In vitro organ slice models are valuable tools to predict drug metabolism and are used to select lead compounds for further development. However, these models also present challenges. Many potential drug candidates are rejected in drug discovery because of undesirable in vivo pharmacokinetics. In vitro tools can serve to predict pharmacokinetics, enabling selection of lead compounds with favorable properties. A major advantage of the use of in vitro models is the opportunity to use human tissue for predictions, overcoming difficulties that may occur with extrapolation of animal data because of large interspecies differences in metabolism. Until recently, liver-derived models were solely used for this purpose, whereas only a few researchers have included in vitro models from extrahepatic organs into predictions. However, the intestine has especially been proven to be metabolically very active; moreover, its metabolism has been shown to be readily influenced by drug interactions on enzyme and transporter activity. Recently, we have developed an in vitro model using tissue slices from liver, small intestine, colon, kidney, and lung to predict whole body metabolic clearance and showed that, dependent on the substrate used, extrahepatic organs may account for a significant part of metabolic clearance of xenobiotics. An advantage of the use of slices for clearance prediction, which they share with isolated cells like hepatocytes, is that they exhibit the natural interplay between cofactors, transporters, and metabolizing enzymes that is lost when subcellular fractions are used. Unlike in hepatocytes, however, cell-to-cell contacts and normal tissue microarchitecture remain intact in slices, and they are prepared according to a rapid, reproducible, and relatively simple method from various organs and animal species. However, potential drawbacks also exist, including reported poor penetration of high turnover drugs into the inner cell layers of slices and loss of metabolic capacity during prolonged incubation. These results show that drawbacks of the use of slices for clearance prediction are largely surmountable. Provided that thin liver slices and physiological albumin concentration are used, whole body metabolic clearance is predicted with acceptable accuracy with organ slices. These results emphasize the applicability of organ slices in this field of research.
in vivo organ clearance is directly related to the free fraction of the compound in plasma, which has been confirmed in vitro (Shibata et al., 2000; Bachmann et al., 2003), in some publications it is reported that in vivo clearance is underpredicted in vitro when intrinsic clearance is corrected for plasma protein binding (Ludden et al., 1997; Blanchard et al., 2004).

In the present study, we empirically identified and designed strategies to eliminate potential sources of underprediction of in vivo clearance using tissue slices of rat lung, intestine, kidney, and liver. Intrinsic clearance of model compounds with variable intrinsic liver clearance [7-hydroxycoumarin (7-HC), testosterone, warfarin, 7-ethoxycoumarin (7-EC), midazolam, haloperidol, and quinidine] was compared between liver slices of various thicknesses and with freshly isolated hepatocytes. In this manner, the extent to which cells in the inner layers of liver slices take part in metabolic conversion of high and low clearance drugs was examined. Intrinsic slice metabolic clearances of 7-HC, testosterone, and 7-EC in organ slices also were determined after various periods of preincubation (0, 4, 8, and 24 h) to assess the stability of metabolism during prolonged culturing. In addition, organ slices were incubated with model compounds either without or in the presence of albumin in the incubation medium to determine the influence of the free fraction on calculated organ clearances. Finally, an improved in vitro method using organ slices for whole body metabolic clearance prediction is proposed.

Materials and Methods

Chemicals. Quinidine, haloperidol, 7-HC, warfarin, testosterone, Leibovitz’s L-15 medium (L-15), insulin, trypan blue, and low gelling temperature agarose (amphotericin B), and penicillin/streptomycin were products from Invitrogen (Carlsbad, CA). Liver perfusion medium, liver digest medium, and Henseleit buffer.

