# Comparison of the Hepatic Uptake Clearances of Fifteen Drugs with a Wide Range of Membrane Permeabilities in Isolated Rat Hepatocytes and Perfused Rat Livers

Seiji Miyauchi,[1] Yasufumi Sawada,[2] Tatsuji Iga,[2] Manabu Hanano,[3] and Yuichi Sugiyama[4,5]

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The hepatic uptake clearances of 15 ligands with a wide range of permeabilities were determined in rats using two techniques: centrifugal filtration with isolated hepatocytes and the multiple indicator dilution (MID) method with isolated perfused livers. Some of the uptake clearance values were taken from the literature. Uptake clearance values obtained from isolated hepatocytes were extrapolated to that per gram liver ($PS_{inf,cell}$), assuming that 1 g of liver has $1.3 \times 10^8$ cells. The values of $PS_{inf,cell}$ varied from approximately 0.1 to 72 (mL/min/g liver). The values of $PS_{inf,cell}$ were similar to those ($PS_{inf,MID}$) determined by the MID method for ligands with uptake clearances below approximately 1 mL/min/g liver. However, for the ligands with larger uptake clearances, the $PS_{inf,MID}$ values were lower than the $PS_{inf,cell}$ values and appeared to reach an upper limit (approx. 15–20 mL/min/g liver). The $PS_{inf,cell}$ values of 1-propranolol, tetraphenylphosphonium ($TPP^+$), and diazepam were 72, 43, and 22 mL/min/g liver, respectively, whereas their uptake clearances ($PS_{inf,MID}$) determined by the MID method were 4 to 10 times lower. One of the possible mechanisms for this discrepancy is that an unstirred water layer, which may exist in Disse's space in isolated perfused livers (and probably under *in vivo* condition), limits the hepatic uptake rate of ligands with extremely high membrane permeabilities.

KEY WORDS: hepatic uptake clearance; rat hepatocytes; perfused rat liver; unstirred water layer.

## INTRODUCTION

Clearance concepts (1–3) can serve to predict from *in vitro* metabolic experiments the metabolic activity in the intact liver. For such predictions, one must consider enzymatic activity (4), hepatic blood flow (3), and unbound fraction in blood (3,5). However, when equilibrium between blood and hepatocytes is slow, transmembrane permeability must also be considered in predicting metabolic ability of the intact liver (6,7). We have previously demonstrated that the assumption of a rapid equilibrium does not always hold even for the lipophilic drug, 4-methylumbelliferone (7).

To estimate the membrane permeability of a ligand in the liver, the following techniques have been used: (a) centrifugal filtration using isolated hepatocytes (8), (b) multiple indicator dilution (MID)[6] in isolated perfused livers (9), and (c) tissue sampling after single injection *in vivo* (10). Although initial uptake rate in isolated hepatocytes can easily be determined, care must be taken in interpreting the results since isolated hepatocytes lack cell polarity and anatomical architecture such as capillaries, Disse's space, and bile canalicules (11).

On the other hand, the MID method has an advantage that the membrane permeability can be determined with hepatic spatial architecture maintained. It may be of value if membrane permeability in the more physiological perfused liver can be predicted utilizing *in vitro* uptake data obtained by a convenient method with isolated hepatocytes. In the present study therefore, we compared the hepatic uptake clearances ($PS_{inf}$) of various ligands with different permeabilities using the two techniques.

## METHOD

*Chemicals.* $^{14}$C-Inulin (3.22 μCi/mg), $^3$H-cholic acid ($^3$H-CA; 16 Ci/mmol), $^3$H-digoxin ($^3$H-DIG; 10.1 Ci/mmol), $^3$H-inulin (225 mCi/g), $^3$H-ouabain ($^3$H-OUA; 22.1 Ci/mmol), and $^3$H-*l*-propranolol ($^3$H-l-PR; 26.6 Ci/mmol) were purchased from New England Nuclear Corp. (Boston, MA). $^3$H-Diazepam ($^3$H-DIZ; 82.3 Ci/mmol), $^3$H-tetraphenylphosphonium ($^3$H-$TPP^+$; 26 Ci/mmol), and $^3$H-vinblastine ($^3$H-VBL; 23 Ci/mmol) were purchased from Amersham International Ltd. (Buckinghamshire, England). $^{14}$C-Cefodizime ($^{14}$C-CFZ; 20 mCi/mmol) was kindly supplied by Taiho Pharmaceutical Co. (Tokushima, Japan). Bovine serum albumin (BSA; Fraction V) was purchased from Sigma Chemical Co. (St. Louis, MO). 1-PR was kindly supplied by ICI pharmacy (Tokyo). All other chemicals were commercial products of analytical grade.

