Sahin, Selma, and Malcolm Rowland. Estimation of specific hepatic arterial water space. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G228–G236, 1998.—The aim of this study was to estimate the specific arterial water space and associated blood flow using statistical moments of the frequency versus time outflow profile, with a model with specific spaces for hepatic arterial (HA) and portal venous (PV) flows in parallel with a common space. Studies were performed in the in situ dual-perfused rat liver (n = 6–10), using Krebs-bicarbonate buffer with constant PV flow (12 ml/min) and various HA flow rates (3–6 ml/min). An impulse input-output technique was employed, varying the route of input, using [14C]urea as the reference indicator. Regardless of flow conditions, the frequency outflow profile after HA input was flatter and broader and the mean transit time longer than after PV input. Excellent recovery of marker was obtained in all cases. Applying the above model, the specific arterial space was estimated to be 9.7 ± 2.3 of total water space and receives ~17% of the HA flow, with the remainder mixing with portal blood in the common space. The estimated total water content of liver (0.67–0.72 ml/g liver) agrees well with that determined by desiccation (0.72 ± 0.01 ml/g liver).

Liver preparation was used under a variety of HA flow rates to determine the existence of such a specific space. An impulse input-output response technique was employed with [14C]urea chosen as the reference indicator for the estimation of this space.

MATERIALS AND METHODS

Materials

[14C]urea (7.3 mCi/mmol) was obtained from Sigma Chemical and used without further purification. All other chemicals were of analytical grade (BDH Chemicals).

Perfusion Procedure

Male Sprague-Dawley rats were used as liver donors (319.0 ± 19.3 g, wet liver wt 13.5 ± 0.8 g, mean ± SE; n = 10). The perfusate consisted of Krebs-bicarbonate buffer containing 3 g/l glucose and 6 mg/l taurocholic acid. Single-pass dual perfusion of rat livers was performed as previously described (34). Briefly, after introduction of anesthesia, the bile duct was cannulated and loose ligatures were placed around the PV, ensuring exclusion of the HA. At this point, the abdominal contents were deflected to the animal’s right and all branches of the celiac artery (i.e., left gastric artery, lineal artery) were tied very close to their junctions to the celiac artery. Only the HA was left patent after ligation of the gastroduodenal artery. After cannulation of the PV with a 16-gauge catheter (Argyle Medicut, OD 1.7 mm × 45 mm), the cannula was immediately connected to the tubing and the perfusion then started. Exsanguination of the liver was facilitated by inserting a PE-50 tubing into the thoracic vena cava via the heart. The HA was cannulated indirectly through the celiac artery using an 18-gauge (Argyle Medicut, OD 1.7 mm × 45 mm) or 20-gauge (Argyle Medicut, OD 1.1 mm × 45 mm) catheter. The second perfusion was then started, and the arterial cannula was fixed in place using tissue adhesive (Vetbond, 3M Animal Care Products). At the end of surgery all loose ligatures were tied securely, and then the HA cannula was connected to a mercury manometer (Fisons Scientific Equipment) by a side arm anterior to the arterial cannula, to monitor the perfusion pressure continuously. All operative procedures were completed within 20–30 min without interruption of flow to the liver. The exposed liver was kept moist with saline and covered with a piece of Parafilm to reduce dehydration. The preparation was then placed into a cabinet maintained at a temperature of 37 ± 2°C and stabilized for 20–30 min, using protein-free perfusate before the injection of [14C]urea. Viability of the liver was assessed from measurement of bile flow, perfusate recovery, and HA pressure and from gross appearance.

Desiccation of Liver

To obtain a physical estimate of total water content, the liver was quickly removed without exsanguination at the end of each experiment, weighed, cut into small pieces to increase the surface area for evaporation, and dried to a constant weight in an oven at 45°C. The liver was monitored up to 2 wk to ensure achievement of a constant weight.
Experimental Procedure

After the stabilization period, two different perfusion modes, dual and single, were utilized in the same liver preparation. Regardless of the perfusion mode, the perfusate was delivered into the PV at a constant flow rate (12 ml/min), whereas the HA flow rate (3, 4.5, and 6 ml/min) was varied. During the stabilization period all the livers were perfused bivascularly and then allocated into one of three groups. In group A (n = 5 livers), the HA flow was increased stepwise and then stopped so that the perfusion was via the PV only. In group B (n = 3 livers), the HA flow was decreased stepwise and then stopped. In group C (n = 2 livers), initially the liver was perfused only through the PV and then HA flow was increased stepwise.