Preparation of Precision-Cut Organ Slices. Male Wistar rats (body weight between 300 and 350 g) (Harlan, Horst, The Netherlands) were housed in standard cages with food (Harlan chow) and tap water available ad libitum. They were anesthetized with isoflurane and N2O/O2 before organs were isolated. The liver was perfused in situ with prewarmed (37°C) and pregassed (95% O2/5% CO2) Krebs-Henseleit buffer. For the isolated hepatocyte studies, male Sprague-Dawley rats (200–220 g) from Charles River Italia (Calco, Italy) were maintained under a 12 h light/dark cycle in standard cages and bedding with free access to standard commercial food pellets and tap water. Although a different rat strain was used for the hepatocyte studies than for the slices (because these studies were performed in different laboratories), we do not expect this to influence the experimental outcome because previous (unpublished) studies with microsomes from Wistar and Sprague-Dawley rats within our laboratories did not show differences in metabolic rates of 20 tested compounds (including warfarin, quinidine, midazolam, and testosterone) between the two strains. After i.p. anesthesia with sodium thiopental (100 μg/kg), the liver was perfused in situ with prewarmed (37°C) and pregassed (95% O2/5% CO2) liver perfusion medium for about 5 min and then with prewarmed (37°C) and pregassed (95% O2/5% CO2) liver digest medium for about 10 min. Then the liver was excised and hepatocytes were harvested, by removing the capsule, into L-15 (4°C), and centrifugation (four times at 50g for 5 min at 4°C), the viability of the hepatocytes was determined using the trypan blue (0.4%) exclusion method. Only preparations with viability greater than 80% were used. Hepatocytes were resuspended in L-15 at a trypan blue viable concentration of 10⁶ cells/ml.
Validation of Clearance Prediction with Organ Slices

Incubation of Hepatocytes. Hepatocyte incubations were performed in 24-well plates under continuous shaking at 37°C under room atmosphere. In each well, 10⁶ hepatocytes were incubated in a final volume of 1 ml of L-15 containing 1 μM insulin and 2 mM glutamine. The amount of solvent dimethyl sulfoxide present in the incubation was 0.1%. From each well containing hepatocytes, an aliquot of 80 μl of the incubation suspension was taken at 0, 5, 10, 20, 30, 45, 60, and 90 min.

Sample Analysis. Medium samples taken from the incubations without albumin were immediately mixed with an equal amount of ice-cold methanol (slices) or acetonitrile (hepatocytes). In case albumin was used in the incubation medium, samples were mixed at a ratio of 1:4 with methanol/acetonitrile to precipitate all the protein. The medium/solvent mixture was then centrifuged (700g for 20 min at 4°C) and stored at −20°C until analysis. The supernatants then were used for liquid chromatography/mass spectrometry (LC-MS) analysis. To exclude substrate depletion by unspecific binding of compounds to the slice, in some cases substrate concentrations in slice incubations were also determined at the end of incubation in the slice/medium homogenate (obtained by sonification), which was then mixed with methanol and centrifuged (2 min, 2000g). Because in previous studies testosterone was shown to bind to the slices, the latter approach was used as standard for this compound. The protein content of the slices, the latter approach was used as standard for this compound. The protein content of the slices, suspension samples were taken in all cases.

LC-MS Analysis. The samples were analyzed by LC-MS using a turbo ion spray source and a triple quadrupole API 2000 instrument (PerkinElmer Life and Analytical Sciences, Boston, MA). A 4.6 (inner diameter) × 12.5 mm C8 column (Agilent Technologies, Palo Alto, CA) was applied using a mobile phase containing 10 mM ammonium formate (pH 4.0) and acetonitrile increasing from 5% to 95% within 0.4 min and then back to 5% in 1.4 min. The flow rate was 1.5 ml/min for the first 0.2 min to equilibrate the column quickly, and the flow rate was reduced to 0.6 ml/min 0.2 min after injection. The eluent from the column was led into the mass spectrometry system only between 0.3 min and 1.05 min after injection of the sample. The ion spectra were acquired previously (Houston, 1994; De Kanter et al., 2004). Briefly, CLint was calculated by comparing the peak areas with authentic standards of each compound.