*Liver Perfusion Study (Multiple Indicator Dilution Study).* All of the isolated liver procedures were the same as reported previously (7). The perfusate consisted of 20% (v/v) washed bovine erythrocytes and 2 or 3 g/dL BSA in Krebs–Ringer bicarbonate buffer (pH 7.4). The perfusate flow rates were 12–16 mL/min. After a stabilization period of 10–20 min, a 200-μL mixture of $^{14}$C inulin (0.3 μCi), as an extracellular reference, and a test substance [$^3$H-CA, 15 μCi (0.9 nmol); $^3$H-DIG, 15 μCi (1.5 nmol); $^3$H-DIZ, 30 μCi (0.4 nmol); $^3$H-OUA, 5 μCi (0.2 nmol); $^3$H-$TPP^+$, 30 μCi (1.2 nmol); $^3$H-VBL, 15 μCi (0.7 nmol)] was simultaneously injected as a bolus into the portal vein. The 200-μL injection solution was made by mixing washed erythrocytes with Krebs–Ringer buffer containing the test substance, with the extracellular reference. In the case of cefodizime injection, a

[1] Department of Biophysics and Physicochemistry, Faculty of Pharmaceutical Sciences, University of Hokkaido, Kita-12 Nishi-6, Kita-ku, Sapporo 060, Japan.
[2] Department of Pharmacy, University of Tokyo Hospital, Faculty of Medicine, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.
[3] College of Pharmacy, Nihon University, 7-7-1 Narawashinodai, Funabashi City 274, Japan.
[4] Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.
[5] To whom correspondence should be addressed.

[6] Abbreviations used: BSP, bromosulfophthalein; 4-MU, 4-methylumbelliferone; 4-MUG, 4-MU glucuronide; 4-MUS, 4-MUS sulfate; TCA, taurocholate; SA, salicylic acid; TBA, tolubutamide. See Fig. 2 legend for others.

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200-μL mixture of $^3$H-inulin (10 μCi) and $^{14}$C-CFZ (2 μCi, 100 nmol) was injected as a bolus.

After injection, the total effluent from the hepatic vein was collected at 0.5- or 1.0-sec intervals for 30 sec using a turntable. The effluent dilution curves were expressed as outflow fraction per milliliter of perfusate. The dilution curves were corrected for the longer vessel and catheter transit time (7). The longer vessel transit time used in the present analysis was 1.9 ± 0.2. The radioactivities from $^3$H and $^{14}$C in the collected samples were determined in a liquid scintillation spectrophotometer (Model 3255, Packard Instruments Corp., Downers Grove, IL).

*Analysis of Multiple Indicator Dilution (MID) Curves.* We determined the uptake clearance ($PS_{inf}$) utilizing the distributed model developed by Goresky *et al.* (9). The relationship between the outflow fractions of a test substance and its appropriate extracellular reference [$C_s(t')$ and $C_{ref}(t')$, respectively] was expressed as the natural logarithm of the ratio [$C_{ref}(t')/C_s(t')$] vs time. The initial slope calculated by a linear regression analysis of this plot reflects the uptake rate constant ($K_1'$) (12). Using this value as the initial value, $K_1'$ was calculated by fitting the $C_{ref}(t')$ and $C_s(t')$ to the distributed model with the iterative nonlinear least-squares method. The details of the fitting were reported previously (7,13). The hepatic uptake clearance ($PS_{inf,MID}$) for a total ligand can be calculated by the following equation:

$$PS_{inf,MID} = K_1' * V_{ext} \quad (1)$$

where $V_{ext}$ represents the distribution volume, that is, the volume accessible to the extracellular reference during its passage through the liver, which can be estimated by multiplying the plasma flow rate by its mean transit time (7,13).

*Uptake Study of Isolated Hepatocytes.* Male rats (180–220 g) given free access to food and water were used in the same procedure as reported previously (14). The viability for each experiment was determined by the trypan blue exclusion test; the value obtained usually ranged from 95 to 98%.

A temperature-controlled chamber (5 mL), equipped with a stirring device, was used to measure the initial uptake rates of drugs (DIG, 1-PR, $TPP^+$, VBL). The medium was continuously oxygenated with humidified gas (95% $O_2$, 5% $CO_2$). After a 10-min preincubation of the cells (2.4–3.6 × $10^6$ cells/ml) at 37°C in buffer containing the same concentration of BSA in the incubation medium as that used in the MID experiments, an aliquot (50 μL) of labeled drug was added to start the uptake under stirred conditions. The final concentrations of labeled substances were as follows: 0.05 μCi/mL (5 n*M*) $^3$H-DIG, 0.05 μCi/mL (1.9 n*M*) $^3$H-l-PR, 0.05 μCi/mL (1.9 n*M*) $^3$H-$TPP^+$, and 0.05 μCi/mL (2.2 n*M*) $^3$H-VBL. The incubation medium used in the uptake experiment was Hanks' buffer containing 10 m*M* HEPES, pH 7.4. Uptake of substance was terminated by the centrifugal filtration method (14). Total $^3$H and $^{14}$C radioactivities in hepatocytes were determined.