After each alteration in the arterial flow the preparation was allowed to stabilize for ~10 min. Under each condition, a rapid bolus dose of [14C]urea (50 µl, 0.073 ± 0.036 µCi) was introduced, in a random order, into the injection port (extension set with T piece and Luer Lok; Venisystem, Abbott) of either the PV or HA, using a 100-µl Hamilton syringe with its tip positioned after the inflow of the perfusate to ensure adequate mixing, and then, after an appropriate interval (~5–10 min), into the alternate vessel. The injection solution also contained a small quantity of Evans blue dye to visually detect the efficiency of the injection. Immediately after an injection, the total effluent was automatically collected at 2- or 3-s intervals into wells of a locally made motor-driven carousel with 57 sampling holes for 2 or 3 min and thereafter (into test tubes) at increasing time intervals for a further 2 min. The radioactivity in 200 µl of outflow perfusate was determined on an LKB Wallac 1409 Rackbeta liquid scintillation counter with results expressed as dpm.

Estimation of Nonhepatic Region Transit Time

The experimental system has two different regions, the liver and nonhepatic components. The input cannula and outflow tubing account for the nonhepatic region. The time delay in this region was determined in the absence of the liver. The PV and HA cannulas were connected and secured to the outflow tubing, and then the system was perfused with Krebs-bicarbonate buffer at different flow rates. Under each flow condition, a bolus dose of [14C]urea (50 µl, ~0.027 µCi) was administered into either the PV or HA cannula, and then after an appropriate interval (~5 min) into the alternate vessel. The total outflow was collected automatically at 1-s intervals for 30 s. The estimated mean transit times (MTT) in the nonhepatic region (MTTNH) of the arterial and venous systems were 2.9 and 2.6 s, respectively, for a total flow rate of 15 ml/min, 2.2 and 2.4 s, respectively, for a total flow rate of 16.5 ml/min, and 2.1 and 2.2 s, respectively, for a total flow rate of 18 ml/min. In the absence of HA flow, MTTNH for the venous system was 3.3 s.

Data Analysis

The frequency output (f(t), s⁻¹) of the injected radiolabeled material at the midpoint of the sampling interval was calculated using the following equation

\[ f(t) = \frac{C(t) \cdot \dot{Q}}{D} \tag{1} \]

where \( C(t) \) is the concentration of radioactivity, \( \dot{Q} \) is the total perfusate flow (ml/s), and \( D \) is the injected dose (in dpm). A lag time, corresponding to the average transit time of [14C]urea in the nonhepatic region of the experimental system, was simply subtracted from the midtime of the sampling interval. The moments of the frequency outflow against midtime profiles were estimated by linear numerical integration, and then the parameters related to these moments (e.g., normalized variance (CV²)) were calculated using the following equations

\[ AUC = \int_0^\infty C(t) \cdot dt \tag{2} \]
\[ MTT = \frac{\int_0^\infty t \cdot C(t) \cdot dt}{AUC} \tag{3} \]
\[ VTT = \frac{\int_0^\infty t^2 \cdot C(t) \cdot dt}{AUC} - \left(\frac{MTT}{2}\right)^2 \tag{4} \]

where AUC is the area under the concentration versus time profile, MTT is the average time taken for a molecule to pass through the organ, and VTT is the variance of transit times, which is the temporal spreading or dispersion within the organ. These equations assume that \( Q \) is constant throughout and that solute is not eliminated, which is the case for urea. Theoretically, to obtain the VTT within the liver correction should be made for the VTT within the nonhepatic part of the system. In practice, for urea this correction proved so small as to be inconsequential.

CV² is given by

\[ CV^2 = \frac{VTT}{(MTT)^2} \tag{5} \]

CV² is a dimensionless parameter and has been used as a measure of relative dispersion of compound within the liver (32).

The recovery (F) is given by

\[ F = \frac{AUC \cdot \dot{Q}}{D} \tag{6} \]

The frequency outflow versus midtime profiles for each condition were transformed to the corresponding dimensionless plots by multiplying the frequency with, and dividing the time by, the relevant MTT.