Protein Determination. Protein content of the samples was determined using a Bio-Rad protein assay dye reagent and a BSA standard curve. Slices protein was either determined (from the albumin-free incubations) after dissolving the precipitate left after methanol extraction (when homogenate samples were taken for LC-MS analysis, see above) or in slices taken at the end of incubation (when medium samples were used for LC-MS analysis, see above). Either the precipitate or the slices were dissolved in 5 M NaOH at 37°C for 30 min, sonificated, and subsequently diluted to 0.1 M NaOH. The Bio-Rad reagent then was added, and finally the protein content was determined by an enzyme-linked immunosorbent assay reader (ThermoMax microplate reader, Molecular Devices, Sunnyvale, CA) at 450 and 650 nm.

Calculation of Clearance. The intrinsic clearance (CLint), which is defined as clearance without the physiological limitation by blood flow or plasma protein binding, such as is the case in the current in vitro situations, was determined according to the substrate depletion method as described previously (Houston, 1994; De Kanter et al., 2004). Briefly, CLint was calculated by multiplying the slope (k) of the natural logarithm of the concentration of compound remaining against time with the incubation volume and dividing this value by the number of hepatocytes in the incubation volume (eq. 1) or the protein content of the slices (eq. 2):

\[ \text{CL}_{\text{int}} = \frac{-k \cdot \text{volume}}{\text{number of hepatocytes}} \]  
\[ \text{CL}_{\text{slic}} = \frac{-k \cdot \text{volume}}{\text{slic protein content (mg)}} \]

For all the compounds it was found that the slope of the natural logarithmic concentration of compound remaining against time was constant when the initial concentration was 2 μM. This implies that the (overall) apparent metabolic \( K_m \) was \( \gg 2 \mu M \).

If in previous slice experiments metabolic rates had appeared to be constant and no aspecific adhesion to the slices had occurred, samples were taken only at the end of incubation instead of at different time points. Then \( k \) was determined from the slope of the natural logarithm of the initial concentration (in this case substrate incubated without a slice) and the end-concentration in the medium (or slice homogenate in the case of testosterone) against time.

CLorg obtained in this way (in ml/min/10⁶ cells for hepatocytes or ml/ min/mg protein for slices) then was up-scaled to obtain the whole organ intrinsic clearance (CLorg, int) by multiplying with a scaling factor. Scaling factors for slice data were calculated by multiplying the organ weight (per kilogram body weight) with the protein content (in milligrams) per gram organ wet weight (Table 1), thus obtaining the total organ protein content per kilogram rat. Scaling factors differ somewhat from previous data published by our laboratory (De Kanter et al., 2004) because of differences in procedures of solubilizing the protein from slices and organs. From data obtained from the hepatocyte incubations (ml/min/10⁶ cells), intrinsic organ clearance (per kilogram body weight) was calculated assuming a hepatocellularity of 109 million hepatocytes/g liver (Carlile et al., 1997) and 39.9 g liver/kg body weight, which was determined previously in our laboratory (see Table 1).

To calculate metabolic organ clearance (CLorg, int) from CLint, org the organ blood flow and the unbound fraction of the compound in plasma in vivo \((f_u)\) and the unbound fraction of the compound in the medium \((f'_u)\) in vitro were considered according to eq. 3, which is based on the venous equilibration model (or well-stirred model).

\[ \text{CL}_{\text{org}} = \frac{\text{blood flow} \times f_u f'_u \times \text{CL}_{\text{int, org}}}{\text{blood flow} + f_u f'_u \times \text{CL}_{\text{int, org}}} \]  

From individual organ clearances, the whole body clearance was calculated as described previously, taking into account the prehepatic extraction ratio on liver clearance (De Kanter et al., 2004).

