KINETICS OF DRUG METABOLISM IN RAT LIVER SLICES: IV. COMPARISON OF ETHOXYCOUMARIN CLEARANCE BY LIVER SLICES, ISOLATED HEPATOCYTES, AND HEPATIC MICROSOMES FROM RATS PRETREATED WITH KNOWN MODIFIERS OF CYTOCHROME P-450 ACTIVITY

DAVID J. CARLILE, NANCY HAKOOZ, AND J. BRIAN HOUSTON

School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester, United Kingdom

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ABSTRACT:

To evaluate the theory that within precision-cut liver slices intercellular transport occurs in parallel with cellular metabolism and to illustrate the constraints this places on clearance predictions, the kinetics of ethoxycoumarin O-deethylation have been determined under varying conditions of hepatic cytochrome P-450 activity. Liver slices, isolated hepatocytes, and microsomes were obtained from rats treated with the inducers phenobarbital (PB) and β-naphthoflavone (βNF) and the inhibitor aminobenzotriazole (ABT). In hepatocytes and microsomes, a two-site kinetic model with a high-affinity, low-capacity site and an unsaturated low-affinity, high-capacity site described the hydroxycoumarin formation. There were marked increases in V_max (2- to 5-fold and 50- to 70-fold for PB and βNF, respectively) in both systems and in CL_int (3- and 9-fold for PB and βNF, respectively) in hepatocytes and substantial decreases in both parameters (3-8 and 12-23% of control, respectively) in ABT hepatocytes and microsomes. A qualitatively similar response was evident in slices obtained from livers of rats treated with phenobarbital and ABT, but although slices from βNF livers produced high metabolic rates (comparable to slices obtained from livers of rats treated with phenobarbital), these showed a linear increase with substrate concentration without indication of a high-affinity site. The intrinsic clearance parameters were scaled to full liver capacity using hepatocellular and microsomal recovery indices to allow direct comparison of these responses. The slice system consistently underestimated the effects of the modifiers. When compared with hepatocytes, estimates of 30, 15, and 1% for ABT, PB, and βNF, respectively, were observed and the degree of underestimation was dependent on the magnitude of intrinsic clearance and was consistent with the above theory.

Precision-cut liver slices offer an alternative in vitro approach to the use of intact cells or subcellular fractions for investigating drug metabolism. There is a growing body of literature indicating the value of this system for identifying routes of metabolism (Harris et al., 1994; Prueksaritanont et al., 1995; Bach et al., 1996; Ekins, 1996), and there is good accord, on a qualitative level, between the metabolites formed by slices and those formed by other in vitro systems as well as in vivo. Recently we have explored another potential application of liver slices, namely, their utility in predicting rates of metabolism (Worboys et al., 1995, 1996a,b, 1997). Freshly isolated hepatocytes in suspension and, to a lesser extent, hepatic microsomes provide accurate predictions of in vivo intrinsic clearance (CL_int) and hepatic clearance (Huangson, 1994; Houston and Carlile, 1997). However, there are a number of additional advantages to the use of precision-cut liver slices for prediction work. In addition to maintaining intact architecture, their preparation is simpler, quicker, and does not involve the use of proteolytic enzymes.

We have found that liver slices, in comparison with isolated hepatocytes in suspension, consistently underpredict CL_int when both parameters are normalized for their respective hepatocellularity (Worboys et al., 1996a). Thus, either there are only a limited number of hepatocytes in the slice that contribute to clearance or not all hepatocytes are operating at their full metabolic potential. Furthermore, the degree of this underprediction appears to be related to the magnitude of the CL_int value; high-clearance drugs are underpredicted to a larger degree than low-clearance drugs. Direct evidence is available that indicates that this phenomenon is dependent on the extent to which the drug distributes within the slice during the incubation period (Worboys et al., 1997). Thus, there is a need to consider rates of intercellular transport and intracellular metabolism as parallel processes when interpreting metabolite production by liver slices.