Total Water Volume of Liver

Total water volume of the liver (VTW) was calculated by two different methods, physical and indicator dilution. The physical water volume of the liver was estimated by desiccation. The difference in weights (volume) of the wet (Wwet) and dry (Wdry) livers represents VTW, assuming the density of water to be 1.

\[ VTW = W_{wet} - W_{dry} \tag{7} \]

Subsequently, results for this and all other volume terms were expressed in milliliters per gram of wet liver weight. The volume (VT) based on the indicator-dilution method, for both single- and dual-perfusion modes, was estimated as follows for single perfusion

\[ V_T = \dot{Q} \cdot MTT \tag{8} \]

and for dual perfusion (9)

\[ V_T = \dot{Q}_{HA} \cdot MTT_{HA} + \dot{Q}_{PV} \cdot MTT_{PV} \tag{9} \]
where $MTT_{HA}$ and $MTT_{PV}$ are the total transit times of solute through the liver after injection into the HA and PV, respectively, $Q_{HA}$ is the HA flow rate, and $Q_{PV}$ is the PV flow rate.

Estimation of Specific Water Space Associated With Hepatic Artery

Three different methods were used to estimate the specific water space associated with the HA ($V_{sa}$).

Desiccation method. The assumptions made in this method are as follows. 1) $V_{TW}$ represents the sum of the common space ($V_c$) and specific arterial space ($V_{sa}$). 2) In the absence of HA flow, PV flow does not have access to the $V_{sa}$.

On the basis of these assumptions, $V_{sa}$ is then given by

$$V_{sa} = V_{TW} - V_{PVs} \quad (10)$$

where $V_{PVs}$ is the apparent volume of distribution obtained from the single (PV)-perfused rat liver preparation and estimated as the product of the corresponding $MTT$ and $Q_{PV}$.

Serial model. This model was originally described by Field and Andrews (9) for the estimation of the specific arterial vascular space. Briefly, the total hepatic water space is considered to consist of three separate spaces, two specific spaces supplied solely by the HA and PV, each connected serially to a common space (Fig. 1). The model is based on several assumptions. To quote these investigators, "The common space receives mixed blood and the transit time of a solute through this space is the same for both the PV and HA. Even if the total space changes, the proportion of the total vascular space represented by any one of the specified spaces is assumed to remain constant." Therefore, this model predicts a linear relationship between the fractional arterial space ($V_{HA}/V_T$) and the fractional arterial flow ($Q_{HA}/Q_T$) expressed by

$$V_{HA} = a \frac{Q_{HA}}{V_T} + b \quad (11)$$

where $a$ is the slope of the regression line and the intercept on the ordinate ($b$) represents the fraction of the hepatic water space supplied solely by the artery; $Q_T$ is the total flow rate ($Q_{HA} + Q_{PV}$); and $V_T$ is the total volume of the liver calculated using Eq. 9.

Parallel model. In this model the liver is envisaged as comprising a common space and two specific water spaces. Unlike the serial model, here the specific spaces are arranged in parallel with the common space (Fig. 2). In this model the following assumptions are made. 1) The common space is supplied by both portal and arterial streams. 2) Regardless of the route of administration (HA or PV), the transit time of solute through the common space for a given flow rate is the same. 3) The fractional flows of HA and PV to the common and respective specific water spaces are independent of alterations in $Q_{HA}$ and $Q_{PV}$. 4) The specific HA water space remains constant regardless of the $Q_{HA}$ used.

On the basis of these assumptions, the following equation holds after HA input (see APPENDIX)

$$MTT_{HA} = \frac{V_{sa}}{Q_{HA}} + \frac{V_c}{(Q_c + Q_d)} (1 - f_3) \quad (12)$$

where $MTT_{HA}$ is the total transit time of the solute through the liver after injection into the HA, $Q_2$ and $Q_4$ are the flow rates to the common space from PV and HA inputs, respectively, and $f_3$ is the fraction of HA flow perfusing the specific space. Assuming that there is no specific portal space ($Q_2 = Q_{PV}$), recasting Eq. 12 into experimental variables then yields

$$MTT_{HA} = \frac{V_{sa} * W}{Q_{HA}} + \frac{MTT_{PVs} * Q_{PVs} (1 - f_3)}{[Q_{PV} + (1 - f_3) * Q_{HA}]} \quad (13)$$

