounts per unit sinusoidal space, normalized to the injected dose

\[
u = \frac{1}{q_{0} (1 + \beta)} \begin{pmatrix}
1 + \gamma k_{rp} \\
\beta c_{r} \\
\theta c_{i}
\end{pmatrix}
\]
With these definitions, the system of partial differential equations can then be written concisely as

\[
\frac{\partial \mathbf{u}}{\partial t} + W \frac{\partial \mathbf{u}}{\partial x} = \mathbf{A} \mathbf{u}
\]  

(B9)

The initial conditions become

\[
\mathbf{u} = \delta(t) \mathbf{u}_0, \quad x = 0
\]  

(B10)

where

\[
\mathbf{u}_0 = \frac{1}{F(1 + \lambda \beta)} \begin{bmatrix} 1 + \gamma \\ \lambda \beta \\ 0 \\ 0 \end{bmatrix}
\]  

(B11)

The response of the whole organ will be obtained by integrating over all flow paths with different transit times. If \( n(x) \) is the distribution of flow paths [that is, \( n(x)dx \) is the proportion of the flow with transit times between \( x \) and \( x + dx \)], then the concentrations at the outflow of the whole liver will be the flow-weighted average of the responses of single sinusoidal paths (18), according to the integrals

\[
\mathbf{h}(t + t_0) = W \int_0^\infty n(x)\mathbf{u}dx
\]  

(B12)

where \( \mathbf{h}(t) \) is a vector function whose elements are the total amounts per unit sinusoidal plasma space.

We now perform Laplace transformation with respect to \( x \).

\[
\tilde{\mathbf{u}}(s) = \int_0^\infty e^{-st} \mathbf{u}dx
\]  

(B13)

where \( S \) is the Laplace variable. Equation B9 then becomes

\[
\frac{d\tilde{\mathbf{u}}}{dt} + sW\tilde{\mathbf{u}}(s) = \mathbf{A}\tilde{\mathbf{u}}
\]  

(B14)

which has the solution

\[
\tilde{\mathbf{u}}(s) = e^{sA - sW} \mathbf{u}_0
\]  

(B15)

For the definition of an exponential with a matrix-valued exponent, see APPENDIX D. The transit time distribution was approximated by a sum of \( n \) exponential terms, as follows

\[
n(x) = \sum_{i=1}^{n} \alpha_i e^{\lambda_i x}
\]  

(B16)

where the parameters \( \alpha_i \) and \( \epsilon_i \) are arbitrary and have no physical meaning. Thus the parameters \( \alpha_i \) and \( \epsilon_i \) may be determined from the reference curve.

Substitution of Eq. B16 into Eq. B12 yields

\[
\mathbf{h}(t + t_0) = W \sum_{i=1}^{n} \alpha_i \int_0^\infty \mathbf{u} e^{\lambda_i x} dx
\]  

(B17)

and substitution of Eq. B13 yields

\[
\mathbf{h}(t + t_0) = W \sum_{i=1}^{n} \alpha_i \tilde{\mathbf{u}}(\lambda_i)
\]  

(B18)

Further substitution of Eq. B15 yields

\[
\mathbf{h}(t + t_0) = W \sum_{i=1}^{n} \alpha_i e^{sA - sW} \mathbf{u}_0
\]  

(B19)

When the elements of the matrices \( \mathbf{A} \) and \( \mathbf{W} \) and the values for \( \alpha_i \) and \( \epsilon_i \) are known, the outflow profiles contained in \( \mathbf{h} \) can be calculated.

For labeled erythrocytes as the vascular reference, we obtain the single-element matrices \( \mathbf{A}_o = (0) \) and \( \mathbf{W} = (1) \). The response of the whole liver is then simply

\[
\mathbf{h}_{RBC}(t) = \sum_{i=1}^{n} \alpha_i e^{it - \epsilon_i}
\]  

(B20)

We use the following multiexponential approximation for the erythrocyte outflow profile

\[
\mathbf{C}_{RBC}(t + t_0) = \frac{1}{F} \sum_{i=1}^{n} \alpha'_i e^{it}
\]  

(B21)

where \( \alpha'_i \) are empirical coefficients. Because the upslope is smooth, the following restriction is introduced

\[
\sum_{i=1}^{n} \alpha'_i = 0
\]  