The amounts taken up by hepatocytes were corrected for the adherent water film (2.2 μL/mg protein) and were expressed as the ratio of the concentration in the intracellular space [cellular volume ($V_{cell}$), 5.2 μL/mg protein] to that in the medium. The adherent water volume and intracellular volume were determined using $^{14}$C-inulin and $^3H_2O$, respectively (15). Protein was determined with protein assay kits (Bio-Rad Co. Ltd., Tokyo), using BSA as a standard. The initial slope (tan α) of uptake (expressed as C/M ratio) was obtained from a regression analysis of the linear portion in an uptake time course.

Furthermore, we calculated the hepatic uptake clearance ($PS_{inf,cell}$) for a total ligand as (tan α) * $V_{cell}$, after converting the $V_{cell}$ value to that per gram of liver, assuming that 1 mg of protein contains 1.1 × $10^6$ cells and 1 g of liver contains 1.3 × $10^8$ cells (16). Hence, $PS_{inf,cell}$ is expressed as follows:

$$PS_{inf,cell}\ (\text{mL/min/g liver}) = \frac{\tan\alpha\ (\text{min}^{-1}) * V_{cell}\ (\text{mL/mg protein}) * (1.3 \times 10^8)\ (\text{cells/g liver})}{(1.1 \times 10^6)\ (\text{cells/mg protein})} \quad (2)$$

## RESULTS

*MID Studies.* Figure 1 shows the typical dilution curve patterns. The dilution curves of CA and DIZ reached their peaks earlier than those of the extracellular reference (inulin), and the peak heights were much smaller, indicating that these substances were rapidly taken up into hepatocytes. In contrast, the dilution curves of OUA were similar to those of the extracellular reference, suggesting a slow uptake into hepatocytes. The differences in the uptake clearances ($PS_{inf,MID}$) can be more clearly seen in the ratio plots in Fig. 2 of these and other ligands obtained previously in our laboratory (17–19). A comparison of the initial slopes of ratio plots among the ligands examined indicates that 1-PR and CFZ have the maximum and minimum uptake clearances, respectively. L-PR is taken up by a perfused liver approximately 50 times faster than CFZ. For $TPP^+$, CA, OUA, and CFZ, the initial uptake phase continues for a relatively long time. On the other hand, the ratio plots of other ligands have peaks around 10–15 sec, indicating that an appreciable efflux occurs at the time. In Table I, the $PS_{inf,MID}$ values thus obtained from the present experiments are listed together with those obtained previously.

*Uptake Studies into Isolated Hepatocytes (Table II).* In Fig. 3, the uptake time courses for the ligands examined in the present study are depicted. The initial uptake phases of 1-PR and DIZ ceased at about 15 sec, suggesting a rapid efflux from hepatocytes, whereas those of DIG, $TPP^+$, and VBL proved linear, at least up to 1 min. The fact that the efflux starts at earlier times for the above ligands is compatible with result from the MID experiment except for VBL (Figs. 2 and 3). 1-PR and CFZ have the maximum and minimum uptake rates, respectively, and the uptake clearance of 1-PR is more than 400 times larger than that of CFZ.

*Correlation between the $PS_{inf,cell}$ and $PS_{inf,MID}$ Values.* Figure 4 shows a comparison of the uptake clearances obtained using isolated hepatocytes ($PS_{inf,cell}$) and liver perfusion ($PS_{inf,MID}$). Up to approximately 1 mL/min/g liver, the $PS_{inf,cell}$ values showed good agreement with the $PS_{inf,MID}$ values. In contrast, as the uptake clearances get larger, the $PS_{inf,MID}$ values get smaller than the $PS_{inf,cell}$ values and appear to reach an upper limit (approx. 15–20 mL/min/g liver).