where $V_{sa} * W$ is the volume of the specific arterial space per gram of liver, $W$ is liver weight, $MTT_{PVs}$ and $Q_{PVs}$ are the MTt and corresponding $Q_{PV}$ after PV administration when operating in the single PV-perfusion mode, and $Q_{PV}$ is the portal flow rate when operating in the dual-perfusion mode. In the current experiment the PV flow rate was maintained the same in both the single- and dual-perfusion modes. Mean values of $V_{sa} * W$ and $f_3$ were estimated by fitting Eq. 13 to the entire experimental data using mixed-effect regression analysis (NONMEM; Ref. 4), with equal weighting of the data.

Estimation of HA Perfusion Resistance

The HA resistance ($R_{HA}$, mmHg·ml⁻¹·min⁻¹) of the preparation was determined from the HA perfusion pressure ($P_{HA}$, mmHg) and $Q_{HA}$ (ml/min; Ref. 37) given by

$$R_{HA} = P_{HA}/Q_{HA} \quad (14)$$

Statistical Analysis

The results are presented as means ± SE and compared by means of a one-way analysis of variance. A $P < 0.05$ was taken as significant.

RESULTS

Hemodynamic Parameters

During perfusion, the liver maintained its uniform light brown color and stable bile production (5.7 ± 0.5 µl/min). Regardless of the flow conditions, the volumetric recovery of the total effluent was always >95% (96–98%) of the input flow rate. The HA perfusate pressure increased with an increase in the HA flow (i.e.,
57 ± 4, 83 ± 12, and 96 ± 13 mmHg for Q_HA of 3, 4.5, and 6 ml/min, respectively). However, for a given flow rate, the pressure remained stable on commencement of the injection, indicating the constancy of the flow rate during the administration. The arterial resistance remained relatively constant (17.2 ± 2.6 to 19.8 ± 3.1 mmHg·ml⁻¹·min⁻¹) irrespective of the Q_HA employed.

Outflow Profiles and Tracer Transit Times

Representative frequency (f) outflow versus midterm profiles for [¹⁴C]urea after injections into the PV and HA under different flow conditions are depicted in Figs. 3–5.

After HA injection, urea emerged slightly earlier but the peak was diminished (e.g., maximum frequency (f_max) = 0.021 ± 0.002 s⁻¹ for HA and 0.028 ± 0.002 s⁻¹ for PV) and the curve was flatter and broader than after PV input (Fig. 3). With an increase in HA flow rate, the outflow profiles after intra-arterial injections were displaced to earlier times (time to reach maximum frequency (t_max) from 27 to 23 s) and gave slightly higher peaks (e.g., f_max from 0.020 to 0.023 s⁻¹; Fig. 4A). In contrast, the effect of increased HA flow on the outflow profiles after PV input was not that pronounced; although the fractional output at the peak increased slightly (e.g., f_max from 0.029 to 0.032 s⁻¹) with an increase in HA flow, the curves peaked almost at the same time (e.g., t_max ~ 21 s; Fig. 4B). Figure 5A shows the urea profiles normalized to the corresponding MTT in the same liver preparation after HA administrations. Regardless of the perfusate flow rate (15, 16.5, and 18 ml/min) after normalization, in each case, the HA profiles were superimposable. Similar observations were also observed for the PV profiles (Fig. 5B).

The MTT after arterial administration was longer than that after venous administration (Table 1). With an increase in Q_HA, the MTT obtained after HA injection was decreased. In the case of PV injections, the MTT were comparable irrespective of both perfusion mode and flow rate. The CV² for labeled urea was very similar whether injection was into the HA or PV, indicating that relative spreading of urea within the liver is independent of the route of administration. Excellent recovery of [¹⁴C]urea was obtained for all cases.

Total Water Volume of Liver

Estimates of V_TW by two different methods are depicted in Fig. 6. In the desiccation experiment, a constant dry weight was achieved within a week. The values obtained by desiccation (0.72 ± 0.01 ml/g, n = 10
livers) and transit time methods after dual perfusion under different flow conditions 

\[
\begin{align*}
0.67 \pm 0.03 \text{ ml/g (n = 10)} & \quad \text{for } Q_1, \\
0.71 \pm 0.03 \text{ ml/g (n = 8)} & \quad \text{for } Q_2, \\
0.67 \pm 0.06 \text{ ml/g (n = 6)} & \quad \text{for } Q_3,
\end{align*}
\]

where \( Q_1 = 15.0 \text{ ml/min}, Q_2 = 16.5 \text{ ml/min}, \) and \( Q_3 = 18.0 \text{ ml/min} \) were in good agreement. In contrast, the value based on the transit time after single perfusion was only 74% of the physical volume.