Determination of ATP Content and Histomorphological Examination of Slices. For the experiments determining the influence of slice thickness on clearance, the ATP content of liver slices with various thicknesses was determined as described previously (de Kanter, 2005) with the only difference that the slice homogenate was centrifuged (at 16,000g for 2 min), and the supernatant was used for measuring the ATP content with a luminometer (Luminocounter; PerkinElmer Life and Analytical Sciences) instead of the homogenate. ATP content was determined relative to the protein content of the slices.

For histomorphological examination, slices were fixed in 70% ethanol and further processed as described previously (de Graaf et al., 2000). Sections were stained with H&E. The number of cell layers of which slices consisted was estimated by counting the number of cells in their cross-sections.

Results

Influence of Slice Thickness on Clearance. Intrinsic organ clearance of model compounds was determined with liver slices of several thicknesses and with hepatocytes in suspension. As shown in Fig. 1, there is a strong relationship between the wet weight and the protein content of liver slices (Fig. 1A) and the wet weight and the number of cell layers in a slice, as determined on histomorphological examina-

### Table 1

<table>
<thead>
<tr>
<th>Organ Weight</th>
<th>Organ Protein Content</th>
<th>Scaling Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/kg body weight</td>
<td>mg/g organ weight</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>39.9</td>
<td>141</td>
</tr>
<tr>
<td>Lung (agarose filled)</td>
<td>18.4</td>
<td>9</td>
</tr>
<tr>
<td>Kidney</td>
<td>7.7</td>
<td>170</td>
</tr>
<tr>
<td>Small intestine</td>
<td>18.9</td>
<td>120</td>
</tr>
<tr>
<td>Colon</td>
<td>2.9</td>
<td>122</td>
</tr>
</tbody>
</table>

*a* Determined by measuring the protein content of an organ sample with a known wet weight.  
*b* Calculated by multiplying organ weight with the protein content per gram organ.
tion (Fig. 1B). Therefore, wet weight was further used as a measure for slice thickness. As shown in Fig. 2, for all the compounds with a high (≥blood flow) liver intrinsic clearance (midazolam, 7-EC, 7-HC, testosterone, quinidine, and haloperidol), CLint, liver was significantly lower (1.5–4 times) when calculated from slices with a “standard format” (9–12 mg wet weight/11 cell layers) than when thin slices of 2 to 5 mg (5 cell layers = ~100 μm) were used.

CLint, liver calculated for these compounds with (thin) liver slices were not significantly different from those obtained with hepatocytes, indicating that metabolism was not limited by penetration of the compounds into these thin slices. CLint, liver of warfarin, a low turnover compound with CLint, liver < liver blood flow, was not different in slices of various thicknesses. Metabolism of warfarin was too slow to be measured during the 6-h culture period with hepatocytes in suspension. As indicated by slice ATP levels (Fig. 3), decrease of ATP content per milligram protein than the thinnest slices, but this difference was not statistically different, although they exhibited a lower intrinsic clearance of most compounds.

**Metabolic Activity during Prolonged Culturing.** Intrinsic clearance of 7-HC, testosterone, and 7-EC by organ slices was determined after various periods (0, 4, 8, or 24 h) of preincubation. It appeared that intrinsic metabolic clearance of 7-HC (Fig. 4A), which is conjugated with glucuronide and sulfate, was unchanged during at least 24 h of incubation of liver, kidney, colon, and lung slices. Only in small intestinal slices was the clearance reduced to about 50% of initial values after 24 h of preincubation.

Testosterone, which in rat is metabolized via several cytochrome P450 isoenzymes (Arlotto et al., 1991) and 17β-hydroxysteroid dehydrogenase (Farthing et al., 1982), was cleared at constant rates in all the organs after various periods of preincubation (Fig. 4B). In contrast, slice intrinsic clearance of 7-EC (Fig. 4C) decreased to significantly lower values after 8 h (liver) and 24 h (liver and lung) of preincubation. In colon and small intestinal slices, metabolic rates tended to decrease as well, although the decrease was not statistically significant.