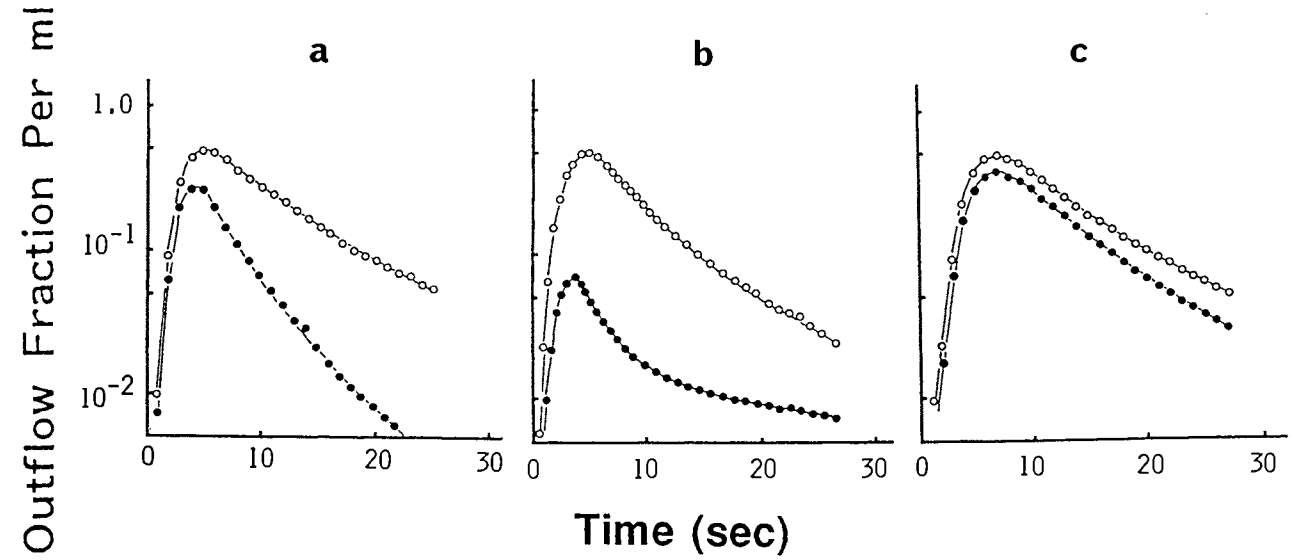


Fig. 1. Normalized venous outflow dilution curves for various ligands: (a) cholic acid; (b) diazepam; (c) ouabain. (○) Labeled inulin; (●) labeled ligand.

## DISCUSSION

The major purpose of this paper is to examine whether hepatic uptake clearance in perfused livers can be predicted from that determined *in vitro* by a convenient method with isolated hepatocytes. Using various ligands with a wide range of permeabilities, we compared the hepatic uptake clearances obtained from the two techniques: centrifugal filtration with isolated hepatocytes (8,14,15) and the multiple indicator dilution (MID) method in isolated perfused livers (9,11,12). In the present study, we used incubation medium and perfusate containing 2–3% BSA to unify unbound fractions of ligands in the two experimental systems.

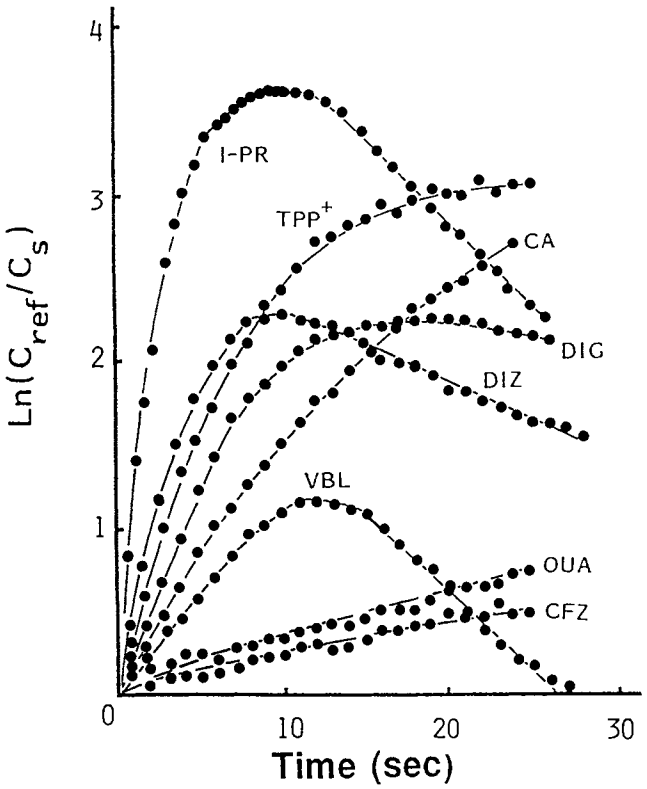


Fig. 2. Plot of the natural logarithm of the ratio (reference outflow fraction per mL/substance outflow fraction per mL) vs time. The ratio plot for 1-PR is from Ref. 18. CA, cholic acid; DIG, digoxin; DIZ, diazepam; 1-PR, 1-propranolol; $TPP^+$, tetraphenylphosphonium; VBL, vinblastine; CFZ, cefodizime; OUA, ouabain.