### Specific HA Water Space

The results for \( V_{sa}^* \) and the fractions of HA flow perfusing this space and the common space are summarized in Table 2.

#### Table 1. Results obtained after bolus injections of \(^{14}C\)urea into portal vein and hepatic artery of dual perfused and into portal vein of single perfused rat liver preparation

<table>
<thead>
<tr>
<th>Perfusion Mode</th>
<th>( Q_T, \text{ml/min} )</th>
<th>Route</th>
<th>( n )</th>
<th>MTT, s</th>
<th>CV(^2)</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dual</td>
<td>15.0</td>
<td>HA</td>
<td>10</td>
<td>47.7 ( \pm 3.0 )</td>
<td>0.44 ( \pm 0.02 )</td>
<td>92.1 ( \pm 3.2 )</td>
</tr>
<tr>
<td>16.5</td>
<td>HA</td>
<td>10</td>
<td>41.9 ( \pm 3.1 )</td>
<td>0.43 ( \pm 0.02 )</td>
<td>94.4 ( \pm 3.1 )</td>
<td></td>
</tr>
<tr>
<td>18.0</td>
<td>HA</td>
<td>8</td>
<td>35.5 ( \pm 2.0 )</td>
<td>0.34 ( \pm 0.02 )</td>
<td>106.2 ( \pm 3.3 )</td>
<td></td>
</tr>
<tr>
<td>16.5</td>
<td>PV</td>
<td>10</td>
<td>34.5 ( \pm 3.2 )</td>
<td>0.38 ( \pm 0.01 )</td>
<td>106.4 ( \pm 3.3 )</td>
<td></td>
</tr>
<tr>
<td>18.0</td>
<td>PV</td>
<td>8</td>
<td>35.5 ( \pm 2.0 )</td>
<td>0.34 ( \pm 0.02 )</td>
<td>106.2 ( \pm 3.3 )</td>
<td></td>
</tr>
<tr>
<td>12.0</td>
<td>PV</td>
<td>10</td>
<td>36.4 ( \pm 2.8 )</td>
<td>0.45 ( \pm 0.03 )</td>
<td>99.3 ( \pm 2.8 )</td>
<td></td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE; \( n \), no. of liver preparations. HA, hepatic artery; PV, portal vein; \( Q_T \), total flow rate [sum of constant PV flow rate \( Q_{PV}; 12 \text{ ml/min} \) and variable HA flow rate \( Q_{HA}; 3, 4.5, \) and 6 ml/min]; MTT, mean transit time; CV\(^2\), normalized variance.

Desiccation method. This method showed considerable variation, with results ranging from 12.6 to 43.2% and an average value of 29.8 \( \pm 4.4\% \) \( \text{mean} \pm \text{SE}, n = 9 \) livers of the total water space. Only 1 of 10 experiments was rejected because of a negative value obtained for \( V_{sa}^* \). The results differed significantly from both the serial and parallel models \((P < 0.001)\).

Serial model. The \( V_{sa}^* \) calculated by this method provided a mean value of 13.7 \( \pm 4.6\% \) of the total water space.

Parallel model. This model yielded a \( V_{sa}^* \) of 0.070 \( \pm 0.014 \text{ ml/g liver} \) corresponding to 9.7 \( \pm 1.9\% \) of the total water space estimated from desiccation. The fraction of HA flow that perfuses the specific space \( f_3 \) was 0.173 \( \pm 0.074 \), with the remainder supplying the common space.

The values of the specific arterial space obtained from the serial and parallel models agreed well, with no significant difference between them.