**Influence of Albumin on Clearance Prediction.** Clearance is limited in vivo by protein binding because only the free fraction (fu) of a given compound can be taken up by the cell and metabolized. Therefore, intrinsic clearances in vitro should be corrected for protein binding of a compound in vivo when no protein is present in the incubations. This also means that for compounds that in vivo predominantly bind to albumin, intrinsic clearances found in vitro in the presence of 4% albumin should be lower, proportionally to fu, than those of albumin-free incubations. In Fig. 5, intrinsic clearances of six model compounds from incubations with liver slices in the presence of 4% albumin are shown as a fraction of those obtained from albumin-free incubations (set to 1). According to the foregoing, this fraction should be equal to the literature fu in vivo. In fact, this is the case for testosterone, quinidine, and 7-EC, but not for haloperidol, 7-HC, and midazolam. For the latter compounds, intrinsic clearances were approximately 3 times higher than expected based on protein binding data from literature. Correspondingly, liver clearances were also higher for these compounds when calculated from intrinsic clearances obtained from incubations in the presence of 4% albumin.

Strikingly, the whole body clearance, calculated with the venous equilibrium model from albumin-free incubations with the different organ slices, was underestimated for the same compounds (haloperidol, 7-HC, and midazolam), whereas the whole body clearance of testosterone, quinidine, and 7-EC was correctly predicted (Fig. 6). Importantly, when whole body clearance was calculated from organ slice incubations (using thin liver and kidney slices) with 4% albumin, predicted values were in the desired range of 0.5- to 2-fold in vivo values for all the compounds under study.

![Fig. 1](image-url)  
**Fig. 1.** Relation between slice wet weight and protein content (A) and slice wet weight and the number of cell layers in the slice cross-section (B). Values represent the mean of three (2- to 5-mg slices) to eight (all the other groups) experiments, three to five slices per experiment (±S.E.M.).
Fig. 2. CL\textsubscript{int, liver} of model compounds quinidine (A), midazolam (B), 7-EC (C), haloperidol (D), testosterone (E), 7-HC (F), and warfarin (G) calculated with hepatocytes or slices of several thicknesses. Bars represent the mean of three to eight experiments (+S.E.M.), three to five slices per substrate per experiment, one hepatocyte incubation per substrate per experiment. Slices were incubated with 1 μM substrate for 1 h (testosterone), 3 h (7-HC, 7-EC), 6 h (quinidine, haloperidol, and midazolam), or 24 h (warfarin). Hepatocytes were incubated for 6 h. Neither slices nor hepatocytes were preincubated. Medium samples were taken at various time points and/or at the end of incubation. *, values differ significantly (p < 0.05) from those obtained with the thinnest slices; **, values differ significantly with p < 0.01. ND (not detectable): CL\textsubscript{int, liver} was lower than 5 ml/min/kg, but the exact amount could not be determined because this compound was hardly metabolized by hepatocytes.
Discussion

The major advantage of using precision-cut slices for prediction of in vivo metabolic clearance is that metabolically active extrahepatic organs can easily be taken into account besides the liver. A disadvantage of slices is that not all the cells are in direct contact with the medium containing the drug, making it necessary for the drug to travel through the slice into the inner cell layers. This phenomenon may not play a role in intestinal and lung slices because metabolizing cells are in direct contact with the easily permeable agarose gel, but metabolism may be limited to the outer cell layers in kidney and liver slices. Intrinsic clearances of drugs were reported to be structurally lower in liver slices than in hepatocytes (Worboys et al., 1997). It was suggested that this could be partly because of poor penetration of oxygen into the slice. However, although in the present study we also found, for most of the tested compounds found, a 2- to 4-fold lower CLint/mg protein in thicker slices than in thinner slices (and hepatocytes), ATP
content of thick (approximately 20-cell layer) liver slices was not lower than that of thin (5-cell layer) slices. Moreover, the intrinsic clearance of warfarin was not different in thick and thinner slices, and no signs of cell damage in the inner cell layers of slices were observed histomorphologically (data not shown). Thus, comprised metabolic function by lack of oxygen seems not to be the cause of lower clearance in slices. It seems more likely that metabolism of lipophilic high and intermediate turnover drugs like testosterone, haloperidol, midazolam, quinidine, 7-EC, and 7-HC is limited by delayed penetration of the drug into thicker slices, preventing establishment of equilibrium concentrations. However, the solution for this problem seems obvious: when thin slices (2–5 mg wet weight/100-μm thickness/4- to 6-cell layers) were used, CLint was found to be equal or higher than in hepatocytes, thus preventing underprediction.