(B22)

To account for the influence of the injection and collection devices, Eq. B21 is substituted into Eq. A4 from APPENDIX A. The result of this substitution is of the form of Eq. B20 with the following coefficients

\[
\alpha_o = \frac{\alpha'_i \left( \alpha_{cath} + \epsilon_i \right)}{\alpha_{cath}}
\]  

(B23)

The observable outflow profile of lactate \( \mathbf{C}_{Lac}(t) \) (outflow concentration normalized to dose) is the sum of the plasma and erythrocyte contents. When calculated per milliliter of blood, the latter becomes the sum of the first two elements of \( \mathbf{h}(t) \), \( h_o(t) \) and \( h_i(t) \)

\[
\mathbf{C}_{Lac} = \frac{1}{1 + \beta} \left[ h_o(t) + h_i(t) \right] * \mathbf{C}_{cath}(t)
\]  

(B24)

According to Eq. B19, \( h_i(t) \) and \( h_o(t) \) are sums of exponential functions, and according to Eq. A3, \( \mathbf{C}_{cath}(t) \) is a single exponential function. \( \mathbf{C}_{Lac}(t) \) can then be evaluated analytically using the convolution formula for exponential functions

\[
e^{at} * e^{bt} = \frac{e^{at} - e^{bt}}{a - b}
\]  

(B25)

Similarly, for the product, bicarbonate/CO₂

\[
\mathbf{C}_m = \frac{1}{1 + \beta} \left[ h_m(t) * \mathbf{C}_{cath}(t) \right]
\]  

(B26)

where \( h_m(t) \) is the third element of \( \mathbf{h}(t) \).

The hepatocyte influx permeability-surface area product is obtained by multiplying the value obtained for \( k_{ph} \) with the space of distribution of lactate in the plasma

\[
P_{in}S = F(1 - Hct)(t_{suc} - t_0)k_{ph}
\]  

(B27)

where \( F \) is blood flow rate, \( Hct \) is hematocrit, and \( t_{suc} \) is sucrose mean transit time.

APPENDIX C

Analytical Solutions for a Single Sinusoid

An analytical solution of the system of partial differential equations B1a–B1d with initial conditions B5a–B5b was found by using Laplace transformations. The strategy was similar to that used in the case of no barrier between plasma
and parenchymal cells (18). Details of the solution procedure have been deposited.\(^1\) The solution, representing the concentrations at time \(t\) at the outflow of a sinusoid of length \(x\), is given below.

a) when \(t < x\)

\[
c_p = c_\gamma = 0 \quad (C1)
\]

b) in the time interval \(x \leq t \leq (1 + \gamma)x\)

\[
c_p = \tilde{c}_\gamma e^{-k_{ph} + k_{pr} x} \left[ t - (1 + \gamma)x \right]
+ E(t) \left[ \tilde{c}_0 \frac{\zeta_i (t - x)}{2 \sqrt{(1 + \gamma)x - t}} l_1[\zeta_i (t - x) (1 + \gamma)x - t] + \tilde{c}_0 \frac{\zeta_i (t - x)}{2 \sqrt{(1 + \gamma)x - t}} l_1[\zeta_i (t - x) (1 + \gamma)x - t] \right]
+ E(t) \int_x^{(1 + \gamma)x} e^{(t - \tau) \beta} \frac{\phi \sqrt{\gamma} x}{2 \sqrt{(1 + \gamma)x - t}} l_1[\phi \sqrt{\gamma} x (t - (1 + \gamma)x)] l_1[\phi \sqrt{\gamma} x (t - (1 + \gamma)x)] d\tau
\]

(c) when \(t > (1 + \gamma)x\)

\[
c_p = e^{-(1 + \gamma)(k_{ph} + k_{pr} x) t} e^{-(k_{ph} + k_{pr} x) x} \frac{\phi \sqrt{\gamma} x}{2 \sqrt{(1 + \gamma)x - t}} l_1[\phi \sqrt{\gamma} x (t - (1 + \gamma)x)]
+ E(t) \int_x^{(1 + \gamma)x} e^{(t - \tau) \beta} \frac{\phi \sqrt{\gamma} x}{2 \sqrt{(1 + \gamma)x - t}} l_1[\phi \sqrt{\gamma} x (t - (1 + \gamma)x)] l_1[\phi \sqrt{\gamma} x (t - (1 + \gamma)x)] d\tau
\]

where \(l_0\) and \(l_1\) are modified Bessel functions of the first kind and order 0 and 1, respectively.