### Fig. 5

A: dimensionless outflow profiles of \(^{14}C\)urea from a representative liver obtained after bolus administration into HA of a dual-perfused liver preparation with arterial flow rates of 3.0 (HA1), 4.5 (HA2), and 6.0 (HA3) ml/min. B: dimensionless outflow profiles of \(^{14}C\)urea from a representative liver obtained after bolus administration into PV (12 ml/min) of a single (PVs)-perfused rat liver and dual-perfused liver preparation with arterial flow rates of 3.0 (PV1), 4.5 (PV2), and 6.0 (PV3) ml/min. MTT, mean transit time.

### Fig. 6

Total water volume of liver estimated from desiccation (DESIC) and MTT methods after single (PVs) and dual perfusion with total flow rates of 15.0 (VT1), 16.5 (VT2), and 18.0 (VT3) ml/min.
In the present study, [14C]urea was administered via the PV, the MTT were very similar regardless of both the perfusion modes (single or dual) and Q˙HA, indicating that distribution of PV-administered urea is minimally affected by the presence of HA inflow. This observation is in accordance with Pang et al. (27), who recently reported unchanged vascular, interstitial, and cellular distribution volumes after PV administrations for different HA-to-PV flow ratios.

**Total Water Volume of Liver**

The desiccation method offers a direct approach for estimation of the absolute amount of water in the whole body (8, 20, 36) and in various tissues, including liver (11) and heart (10). Whole body studies (8, 20, 36) suggest that the isotope-dilution method, using tritiated water, overestimates the total water content of the body compared with the desiccation method. Nevertheless, Goresky (12) found a good agreement between these two methods in the PV-perfused dog liver. The current study extends this knowledge to the dual-perfused liver preparation. Desiccation of the liver was performed by heating for an extended period (~2 wk) at a relatively low temperature (45–50°C) to minimize the possibility of release of other volatile substances in addition to water. In the dual-perfused rat liver, the values obtained from the indicator dilution method (0.67–0.72 ml/g) agreed very well with the value (0.72 ml/g) obtained by desiccation. Regardless of the method employed, the estimates of water volume lie within the range of values reported in the literature (18, 25, 28, 31, 33) and agree well with the tentative value of 75% of liver weight given by Greenway and Stark (14).

In the absence of arterial input, urea is not expected to distribute into the entire water space of the liver because ~10% of the total water space is specific to the arterial input. However, the estimated value (74% of...
total) was less than the expected value (90% of total), probably because of a reduction in the total perfusion rate from 15 to 12 ml/min. An increase in the volume of distribution of tritiated water with an increase in the perfusate flow rate (e.g., from 0.51 to 0.65 ml/g for the flow rates of 15 and 30 ml/min; Ref. 18) clearly shows the effect of perfusion rate on the observed volume of distribution and may be taken as an indication of poor perfusion of the liver at low flow rates. Similar observations were also made by Pang et al. (26).

Specific HA Space

In the current analysis it is assumed that there is no specific portal space, nor, unlike the case of a specific arterial space, is there any support for, or suggestion of, a specific portal space in the literature. Theoretically, the presence of a specific portal space should be detected by predicted differences in the output profiles after PV input operating in the dual- and single-perfusion modes. In practice, we could discern no such difference between these perfusion modes, which could mean that either there is no specific portal space or it is too small to be detected under the experimental conditions. This was not the case for the HA-specific space.

Although the dual-perfusion studies support the idea that a small fraction of the sinusoids remains separate in favor of HA input, the degree of mixing between the HA and PV inflows at the microscopic level is still controversial. Field and Andrews (9) were the first to calculate the proportion of the specific arterial vascular space; from the linear relationship between the fractional HA flow and fractional space, they estimated that 10% of the total vascular space in dog liver is perfused solely by the HA. Their method was later applied by Nakai et al. (Ref. 24; using Evans blue dye), and then by Ahmad et al. (Ref. 2; using labeled red blood cells). Although Nakai et al. (24) failed to demonstrate the existence of such a specific space, Ahmad et al. (2) obtained an almost identical value (11%) in the rat liver as that reported by Field and Andrews (9). More recently, Kassissia et al. (19) took the product of HA flow and the difference between arterial and venous transit times as a close approximation of the specific arterial vascular space, which provides values of 6.7% of the total sinusoidal space in normal liver and 7.8% in the cirrhotic liver. Both Reichen (31) and Pang et al. (27) reported a slightly larger water space (5%) after arterial than after portal administration.