Stability of metabolic rate on culturing is a prerequisite to adequately predict metabolic clearance of low clearance compounds (such as warfarin) in vitro, for which up to 24 h of incubation is required to reliably measure substrate depletion. With hepatocytes in suspension, as used in the present study, viability is not sufficient (approximately 50% after 6 h of culturing) to accurately measure clearance of such compounds. With organ slices, metabolic activity toward testosterone [metabolized via several P450s (Arlotto et al., 1991) and 17β-hydroxysteroid dehydrogenase (Farthing et al., 1982)] was maintained during culturing for 24 h. Conjugation of 7-HC with glucuronide and sulfate was only significantly decreased in small intestinal slices after 24-h preincubation. Metabolism of 7-EC [via CYP1A1 (Zhang et al., 1997), CYP1A2, CYP2A1, and CYP2B1/2 (Bayliss et al., 1994)] decreased on incubation. Calculated over 24 h, the mean activity toward 7-EC was minimally 50% of initial values. This implies that for highly stable compounds, clearance may be underestimated by maximally 50%, depending on the P450 isoform involved. The fact that conjugation reactions, which presumably are highly energy-consuming and require maintained cellular viability, were barely affected implies that slice quality is not the cause of this loss. Loss of P450 activity on culturing has been reported repeatedly using conventional (monolayer) cultures of isolated hepatocytes (Sherratt and Damani, 1989; Rogiers et al., 1990; Wortelboer et al., 1990; McMillan et al., 1991; Bayliss et al., 1994) and has been ascribed to activation of NO-synthesis as a result of collagenase treatment (Lopez-Garcia, 1998) and dedifferentiation of hepatocytes by loss of cell-to-cell or cell-to-matrix interactions. Indeed, culturing of hepatocytes in cocultures with other liver-derived cells and/or in combination with extracellular matrix has expanded the time course in which hepatocytes stay viable (Rogiers et al., 1990) and to some extent has also led to better preserved P450 levels (Koebe et al., 1994; Evans, 1995; Kern et al., 1997). However, damage by collagenase, loss of intercellular communication, and cell-matrix interactions cannot play a role in the loss of P450 activity in the present study and those of others (Wright and Paine, 1992; VandenBranden et al., 1998; Renwick et al., 2000) because no disruptive isolation procedure is used to prepare slices and cellular interactions are presumably maintained. The observed decrease of P450 activity toward 7-EC may be because of the loss of (endogenous or exogenous) inductive stimuli, which are constantly present at basal levels in vivo but not in vitro. Differential sensitivity of P450 isoenzymes to inactivation has been
reported before (Utesch et al., 1991; Renwick et al., 2000) and may be caused by differences in turnover times and/or inducibility.