These conclusions were formulated from data on six drugs selected for study on the basis of differences in both physicochemical properties and clearance values (Worboys et al., 1997). Therefore, it is important to confirm that these experimental findings reflect the general properties of precision-cut slices rather than originating from physicochemical properties of the drugs under investigation. To establish that the above conclusions are not compound-dependent, we have undertaken a series of studies with one particular drug substrate,
7-ethoxycoumarin (EC), using slices from rats treated with certain well documented modifiers of cytochrome P-450 (CYP) activity to generate a range of $CL_{int}$ values. EC is used frequently as a probe substrate (Carlile et al., 1998), its clearance in rat liver slices is intermediate (Worboys et al., 1995), and, at least in microsomes, it responds to the classic inducers phenobarbital (PB) and β-naphthoflavone (βNF) (Boobis et al., 1986; Fry et al., 1992) and the suicide inhibitor aminobenzotriazole (ABT) (Ervine and Houston, 1990). In addition, analogous studies have been carried out in freshly isolated hepatocytes and hepatic microsomes to allow a detailed comparison of the quantitative responses seen in the three in vitro preparations obtained from rats treated with known modifiers of CYP activity. To ensure that valid comparisons are made, based solely on changes in CYP activity, in vitro parameters have been scaled to in vivo units (Carlile et al., 1997) using hepatocellularity and microsomal recovery factors specifically determined for PB, βNF, and ABT treatments.

Materials and Methods

Chemicals. EC, 7-hydroxycoumarin (HC), Earle’s balanced salt solution, Krebs-Henseleit buffer, and β-glucuronidase were purchased from Sigma Chemical Company (Poole, Dorset, UK). All other chemicals were purchased from either BDH (Lutterworth, Leicester, UK) or Sigma.

Animals. Male Sprague-Dawley rats (225–270 g) were obtained from the Biological Services Unit at the University of Manchester. They were housed two to four per cage on sawdust bedding in rooms maintained at a temperature of 20 ± 2°C and allowed free access to water and Chow Rat and Mouse diet. Rats were either untreated (UT) or administered i.p. injections of either PB (80 mg/kg in 0.9% saline, for 3 days), βNF (100 mg/kg in corn oil, for 3 days), or ABT (50 mg/kg in 0.9% saline, one dose). One day before sacrifice, rats were injected with 1 ml of dimethylformamide (final concentration, 0.25%). LC was added to incubation media, which were prepared by mixing the appropriate amount of PB, βNF, and ABT with dimethylformamide. Reactions were performed at either 37°C or 20°C before analysis. All samples were stored at −20°C until analyzed.

Slice Preparation and Incubation. Animals were sacrificed by cervical dislocation, and the liver was quickly excised into ice-cold Earle’s balanced salt solution. Circular columns of tissue were prepared from the liver using a motor-driven borer (180 rpm, 10-mm diameter). Slices were prepared in ice-cold Earle’s balanced salt solution using a Krumdieck Tissue Slicer (Alabama R & D, Munford, AL).

Slices were transferred into Krebs-Henseleit buffer (pH 7.4, room temperature) and floated onto stainless steel wire mesh inserts. Slices were supported in 5 ml of dimethylformamide. Reactions were performed at either 37°C or 20°C before analysis. All samples were sonicated to ensure slice disruption before assay. Rates of formation of HC in slices are expressed per million cells using a slice hepatocellularity of 2.05 × 10⁶ cells per standard slice of 260 μm² (Worboys et al., 1996a). The formation of HC was determined in slices using a Krumdieck Tissue Slicer (Alabama R & D, Munford, AL).

Hepatocyte Preparation and Incubation. Hepatocytes were isolated as described elsewhere (Hayes et al., 1995). Incubation conditions were established that produced linear rates of metabolism with respect to time (10 and 30 min for induced and ABT cells, respectively) and cell density (0.5, 0.1, and 0.05 × 10⁶ cells/ml for ABT, PB, and βNF cells, respectively). EC (final concentration, 0.5–100 μM) was added to Williams Media E (total incubation volume, 3 ml) in dimethylformamide (final dilution, 0.25%). Reactions were terminated by freezing in liquid N₂ and stored at −20°C until analyzed.

Microsomal Preparation and Incubation. Microsomes were prepared by differential ultracentrifugation as described previously (Hayes et al., 1995). Initial studies identified the conditions for linear rates of metabolism with respect to time (4 min for UT, PB, and βNF microsomes and 10 min for ABT microsomes) and protein concentration (0.25 mg/ml for UT, PB, and βNF microsomes and 0.5 mg/ml for ABT microsomes). EC was added to incubations (final volume of 2 ml) in 5 μl of dimethylformamide. Reactions were terminated by the addition of 10 μl of 10 M sodium hydroxide.