The hepatic uptake clearances ($PS_{inf,cell}$) obtained from isolated hepatocytes coincided well with those ($PS_{inf,MID}$) obtained from isolated perfused livers when the values were less than approximately 1 mL/min/g liver (Fig. 4). In contrast, there are great discrepancies between the $PS_{inf,cell}$ and the $PS_{inf,MID}$ values for the ligands with larger $PS_{inf,cell}$ values; that is, the $PS_{inf,cell}$ values overestimate the $PS_{inf,MID}$ values. A cursory examination of this relation (Fig. 4) indicates that the $PS_{inf,MID}$ value appears to have an upper limit (15–20 mL/min/g liver). The existence of the upper limit for uptake in the isolated perfused liver system suggests that the uptake rates for highly permeable ligands might be restricted by the diffusion through an unstirred water layer, possibly existing on the surface of hepatocytes.

Disse's space, that is, the interstitial space of the liver, is occupied by a matrix of fibrillar material (11), which excludes large molecules from increasing proportions of Disse's space as their molecular weights increase. This effect has been simulated *in vitro* by allowing molecular probes to diffuse from buffer into hyaluronic acid gel (20) and collagen (21). Consequently, in Disse's space, the stirring effect of the blood flow can be said to be minimal. Structurally, the hepatocyte itself has finger-like microvilli and an adherent water film (15). These phenomena have suggested the existence of an unstirred water layer in Disse's space under the physiological condition (22,23).

In the present study, we assumed that isolated hepatocytes do not have the unstirred water layer, but strictly speaking, a minimum unstirred water layer directly adjacent to the plasma membrane may exist even under stirred con-

Table I. Multiple Indicator Dilution Analysis

| Drug | Flow (mL/min) | Availability | $K_1'$ ($sec^{-1}$) | $PS_{inf,MID}$ (mL/min/g liver)[a] | Albumin conc. (%)[b] | Drug conc. ($\mu M$) | MW |
|---|---|---|---|---|---|---|---|
| DIZ | 16.5 | 0.16 | 0.45 | 5.9 | 2.0 | Tracer | 285 |
| | (0.1)[c] | (0.02) | (0.05) | (0.7) | | | |
| $TPP^+$ | 12.2 | 0.21 | 0.30 | 4.0 | 2.0 | Tracer | 339 |
| | (0.1) | (0.03) | (0.05) | (0.7) | | | |
| CA | 12.3 | 0.24 | 0.30 | 4.0 | 3.0 | Tracer | 409 |
| | (0.2) | (0.06) | (0.03) | (0.4) | | | |
| DIG | 12.4 | 0.24 | 0.24 | 3.2 | 2.0 | Tracer | 781 |
| | (0.1) | (0.01) | (0.01) | (0.1) | | | |
| VBL | 12.4 | 0.52 | 0.13 | 1.8 | 2.0 | Tracer | 811 |
| | (0.4) | (0.02) | (0.01) | (0.2) | | | |
| OUA | 12.7 | 0.58 | 0.058 | 0.76 | 2.0 | Tracer | 585 |
| | (0.1) | (0.05) | (0.007) | (0.09) | | | |
| CFZ | 12.0 | 0.77 | 0.027 | 0.36 | 2.0 | Tracer | 585 |
| 1-PR (18) | 16.0 | 0.04 | 1.5 | 18.0 | 3.0 | 60 | 259 |
| 4-MU (7) | 16.0 | 0.11 | 1.14 | 12.5 | 3.0 | 100 | 176 |
| TCA (17) | 12.0 | 0.05 | 0.52 | 6.2 | 2.0 | Tracer | 516 |
| TBA (32) | 16.0 | 0.66 | 0.18 | 2.1 | 1.9 | Tracer | 270 |
| SA (32) | 16.0 | 0.61 | 0.13 | 1.6 | 1.9 | Tracer | 138 |
| BSP (43) | 16.0 | ND[d] | 0.045 | 0.65 | 2.5 | Tracer | 815 |
| 4-MUS (14) | 16.3 | 0.82 | 0.0079 | 0.10[e] | 3.0 | 100 | 256 |
| 4-MUG (14) | 16.7 | 0.97 | 0.0042 | 0.52[e] | 3.0 | 100 | 352 |

[a] Uptake clearance, calculated by Eq. (1).
[b] Albumin concentration in the perfusate.
[c] The values in parentheses represent the standard error of three independent experiments.
[d] Not determined.
[e] Obtained by simulating the outflow curve of a test substance, on the basis of the "distributed" model (14).

ditions. According to Barry and Diamond (24), the lowest values of the unstirred water layer measured adjacent to membranes are those obtained for permeation into red blood cells in suspension. Recently, Holland *et al.* (25) have elegantly estimated the unstirred water layer thickness to be as low as 0.6 μm for oxygen permeation through red blood cells under stirred conditions with the stop-flow technique. Therefore, the assumption that the hepatic uptake clearance obtained from isolated hepatocytes is close to the intrinsic membrane permeability may be considered sound and was, thus, used to determine the upper limit of the $PS_{inf,MID}$ values.