In the present study three different methods, desiccation, serial, and parallel methods, were employed for the estimation of specific arterial water space. Of these, the desiccation method is based on the volume difference between the physical and indicator-dilution estimates. Although the method is very easy to perform, two types of error may be associated with its application; one error is the temperature effect on the estimation of physical water volume and the other is the error involved in the estimation of the volume of distribution from application of moment analysis caused by extrapolation and low perfusion rate. The effect of temperature can be easily avoided by desiccating at relatively low temperature (e.g., 50°C) or alternatively by employing another drying method (e.g., freeze drying). The estimates of specific arterial water space using this method were relatively high compared with the estimates obtained with the serial and parallel models. This is attributed to a slight underestimation of urea volume of distribution, caused by the inadequate perfusion of the liver in the absence of HA flow. The other two methods (serial and parallel) are very similar with regard to the assumptions made in the development of the models but differ structurally. In the serial model the specific spaces are serially connected to the common space, whereas in the parallel model these are in parallel with the common space. Although both models consider HA flow segregation, only the parallel model provides an estimate for the degree of the arterial flow segregation. It may be argued that neither a serial nor a parallel model truly reflects reality; however, the PV and HA perfusion studies suggest that some of the arterial blood drains directly into the terminal hepatic venules, thus completely bypassing the sinusoidal bed. Furthermore, some of the vessels draining the peribiliary plexus may form direct connections to the terminal hepatic venule (35). These findings tend to support the existence of a similarly connected specific arterial space.

Agreement between the current results for the specific arterial space (9.7%) and the literature values (10–11%; Refs. 2, 9) is noteworthy. Furthermore, close similarity between the MTT values after PV administration in the absence and presence of the HA input imply that PV input is minimally affected by the presence of the HA flow. This latter observation agrees well with that by Pang et al. (27), who recently reported that the excess space associated with the HA is independent of total-to-arterial flow ratio and that the distributional volumes after PV administration are unaffected by the presence of arterial input. These observations strongly support the assumption made in the development of the parallel model.

Together, these results suggest that regardless of the space of interest and methods used, there are two separate spaces for the HA input; one is a common space shared with the PV, and the second is a specific space. Some authors (see, e.g., Refs. 2, 24) have suggested that separation of these sinusoids is not necessarily anatomic but functional; others (27, 31) have related this excess space almost exclusively to the peribiliary capillary plexus, whereas still others (19) do not distinguish between these characteristics. The PV and HA branches run parallel within the liver, and their terminal branches supply blood to the sinusoids. In the rat, unlike the PV, the HA flow drains into the sinusoids via various pathways including arteriovenous anastomosis, arteriosinusoidal twigs, and the peribiliary capillary plexus (5, 15, 22, 23). Of these, the peribiliary plexus, which receives the majority of its afferents from the HA (15, 16), may provide an additional anatomic space in favor of arterial input. On the other hand, flow in the sinusoids is not unidirectional but can be reversed (3, 21). The angle at which arterioles join the
sinusoids and also sphincter activity (22) determine not only direction of blood flow through the sinusoids but also the degree of mixing (5). Therefore, sinusoids that were previously perfused by both the PV and HA may on another occasion be perfused solely by the HA (i.e., functionally separate sinusoids). All these suggest that both anatomic and functional spaces contribute to the total HA specific space. Nevertheless, the contribution of each to the total cannot be separated using the methods employed in this study. If we assume that 5% of the total specific space is due to peribiliary capillary plexus, as suggested by Reichen (31) and Pang et al. (27), the remainder (~4–5%) is due to functional characteristics of the arterial input.

The knowledge obtained from the existence of a specific HA water space and its flow fraction can be extended to make predictions about the fate of an eliminated substance after arterial administration. Such a prediction may have relevance to the systemic exposure of compounds after HA administration, as sometimes arises during the treatment of hepatic carcinomas, many of which reside predominantly on the arteriolar (1). If the enzyme distribution responsible for elimination is the same as in the common space, the route of administration should have no effect on the disposition of a substance within the liver. In contrast, if there is no enzyme in the specific HA space, up to 17% of the HA dose will escape extraction, on the basis of the assumption that separation of the dose is a function of the flow, the upper limit being reached with compounds whose extraction ratio after PV input is in excess of 0.90–0.95 (2).