Analysis of HC Formation. Incubates were analyzed for HC by fluorescence (Lake, 1987) after hydrolysis of conjugates with 1000 units/ml type H-1 β-glucuronidase containing sulfatase activity in 60 mM sodium acetate buffer, pH 4.5, for 2 h at 37°C.

Determination of Hepatocellularity. To establish whether treatment with the modifiers of CYP activity altered the hepatocellularity of the liver, total protein content of both liver homogenate and freshly isolated hepatocytes were determined using a previously described protocol (Carlile et al., 1997). The liver consists of approximately 65% hepatocytes, which occupy 80% of the cellular volume. However, during the isolation procedure the cellular content increases to >90% hepatocytes (Steinberg et al., 1987). In the intact liver, hepatocytes contain 97% of total protein, and in an isolated cell suspension, >99% of protein is contained in hepatocytes (Steinberg et al., 1987). Thus, the ratio of protein in liver homogenate to protein in the isolated cell suspension provides an accurate measure of hepatocellularity allowing the calculation of clearance expressed as percent of milligrams of protein to values per gram liver and, hence, per standard rat weight of 250 g (SRW; liver weight, 11 g per SRW).

Determination of Microsomal Protein Yield. Microsomal protein yield was used to scale microsomal data to in vivo units. The method, detailed elsewhere (Carlile et al., 1997), involves measurement of the CYP content in both liver homogenate and microsomes. The liver homogenate/microsome CYP ratio (microsomal recovery index) was used to scale the microsomal clearances, expressed per mg of microsomal protein, to values for an intact liver.

Data Analysis. Kinetic parameters for HC production were estimated using eq. 1 for a two-site kinetic model and the SIPHAR package (SIMED; Centers D’études et de Recherches en Statistiques et Informatique Medicale, Paris, France), which uses the Powell nonlinear algorithm and a weighting of 1/Y²(calc). Because the loss of substrate in the in vitro incubations was substantial, the logarithmic mean of the initial and final substrate concentration was used as the independent variable in the curve-fitting procedure.

\[
V = \frac{V_{\text{max}} \cdot S}{K_m + \frac{S}{CL_1} + \frac{S}{CL_2}}
\] (1)

$V_{\text{max}}$ and $K_m$ describe the maximal rate of metabolism and the Michaelis constant for the high-affinity site, respectively. $V_{\text{max}}/K_m$ corresponds to the clearance for this high-affinity site ($CL_1$), and $CL_2$ describes the clearance by a low-affinity site that is not saturated over the concentration range studied. Total $CL_{int}$ was determined from the sum of $CL_1$ and $CL_2$. In the case of the data from βNF slices, no saturation was observed and $CL_{int}$ was obtained from the slope of the linear relationship between HC formation rate and EC concentration.

Hepatocellularity was used to scale the parameters from slice and isolated hepatocyte studies to in vivo units denoted by SRW. The microsomal recovery index was used in a similar manner for microsomal data (Carlile et al., 1997).

Results

In Vitro Kinetic Studies. The formation of HC was determined over a wide concentration range (0.5–100 μM) in slices, isolated hepatocytes, and microsomes from rats treated with either PB, βNF, or ABT. As illustrated for the PB (Fig. 1A) and ABT (Fig. 1B) cases, these profiles could be best described by a two-site model with a high-affinity, low-capacity site and a low-affinity, high-capacity site that was not saturated over the concentration range studied. Total $CL_{int}$ was determined from the sum of $CL_1$ and $CL_2$. In the case of the data from βNF slices, no saturation was observed and $CL_{int}$ was obtained from the slope of the linear relationship between HC formation rate and EC concentration.

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Marked changes in both $V_{\text{max}}$ and clearance terms ($CL_1$ and $CL_2$)
were observed in hepatocytes from treated livers when compared with the corresponding parameters from UT rats (Table 1). The anticipated increases in the induced livers (5- and 70-fold for $V_{\text{max}}$, 3- and 9-fold for $CL_{\text{int}}$ for PB and βNF, respectively) and decreases in the inhibited livers (8 and 23% of UT for $V_{\text{max}}$ and $CL_{\text{int}}$, respectively) for these parameters were observed. Thus, the induction effects observed in cells from βNF rats were more marked than those observed for PB cells. Also, the changes in $K_m$ were minor with the exception of βNF treatment, which resulted in a 5-fold increase.