Based on the assumption that the unstirred water layer exists only in the perfused liver, the uptake clearance obtained from the MID experiment is given by the following equation (19,24):

$$\frac{1}{PS_{inf,MID}} = \frac{1}{PS_m} + \frac{1}{P_{dif}} \quad (3)$$

where $PS_m$ and $P_{dif}$ represent the true uptake clearance across the sinusoidal membrane and the diffusion clearance through the unstirred water layer, respectively. Assuming that binding equilibrium between ligands and albumin exists in the unstirred water layer, $P_{dif}$ in Eq. (3) is given by the following equation (19):

$$D_{dif} = \frac{f_u * A * D_f}{\delta} + \frac{(1 - f_u) * A * D_b}{\delta} \quad (4)$$

where $A$ is the effective uptake area; $\delta$ is the thickness of the unstirred water layer; $D_f$ and $D_b$ are the aqueous diffusion coefficient for unbound and bound ligands, respectively; and $f_u$ is the unbound fraction in the perfusate. The diffusion coefficients of unbound and albumin-bound ligands are approximately $3 \times 10^{-6}$ and $6 \times 10^{-7}$ $cm^2/sec$ (26), respectively, considering that the molecular weights of small ligands and albumin are approx. 500 and 70,000, respectively. Assuming a hepatocyte to be cubical, Bass *et al.* (23) calculated the total effective uptake area to be $0.36 \times 10^3$ $cm^2/g$ liver. For the highly permeable ligands such as 1-PR, $TPP^+$, TCA, and DIZ (Fig. 4), $f_u$ values in the presence of 2–3% BSA ranged from 0.2 to 0.5 (17–19). Assuming the upper limit of the $PS_{inf,MID}$ value to be identical to the $P_{dif}$ value, the $\delta$ values calculated on the basis of Eq. (4) using the above-described parameters range between 18 and 27 μm, any of which would be larger than the width of Disse's space [2 μm (27)] but smaller than the estimates of the thickness of the unstirred water layer reported for other epithelia studied *in vivo* (24). This discrepancy in the $\delta$ value may be explained by the following three reasons. First, we assumed here that the diffusion rates of drugs through Disse's space were governed by aqueous diffusion coefficients. However, it is possible that actual diffusion coefficients ($D_f$ and $D_b$) through Disse's space are much smaller than aqueous diffusion coefficients, since the fibrillar matrix occupies Disse's space (11), causing a higher viscosity in Disse's space. Indeed, Stock *et al.* (28) have demonstrated, utilizing the microcirculation video technique, that the diffusion coefficients reveal little molecular weight dependency and are much smaller than the aqueous diffusion coefficients. Sec-

Table II. Hepatic Uptake Studies Using Isolated Hepatocytes

| Drug | Initial slope ($min^{-1}$)[a] | $V_0$/c (μL/min/$10^6$ cell)[b] | $PS_{inf,cell}$ (mL/min/g liver)[c] | Albumin conc. (%)[d] | Drug conc. (μM) | MW |
|---|---|---|---|---|---|---|
| 1-PR | 108 | 544[e] | 72 | 3.0 | Tracer | 259 |
| | (8)[f] | (41) | (5) | | | |
| $TPP^+$ | 65 | 335[e] | 43 | 2.0 | Tracer | 339 |
| | (5) | (26) | (3) | | | |
| DIG | 14 | 72[e] | 9.4 | 2.0 | Tracer | 781 |
| | (1) | (5) | (0.7) | | | |
| VBL | 6.4 | 33[e] | 4.3 | 2.0 | Tracer | 811 |
| | (0.1) | (1) | (0.2) | | | |
| DIZ (19) | 33 | 169[e] | 22 | 2.0 | Tracer | 285 |
| TCA (44) | —[g] | 71[h] | 9.3 | 2.0 | 10–100 | 516 |
| 4-MU (14) | 7.1 | 37 | 4.8 | 3.0 | 100 | 176 |
| CA (44) | — | 17[h] | 2.2 | 3.0 | 20–200 | 409 |
| OUA (15) | — | 15[h] | 1.9 | 0 | 10–1000 | 585 |
| TBA (19) | 2.5 | 13[e] | 1.7 | 1.9 | Tracer | 270 |
| SA (19) | 2.2 | 11[e] | 1.5 | 1.9 | Tracer | 138 |
| BSP (45) | — | 5.1 | 0.66 | 2.0 | 200 | 815 |
| CFZ (19) | 0.25 | 1.3[e] | 0.17 | 2.0 | Tracer | 585 |
| 4-MUS (14) | 0.17 | 0.88 | 0.11 | 3.0 | 100 | 256 |
| 4-MUG (14) | 0.089 | 0.46 | 0.06 | 3.0 | 100 | 352 |