Protein binding decreases the free concentration and thereby the intracellular concentration of drugs. Because the rate of metabolic conversion of drugs in concentrations under $K_m$ is directly related to the concentration that is “seen” by the metabolic enzyme, metabolic rate is expected to decrease proportionally to the extent of protein binding in the presence of plasma proteins. After adding 4% albumin to the incubation medium, we observed, as expected, that the metabolic rate of model compounds was decreased. For testosterone, 7-EC, and quinidine, this decrease was exactly proportional to their literature $f_u$. However, $CL_{int}$ of midazolam, 7-HC, and haloperidol was much less affected by albumin than was expected based on their extent of protein binding, resulting in an approximately 3 times higher predicted metabolic clearance for the latter compounds in the presence of albumin. Several explanations could account for this observation. First, BSA, which was used in the present study, might not exhibit the same drug-binding properties as rat plasma. This explanation may not be unlikely because plasma also contains other drug-binding proteins, although albumin is the most abundant. Besides this, drugs may bind with affinity to a rat serum albumin other than BSA. To exclude this for the model compounds used, we performed a pilot study in which we compared intrinsic clearance of these compounds in the presence of 20% rat plasma and an equivalent amount of 0.8% BSA. No differences were found, indicating that the use of BSA in the present study was valid (data not shown). Despite this validation, it seems to be more straightforward to use full plasma or serum in future studies (Shibata et al., 2000). However, the latter method may be difficult to standardize because of the presence of other components (e.g., prostaglandins, growth factors) in serum. Future studies need to elucidate whether this may influence the reproducibility and predictive value of the assay.

A second explanation for our results may be that some compounds may nonspecifically bind to the slices, decreasing their free concentration in the incubation medium. Correcting $CL_{int}$ for in vivo $f_u$ could then have introduced an overcorrection. However, no difference in the concentration of either of these compounds between the incubation medium and the medium/slice homogenate at the end of incubation was found (unpublished observations), indicating that binding to slices was not sufficient to influence medium concentration. Protein “contamination,” possibly caused by production of albumin by (liver) slices, “sticking” albumin left after slice preparation (organs were not perfused; therefore, some blood could be left), or release of proteins from dying cells can also be a reason for the culture medium mistakenly being considered as drug-binding protein-free. However, the presence of protein in the medium would imply an impact (proportional to the extent of protein binding) on the clearance of all the tested compounds, which was not the case. Another explanation may be that albumin facilitates (active) transport of a compound into metabolizing cells, thereby (partly) compensating its decreasing effect on intracellular drug concentration. An example is furosemide, of which uptake by specific anion transporters in the kidney is enhanced by albumin binding (Pichette et al., 1996). It has also been shown in vivo that
hepatic extraction of some (lipophilic) compounds (e.g., propanolol) (Gariepy et al., 1992) is not restricted by protein binding. It has been suggested that the possibly enhancing effect of albumin on the uptake of drugs is rather aspecific and might be explained by the fact that albumin helps to bring the drug more “closely” to the cell by ionic interactions with the plasma membrane, thereby shortening the diffusional distance (Blanchard et al., 2004). Albumin also could improve solubility of compounds that have a low solubility in water. However, this explanation seems less likely for current results because clearance of the compound with the lowest solubility (testosterone) was not increased, whereas that of 7-HC (which has a much higher solubility) was. To further unravel the mechanism by which albumin enhances clearance, more research is required.

Extrahaepatic organ clearance of midazolam, haloperidol, and 7-HC was equally affected by the presence of 4% albumin, and it appeared that whole-body metabolic clearance was much better predicted. Clearances of all the tested model compounds predicted in the presence of albumin (and using thin liver and kidney slices) lay within a 2-fold range of in vivo metabolic clearances. Thus, this approach seems promising to adequately predict in vivo clearance.

In conclusion, these results confirm the applicability of slices in metabolic clearance prediction. The application of thin slices eliminates the penetration limitation reported for high clearance drugs in conventional slices. Furthermore, organ slices can be kept metabolically active for sufficient periods of incubation, enabling study of the kinetics of low clearance compounds, although (a slight) underestimation of clearance can be expected in some cases because of down-regulation of certain P450s. In vitro prediction with organ slices of in vivo metabolic clearance is significantly improved for a number of compounds when incubation is conducted in the presence of albumin, resulting in predictions that lay within a 0.5- to 2-fold range of in vivo values.

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