Similar responses were evident in the microsomal preparations from the βNF and ABT livers (Table 2): $V_{\text{max}}$ increased 126-fold and decreased to 3%, $CL_{\text{int}}$ increased 9-fold and decreased to 12% when compared with UT. Both $CL_1$ and $CL_2$ were markedly influenced by βNF and ABT treatment whereas only $CL_2$ was increased in response to PB, despite the increase in $V_{\text{max}}$. As was the case with hepatocytes, there was a statistical significant increase in $K_m$ in the βNF case when compared with UT animals.

In βNF slices only one clearance term could be determined, and this was of a value similar to the UT $CL_{\text{int}}$ (Table 3). Both PB and ABT treatments gave the expected changes in slices for the $V_{\text{max}}$ (7-fold

**FIG. 1.** Relationship between rate of hydroxycoumarin formation and ethoxycoumarin concentration for slices (□), isolated hepatocytes (■), and microsomes (×) prepared from PB-treated (A), ABT-treated (B), and βNF-treated (C) rats.

The inset in A–C represents the formation rate data over the ethoxycoumarin concentration range to 10 μM to demonstrate the high-affinity, low-capacity component. A, for these particular preparations, $V_{\text{max}} = 37.7$ and 96.3 nmol/min/10^6 cells for slices and hepatocytes, respectively, and 174.1 nmol/min/mg for microsomes, $K_m = 11.1$, 2.4, and 5.6 μM for slices, hepatocytes, and microsomes, respectively, and $CL_{\text{int}} = 0.6$ and 13.0 μl/min/10^6 cells for slices and hepatocytes, respectively, and 17.0 μl/min/mg for microsomes. B, for these particular preparations, $V_{\text{max}} = 0.88$ and 4.40 nmol/min/10^6 cells for slices and hepatocytes, respectively, and 9.93 nmol/min/mg for microsomes, $K_m = 0.7, 0.2$, and 3.6 μM for slices, hepatocytes, and microsomes, respectively, and $CL_{\text{int}} = 0.1$ and 0.6 μl/min/10^6 cells for slices and hepatocytes, respectively, and 0.8 μl/min/mg for microsomes. C, for these particular preparations, $V_{\text{max}} = 1095$ nmol/min/10^6 cells for hepatocytes and 4996 nmol/min/mg for microsomes, $K_m = 4.0$ and 10.2 μM for hepatocytes and microsomes, respectively, $CL_{\text{int}} = 9.4$ μl/min/10^6 cells for hepatocytes and 19.1 μl/min/mg for microsomes, and $CL_{\text{int}} = 3.2$ μl/min/10^6 cells for slices.
increase and 67% decrease, respectively) and clearance terms (3-fold increase and 50% decrease, respectively). There were no statistical differences in the $K_m$ values from slices obtained from the UT and PB livers. The effects of the three modifiers on HC production, as measured by $V_{\text{max}}$ and/or $CL_{\text{int}}$, in slices, hepatocytes, and microsomes are summarized in Fig. 2.

**Scaled In Vitro Parameters.** To compare the absolute parameters from each in vitro system in the PB-, βNF-, and ABT-treated states, the parameters $V_{\text{max}}$ and $CL_{\text{int}}$ are scaled to in vivo units using the respective hepatocellularities and microsomal protein indices.

The hepatocellularity of livers from PB-, βNF-, and ABT-treated rats based on protein determination in both freshly isolated hepatocytes and liver homogenate are similar to UT (Table 4). There was no statistical difference between the induced and UT groups for either protein measurement. For the ABT group the protein concentration in the homogenate was reduced when compared to UT; however, this trend was also reflected in the cellular measurement. Therefore, for all treatments the hepatocellularity was maintained at approximately $1.2 \times 10^5$/SRW. Also presented in Table 4 are the microsomal protein recovery indices determined from the ratio of the liver homogenate/microsomal CYP. ABT treatment results in a substantial decrease in CYP content in both homogenate and microsomes of approximately 3-fold. This produces a modest increase in the protein recovery index compared with UT. Although βNF is an inducer of CYP 1A isoforms, there is no overall change in CYP content with this compound, demonstrating that the levels of other CYP isoforms decline. Moreover, there is no change in the microsomal protein yield compared with UT. Also shown in Table 4 are the corresponding data for PB and UT livers from an earlier study (Carlile et al., 1997).