[a] Obtained by regression of the linear portion of the uptake time course.
[b] Uptake clearance per $10^6$ cells.
[c] Obtained by extrapolating the $V_0$/c value to that per gram liver, on the basis of Eq. (2).
[d] Albumin concentration in the incubation medium.
[e] Obtained by multiplying the initial slope by intracellular volume (5.2 μl/mg protein).
[f] The values in parentheses represent the standard error of three independent experiments.
[g] Unavailable.
[h] Obtained by summing up the reported Vmax/Km value and simple diffusion clearance.

ond, this discrepancy might also arise from the oversimplification of the method for estimating the effective uptake surface area, since the arrangement of hepatocytes in the liver may be more complicated than a simple regular packing of cubical hepatocytes along the blood flow path (11). Thus, the effective uptake surface area may be smaller than that calculated by Bass *et al.* (23). Third, the δ values may be larger than the width of Disse's space due to the tortuosity of the diffusion path. To access hepatocyte surfaces, the molecules must diffuse an additional distance through the fibrillar material matrix occupying Disse's space. Schultz and Armstrong (29) determined the true diffusion of the mole-

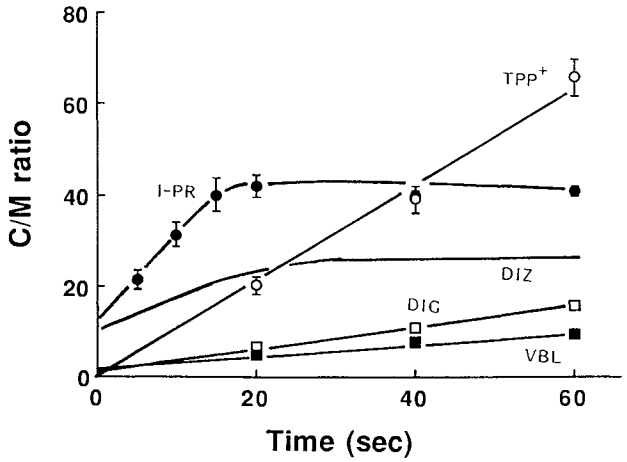


Fig. 3. Time courses of uptake of various substances into isolated hepatocytes. Hepatocytes (2.4–3.6 × $10^6$ cells/mL) were preincubated for 10 min at 37°C prior to the addition of drugs. Data represent the mean ± SE of three experiments. The time course of uptake for DIZ into hepatocytes was obtained in our previous study (19). The initial slope multiplied by the cellular volume (5.2 μL/mg protein) represents the uptake clearance into the hepatocytes.

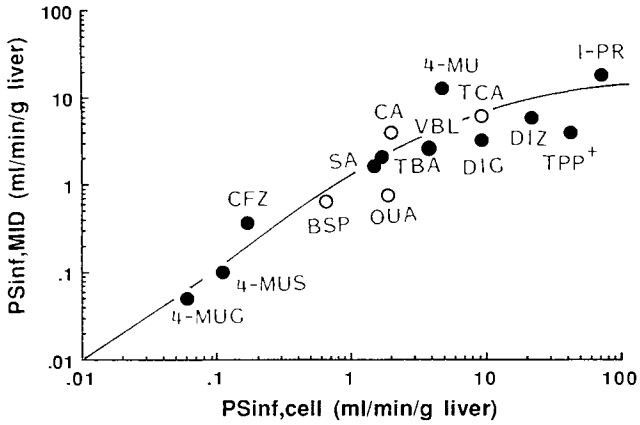


Fig. 4. Comparison of the hepatic uptake clearances determined using perfused livers (MID techique) and isolated rat hepatocytes. Abscissa: the uptake clearance determined with isolated hepatocytes ($PS_{inf,cell}$; mL/min/g liver). Ordinate: the uptake clearance determined with isolated perfused livers ($PS_{inf,MID}$; mL/min/g liver). The filled and open circles represent data obtained in our laboratory and those quoted from experiments in other laboratories, respectively.

cules in the interstitial space by correcting the diffusion distance based on this tissue tortuosity concept.