\[
c_\gamma = E(t) \int_x^{(1 + \gamma)x} e^{(t - \tau) \beta} \frac{\phi \sqrt{\gamma} x}{2 \sqrt{(1 + \gamma)x - t}} l_1[\phi \sqrt{\gamma} x (t - (1 + \gamma)x)] l_1[\phi \sqrt{\gamma} x (t - (1 + \gamma)x)] d\tau
\]

where

\[
\tilde{c}_0 = \frac{q_0 (1 + \beta)}{F} (1 + \lambda \beta) \quad (C4a)
\]

\[
c_\gamma = \frac{2}{\sqrt{1 + \gamma} k_{ph} k_{pr}} \quad (C7)
\]

\[
E(t) = e^{(1 + \gamma)(k_{ph} + k_{pr} x) t} e^{-(k_{ph} + k_{pr} x) x} \frac{\phi \sqrt{\gamma} x}{2 \sqrt{(1 + \gamma)x - t}} l_1[\phi \sqrt{\gamma} x (t - (1 + \gamma)x)] l_1[\phi \sqrt{\gamma} x (t - (1 + \gamma)x)] d\tau
\]
Starting from this solution, the evaluation of the response of the whole liver is analogous to that previously reported for the case of no barrier between plasma and parenchymal cells (18). The numerical results were similar to those obtained as shown in Appendix B, with reasonable accuracy.

Special cases. If erythrocytes are considered impermeable, \( k_{pr} = 0 \) and hence \( c_0 = 0 \) and \( c_0 \), therefore all terms in Eqs. C1a-C2b vanish except the first term of Eq. C1a and the first term of Eq. C2a. This yields

\[
\begin{align*}
\frac{c_p}{F_p} &= \frac{q_0}{F_p} e^{-k_{pr} t} \delta(t - 1 + \gamma x) \\
&+ e^{-(1 + \gamma k_{pr} t)(1 + \gamma x)} e^{-(1 + \gamma k_{pr} t) X} \sqrt{k_{ph} k_{hp} (1 + \gamma x) \sqrt{t - (1 + \gamma x)}} \\
&+ I_2 \sqrt{k_{ph} k_{hp} (1 + \gamma x) \sqrt{t - (1 + \gamma x)}}
\end{align*}
\]

where \( F_p = F/(1 + \beta) \) is plasma flow rate. This equation corresponds to the one describing hepatic galactose uptake (17) and, with \( \gamma = 0 \) and \( k_{ph} = 0 \), to the original equation by Sangren and Sheppard (39).

If hepatocytes are considered impermeable, \( k_{ph} = 0 \) and hence \( \phi = 0 \), therefore Eqs. C3a and C3b and the terms in Eqs. C1a and C1b containing integrals vanish. The resulting equations are equivalent to those formulated previously for the case of thin area, an indicator that penetrates hepatocytes rapidly, if \( \gamma \) is interpreted as the ratio of the total extravascular distribution space to the vascular space (18).

APPENDIX D

Exponentials With Matrix-Valued Exponents

The usual definition for an exponential with a matrix-valued exponent \( X \) is

\[
e^X = I + \frac{X}{1!} + \frac{X^2}{2!} + \frac{X^3}{3!} + \ldots
\]

with an identity matrix \( I \) that has the same dimension as \( X \). Assuming all the eigenvalues of \( X \) are distinct, the following relations hold

\[
X = M \Lambda M^{-1}
\]

and

\[
e^X = Me^\Lambda M^{-1}
\]

where \( \Lambda \) is a diagonal matrix containing the eigenvalues of \( X \), and \( M \) is a matrix whose columns are the eigenvectors of \( X \). These expressions can be evaluated after finding the eigenvalues and the eigenvectors of the matrix \( X \), using standardized numerical methods for finding the eigenvalues and eigenvectors.

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