It is instructive to consider the ratio of the slice/cell parameters as a measure of the consistency between these two in vitro systems (Table 5). For PB the clearance ratio (0.15) is similar to UT (0.14), and for ABT the ratio is higher (0.3), indicating that slice metabolic activity is 15–30% of the isolated cells. For βNF, however, the very low ratio of 0.01 is striking, reflecting the severe underestimation by the slice system of the effects of this inducer. In contrast, the $V_{\text{max}}$ ratios for PB and UT are similar (0.3 and 0.4, respectively), which contrasts with the ABT ratio of approximately 1. Therefore, on the basis of this latter parameter an identical response is seen in both in vitro systems for treatment with the suicide inhibitor but not for treatment with the inducers. Also shown in Table 5 are the ratios of the slice/microsomal parameters. For $V_{\text{max}}$ and $CL_{\text{int}}$, trends are seen that are similar to the above-discussed slice/cell ratios with the exception of the PB case. The poor response to this inducer seen in microsomes in comparison to cells explains the higher values observed for the slice/microsome ratios.

Figure 3 illustrates the relationship between the $CL_{\text{int}}$ for HC formation from EC in isolated hepatocytes and in precision-cut liver slices from PB-, βNF-, and ABT-treated as well as UT livers. Also shown in this figure are previously published $CL_{\text{int}}$ values for caffeine, phenytoin, tobutamide, diazepam (4'-hydroxylation, 3-hydroxylation and N-demethylation pathways), and ondansetron (hydroxylation and N-demethylation pathways). In all cases clearance is expressed per million cells.

**Discussion**

There are several advantages to the use of precision-cut liver slices over that of freshly isolated hepatocytes: in particular, the ease of preparation, the avoidance of proteolytic enzymes, and the maintenance of intact hepatic architecture (Parrish et al., 1995; Bach et al., 1996). The latter feature, however, may also be problematic because it has substantial impact on the interpretation of kinetic studies on drug metabolism. The accessibility of substrate within the slice is controlled by two parallel processes: intercellular transport and intracellular metabolism. Thus, a gradient in drug concentrations will exist between the cells situated on the surface and those in the core of the slice. Under these conditions the rate of metabolism achieved will be an average value arising from this concentration gradient, and the clearance by the slice is lower than that seen for the corresponding number of isolated hepatocytes (Worboys et al., 1996a).

Reduced accessibility of substrate and the existence of concentration gradients within the slice (Worboys et al., 1997) limit the interpretation of the term slice clearance; therefore, an alternative measure of the ability of the slice to metabolize drugs in the absence of any distribution limitation is required. A valuable parameter for this purpose is $V_{\text{max}}$, because this rate measurement is concentration-independent and refers to the inherent metabolic activity of the slice. Comparison of this parameter for six pathways of metabolism, which undergo saturation in both slices and isolated cells, demonstrated that liver slices (of 260-μm thickness) show 35% of full metabolic activity (Worboys et al., 1997). To elucidate further the relationship between metabolic rates by slices and by intact cells, we investigated the kinetics of one particular substrate, EC, in livers from animals treated with various modifiers of CYP activity, specifically, PB, βNF, and ABT. This allows the comparison of parameter values across the three in vitro systems plus an assessment of the sensitivity of each system to a defined change in CYP activity.

Each of these modifiers has been established to markedly alter the rate of $O$-deethylation of EC in hepatic microsomes (Boobis et al., 1986; Ervine and Houston, 1990). The use of both βNF and PB is a classic treatment for induction of CYP isoforms of the 1A and 2B families (Guengerich et al., 1982), and both isoforms are known to be important in HC formation from EC (Ryan and Levin, 1990). ABT generally is regarded as a nonselective inhibitor of CYP activity, at least at high doses. We have confirmed these observations in microsomes and report similar responses to these modifiers in isolated hepatocytes. Both $V_{\text{max}}$ and $CL_{\text{int}}$ are affected in the rank order $\beta$NF > PB > UT > ABT, and the range of values for these parameters in hepatocyte suspension are 1000- and 40-fold, respectively. There was no change in $K_m$ when either PB or ABT was compared with UT; however βNF, surprisingly, showed an increase. It should be remembered that this parameter, particularly in the case of EC, which displays two-site enzyme kinetics, is an empirical parameter describing the data rather than reflecting a specific interaction with a given isoform.