The deterioration of linearity in the initial phase of ratio plots obtained from MID experiments is attributed to the efflux of ligand from hepatocytes (9,12). Figure 2 reveals great differences in the linear periods among the ligands. That is, for $TPP^+$, CA, DIG, OUA, and CFZ, the linear initial uptake phase continued for a relatively longer time, indicating minimal efflux during this time period. For CA, DIG, and OUA, such an inwardly directed concentrated uptake may be due to carrier-mediated active transport (15,30). However, $TPP^+$ is taken up by hepatocytes and immediately sequestered into mitochondria according to the membrane potential (31), a fact which might prevent its efflux from hepatocytes. On the other hand, the ratio plots for 1-PR, DIZ, SA, TBA, and VBL exhibited a decay phase at relatively earlier times (~15 sec; Fig. 2 and Ref. 32), which may suggest a rapid efflux for these ligands. We then compared the efflux properties obtained from the isolated perfused liver system for these ligands with those from the isolated hepatocytes. The uptake by isolated hepatocytes of ligands which were rapidly effluxed by the isolated perfused liver (1-PR, DIZ, SA, and TBA), reached a plateau very rapidly (<30 sec; Fig. 3 and Ref. 19), while the initial uptake phase continued longer for the other ligands (DIG, $TPP^+$, and CFZ) as shown in Fig. 2. Such a comparison advances the possibility that the efflux rate may also be predictable from the transport studies using isolated hepatocytes. On the other hand, for VBL, there existed great difference in the initial linear uptake phase among the two experimental systems. Namely, the initial linear uptake phase of VBL by isolated hepatocytes continued for a relatively long time (at least 1 min) (Fig. 3), whereas its ratio plot earlier exhibited the decay phase (in 10 sec) (Fig. 2). The reason for this discrepancy has yet to be elucidated.

A larger surface area may be available for uptake in isolated hepatocytes compared with the isolated perfused liver, a difference which may explain the great discrepancy between the $PS_{inf,MID}$ and the $PS_{inf,cell}$ values. For an isolated hepatocyte, a ligand may be taken up via the bile canalicular membrane as well as the basolateral domain (33). The basolateral membrane area occupies approximately 75% of the surface area of the hepatocyte (34). Even if the canalicular membrane uptake route is taken into consideration, the discrepancy in the hepatic uptake clearances from the two experiments might still be, at most, 30%. Furthermore, the $PS_{inf,MID}$ values coincided well with the $PS_{inf,cell}$ values for ligands with a low permeability (Fig. 4). Consequently, the difference in effective uptake surface areas between isolated hepatocytes and isolated perfused liver may not fully account for this discrepancy between the $PS_{inf,MID}$ and the $PS_{inf,cell}$ values.

Recently, heterogeneity between centrilobular and periportal cells has been shown (35). Within the acinus, differences exist in oxygen tension, drug metabolizing activities, and concentration of cofactors such as glutathione (36). Furthermore, heterogeneity of uptake clearance along the blood flow path has been demonstrated (37). Such heterogeneity may be a cause for the discrepancy between the hepatic uptake clearances ($PS_{inf,cell}$ and $PS_{inf,MID}$) determined by the two methods, since the $PS_{inf,cell}$ values were calculated by the simple extrapolation considering only numbers of cells per gram liver. However, the analysis based on a tube model by Bass *et al.* (38) suggested that unidirectional hepatic removal rate is independent of lobular distribution of the intrinsic removal ability once the total intrinsic ability in the liver is kept constant. Therefore, lobular heterogeneity may not be a reason for the discrepancy between the $PS_{inf,cell}$ and the $PS_{inf,MID}$ values.

The unstirred water layer concept has been already proposed in the field of intestinal absorption (39), and intestinal absorption rates of highly permeable substances are known to have an upper limit imposed by the presence of an unstirred water layer (40). As discussed in the field of intestinal absorption (39), an unstirred water layer might influence transport parameters also in the liver. Bass and Pond (23) have previously proposed that diffusion in the unstirred water layer may contribute to an albumin-mediated hepatic transport phenomenon. They have demonstrated with an elegant mathematical technique that experimental data exhibiting the so-called "albumin-mediated hepatic transport" phenomenon could be explained by the effect of the unstirred water layer without considering the catalytic dissociation of a ligand from albumin at the hepatocyte surface (23). Most recently, we also found that the albumin-mediated hepatic transport phenomenon was more clearly observed for ligands with a high membrane permeability than for those with a low permeability and supported the contribution of diffusion in the unstirred water layer to this phenomenon (19).

In conclusion, the $PS_{inf,cell}$ values overestimate the $PS_{inf,MID}$ values for highly permeable ligands, because of the diffusion resistance in this unstirred water layer possibly existing in the Disse's space of the liver.

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