APPENDIX

Derivation of Model Equation for Calculation of Specific HA Water Space

In the derivation it is assumed that

\[ \dot{Q}_T = \dot{Q}_{HA} + \dot{Q}_{PV} \]  

(A1)

where \( \dot{Q}_T, \dot{Q}_{HA}, \dot{Q}_{PV} \) are the total outflow and HA and PV flow rates, respectively.

The fractional flows of the common and specific water spaces are given by

\[ f_1 = \frac{\dot{Q}_1}{\dot{Q}_{PV}}; \quad f_2 = \frac{\dot{Q}_2}{\dot{Q}_{PV}} = 1 - f_1; \quad f_3 = \frac{\dot{Q}_3}{\dot{Q}_{HA}}; \quad f_4 = \frac{\dot{Q}_4}{\dot{Q}_{HA}} = 1 - f_3 \]  

(A2)

where \( \dot{Q}_1 \) and \( \dot{Q}_2 \) are the PV flows to the specific portal and common water spaces and \( \dot{Q}_3 \) and \( \dot{Q}_4 \) are the HA flows to the specific arterial and common water spaces, respectively.

Because the derivation of the equations for both arterial and venous injections is the same, only that after HA injection is now considered.

Considering mass balance

\[ D = \dot{Q}_T \cdot \int_0^\infty C_{out\_T} \cdot dt \]  

(A3)

where \( C_{out\_T} \) is the total outflow concentration after injection into the hepatic artery. The net rate of outflow is the sum of that from the specific arterial supply \( \dot{Q}_3 \cdot C_{out\_sa} \) and that from the common space \( \dot{Q}_4 \cdot C_{out\_c} \). So that

\[ \dot{Q}_T \cdot C_{out\_T} = \dot{Q}_3 \cdot C_{out\_sa} + (\dot{Q}_2 + \dot{Q}_4) \cdot C_{out\_c} \]  

(A4)

where \( C_{out\_sa} \) and \( C_{out\_c} \) are the outflow concentrations of the specific arterial and common water spaces after injection into the HA. Integrating Eq. A4 over the interval \( t = 0, \infty \) gives

\[ \int_0^\infty C_{out\_T} = \frac{\dot{Q}_3}{\dot{Q}_T} \int_0^\infty C_{out\_sa} \cdot dt + \frac{\dot{Q}_3 + \dot{Q}_4}{\dot{Q}_T} \int_0^\infty C_{out\_c} \cdot dt \]  

(A5)

The MTT after an HA injection (MTT\(_{HA}\)), after appropriate substitution, is given by

\[ MTT_{HA} = \frac{\int_0^\infty t \cdot C_{out\_T} \cdot dt}{\int_0^\infty C_{out\_T} \cdot dt} \]  

(A6)

By definition, the MTT through the specific HA space (MTT\(_{sa}\)) and common space (MTT\(_{c}\)) are given by

\[ MTT_{sa} = \frac{\int_0^\infty t \cdot C_{out\_sa} \cdot dt}{\int_0^\infty C_{out\_sa} \cdot dt} \]  

(A7)

\[ MTT_{c} = \frac{\int_0^\infty t \cdot C_{out\_c} \cdot dt}{\int_0^\infty C_{out\_c} \cdot dt} \]  

(A8)

Furthermore, after arterial administration, the doses associated with the specific HA and common spaces (D\(_{sa}\) and D\(_{c}\)) are

\[ D_{sa} = f_3 \cdot D = Q_3 \cdot \int_0^\infty C_{out\_sa} \cdot dt \]  

(A9)

\[ D_{c} = (1 - f_3) \cdot D = (Q_2 + Q_4) \cdot \int_0^\infty C_{out\_c} \cdot dt \]  

(A10)

Substituting Eqs. A7–A10 into Eq. A6 then provides

\[ MTT_{HA} = MTT_{sa} \cdot f_3 + MTT_{c} \cdot (1 - f_3) \]  

(A11)

Finally, by replacing MTT\(_{sa}\) and MTT\(_{c}\) with their respective volumes and flow terms, one obtains

\[ MTT_{HA} = \frac{V_{sa}}{Q_{HA}} + \frac{V_{c}}{(Q_2 + Q_4)} \cdot (1 - f_3) \]  

(A12)

where \( V_{sa} \) and \( V_{c} \) are the volumes of the specific HA and common spaces, respectively.

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