For slices, the response to the modifiers was not totally consistent with the response noted in isolated hepatocytes and microsomes (see Fig. 2). In the case of PB similar effects were seen in slices as in the cells, although in absolute terms the $V_{\text{max}}$ and $CL_{\text{int}}$ values were lower in slices than in cells. Because this trend is also seen with UT, the use of the PB/UT ratio as a measure of induction shows that both systems...
give the same quantitative effect (3-fold increase). In contrast, microsomal \( CL \) showed a minimal response to PB induction despite an increase in \( V_{\text{max}} \), the latter being consistent with a previous report (Boobis et al., 1986).

The action of ABT as a suicide inhibitor is well documented and is clearly reflected in the \( CL \) for each in vitro system. The response (as gauged by the ABT/UT clearance ratio) is greater both in isolated cells and microsomes, presumably for reasons linked to limited accessibility of substrate. Whereas the slice \( CL \) is only 15% of the corresponding hepatocellular value in UT (as is the case for PB), this value increases to 30% after ABT treatment. This observation is consistent with the previously reported trend that, for lower clearance drugs, there is closer agreement between the two systems. Slower metabolism is associated with deeper tissue penetration, and, consequently, a higher percentage of cells are able to contribute to the clearance process (Worboys et al., 1997). Thus, the use of the ratio of clearances as a measure of inhibition results in an underestimation of the degree of effect resulting from the ABT treatment.

The \( V_{\text{max}} \) value for HC formation after ABT treatment is essentially identical in slices and in isolated cells. This observation is surprising considering earlier studies discussed above showed a consistent difference between the two systems. However, in the ABT case there appears to be full participation from all hepatocytes in the slice, and \( V_{\text{max}} \) observed is substantially lower (by 5-fold) than the \( V_{\text{max}} \) values reported in the previous study. Further studies are required to establish whether there is a link between low metabolic demands, which occur under ABT inhibition, and the percentage of activity manifest in these particular slices. However, these findings with ABT slices would indicate that the 35% average activity, previously reported for slices under \( V_{\text{max}} \) conditions, results from incomplete metabolic activity by all cells rather than full activity from a reduced number of hepatocytes.

The most dramatic difference between slice and isolated cell kinetics is seen after \( \beta \)-NF treatment. In hepatocytes, as in microsomes, the relationship between rate of HC formation and EC concentration can

**TABLE 2**

<table>
<thead>
<tr>
<th>V(_{\text{max}} ) (pmol/min/mg protein)</th>
<th>( K_{\text{m}} ) (( \mu )M)</th>
<th>CL (( \mu )l/min/mg protein)</th>
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<tbody>
<tr>
<td>ABT</td>
<td>1.2 ± 0.3**</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>PB</td>
<td>112.7 ± 36.5*</td>
<td>3.4 ± 0.6</td>
</tr>
<tr>
<td>( \beta )-NF</td>
<td>5027 ± 2063**</td>
<td>11.1 ± 2.3**</td>
</tr>
<tr>
<td>UT</td>
<td>39.8 ± 9.8</td>
<td>0.9 ± 0.1</td>
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Statistical difference from UT (* \( p < .05 \), ** \( p < .01 \)).

**TABLE 3**

<table>
<thead>
<tr>
<th>V(_{\text{max}} ) (pmol/min/slice)</th>
<th>( K_{\text{m}} ) (( \mu )M)</th>
<th>CL (( \mu )l/min/slice)</th>
</tr>
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<tbody>
<tr>
<td>ABT</td>
<td>2.9 ± 1.0**</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>PB</td>
<td>62.6 ± 37.5**</td>
<td>7.6 ± 5.0*</td>
</tr>
<tr>
<td>( \beta )-NF</td>
<td>83.2 ± 29.9*</td>
<td>14.0 ± 1.5*</td>
</tr>
<tr>
<td>UT</td>
<td>8.7 ± 4.4</td>
<td>1.3 ± 0.8</td>
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Statistical difference from UT (* \( p < .05 \), ** \( p < .01 \)).

* Obtained by linear regression of HC formation rate against substrate concentration.

**Fig. 2.** Comparison of the effects of PB, \( \beta \)-NF, and ABT treatment apparent in slices (■), isolated hepatocytes (□), and microsomes (□). A, ratio of \( V_{\text{max}} \) in treated to that in UT hepatocytes, slices, and microsomes. B, ratio of \( CL_{\text{int}} \) in treated to that in UT hepatocytes, slices, and microsomes.
be described by a two-site model consisting of a high-affinity, low-capacity site and a low-affinity, high-capacity site. This kinetic behavior is absent in the slices from βNF-treated animals. A linear dependence between rate of production and substrate concentration produces a clearance value that is not significantly different from the UT \( V_{\text{max}} \) and \( CL_{\text{int}} \) values for HC formation in slices and hepatocytes from UT and ABT-, PB-, and βNF-treated rats.

In general, slices would appear to be less sensitive in reflecting changes in CYP activity than isolated hepatocytes because of the limited range of clearance values that are apparent in the former in vitro system. As shown in Fig. 3 the range of activity in hepatocytes, based on data from the present study and that published previously for five substrates metabolized by eight pathways of metabolism, covers three orders of magnitude whereas the corresponding range of activity in slices, for the same drugs under the same conditions, is only 50-fold. Furthermore, whereas clearance by both in vitro systems ultimately will be limited by rate of substrate access (either transport into the cell for the hepatocyte and/or between cells for the slice), this limitation occurs far more readily in slices. The present studies, using the same substrate in liver preparations that display a range of CYP activity, confirm the relationship previously demonstrated with six different substrates. Thus, the phenomenon of underprediction by in vitro system and not related to the differing physicochemical properties of the drugs under investigation.

In conclusion, these studies have confirmed the importance of considering substrate accessibility within slices when interpreting metabolic kinetic studies. The changes seen in the rate of HC formation by slices from rats treated with different modifiers of CYP activity can be rationalized, although they are not always predictable from the responses evident in isolated hepatocytes and microsomes. The lower CYP activity evident in ABT-treated livers results in greater accessibility than in the control slice as well as a larger percentage of hepatocytes contributing to slice clearance. In contrast,

### Table 4

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<thead>
<tr>
<th>CYP content of liver</th>
<th>CYP content of microsomes</th>
<th>Protein recovery</th>
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<tbody>
<tr>
<td>( \text{nmol/g liver} )</td>
<td>( \text{nmol/mg protein} )</td>
<td>( \text{mg/g liver} )</td>
</tr>
<tr>
<td>ABT 18.6 ± 2.4**</td>
<td>0.26 ± 0.04**</td>
<td>74.1 ± 16.5*</td>
</tr>
<tr>
<td>βNF 49.0 ± 3.2</td>
<td>0.86 ± 0.09</td>
<td>57.2 ± 3.4</td>
</tr>
<tr>
<td>PB 71.5 ± 9.3*</td>
<td>1.54 ± 0.23**</td>
<td>46.6 ± 1.2**</td>
</tr>
<tr>
<td>UT 49.7 ± 6.8</td>
<td>0.83 ± 0.07</td>
<td>60.1 ± 4.4</td>
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### Table 5

<table>
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<tr>
<th>( V_{\text{max}} )</th>
<th>( CL_{\text{int}} )</th>
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<tr>
<td>( \mu\text{mol/min/SRW} )</td>
<td>( \text{ml/min/SRW} )</td>
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Squares refer to EC values in ABT, UT, PB, and βNF livers, and \( \times \) symbols refer to previous published data on caffeine, tolbutamide, phenytoin, diazepam, and ondansetron (Worboys et al., 1996a).

Statistical difference from UT (* \( p < .05 \), ** \( p < .01 \)).

Data from Carlile et al., 1997.
the higher metabolizing activity resulting from treatment with the CYP inducer βNF results in less accessibility than in the control slice and a lower percentage of contributing hepatocytes. The overall consequences of such changes is to underestimate the degree of effect of these modifiers when only slice data are available. In the case of PB, the effects are more moderate, and the full response of this inducer is manifest in both in vitro systems. These findings will have implications in testing for induction by the approach adopted here, that is, using slices from treated animals. However, they may be of much lesser concern in studies involving the maintenance culture of control slices in the presence of the putative inducing agent (Lake et al., 1996).

References


