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Oxygen Dependence of Omeprazole Clearance and Sulfone and Sulfide Metabolite Formation in the Isolated Perfused Rat Liver[1]

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ABSTRACT

Severe acute hypoxia is known to inhibit markedly the elimination of oxidatively metabolized drugs by the isolated liver. However, little is known of the degree of hypoxia required to produce inhibition of drug elimination by oxidative pathways in the intact organ. This study, in the isolated perfused rat liver, examined the oxygen dependence of the hepatic elimination of omeprazole, a drug which undergoes extensive oxidative metabolism in the rat. The relationship between hepatic oxygen supply and the production of omeprazole's oxidative sulfone and reductive sulfide metabolites was also examined. Rat livers were perfused at 15 ml/min with a perfusate containing 5 μg/ml of omeprazole in a single-pass design. Omeprazole clearance and the formation clearance of the two metabolites were measured in each liver during normal oxygenation, at different levels of hypoxia and after reoxygenation. There was a linear relationship between omeprazole clearance and oxygen delivery over the whole range studied. Production of the sulfone was similarly oxygen-dependent whereas the sulfide was only detectable after a significant reduction in oxygenation. In a further group of experiments the oxygen dependence of omeprazole clearance was shown to not be altered when the concentration of drug was lowered to 1 μg/ml. This study shows that oxygen delivery is a critical determinant of the rate of oxidative drug metabolism in the isolated liver and supports the contention that reductions in hepatic oxygen supply may significantly alter the hepatic disposition of oxidatively metabolized drugs *in vivo*.

One might expect oxidative drug biotransformation to be inhibited by hypoxia, as molecular oxygen is used as a substrate by microsomal oxidases (Jones, 1981). Whereas several studies in the intact liver have confirmed that acute severe hypoxia inhibits markedly drug elimination by hepatic mixed-function oxidases (Jones *et al.*, 1984; Webster *et al.*, 1985; Miller and Oliver, 1986), studies in subcellular systems indicate that inhibition of oxidative metabolism may occur during much milder reductions in hepatic oxygen supply (Jones, 1981). Furthermore, for oxidatively metabolized drugs, the oxygen dependence of the formation of individual metabolites may vary considerably (Takahara *et al.*, 1986) and for some substrates hypoxia may actually enhance metabolism by alternative reductive biotransformation pathways (Smith *et al.*, 1983; Webster *et al.*, 1985). Although a number of studies in isolated hepatocyte preparations have examined the relationship between PO_2 and the rate of oxidative drug metabolism (Jones, 1981), little is known of the relationship in the intact liver in which $[O_2]$ in the various regions of this highly heterogeneous organ is dependent on the rate of oxygen delivery (*i.e.*, on flow rate, PO_2 and hemoglobin content of perfusate).

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The substituted benzimidazole, omeprazole, undergoes extensive oxidative metabolism by the rat liver to at least seven metabolites. Its major metabolic pathways (>50%) involve aromatic hydroxylation and formation of a carboxylic acid and only a small proportion (<20%) of its oxidative metabolites undergo subsequent conjugation (Hoffman, 1986). Two of its metabolites, which can be measured by high-pressure liquid chromatography, are omeprazole sulfone, which is formed by *S*-oxidation, and omeprazole sulfide, which is produced by sulfoxide reduction. The aim of the present study was first to determine in the IPRL the relationship between the degree of hepatic oxygenation and omeprazole clearance, and second to define the oxygen dependency of its elimination by a specific oxidative (sulfone) and reductive (sulfide) pathway.

Methods

Experimental preparation. Livers of nonfasting male Sprague-Dawley rats were removed by standard surgical techniques and perfused initially in a recycling system (Gollan *et al.*, 1981). After a 20-min postsurgical equilibration period, perfusion was switched for the experimental period to a nonrecycling mode at a flow rate of 15 ml/min. The perfusate (1100 ml), consisting of 10% (v/v) washed human red cells, 1% bovine serum albumin and 0.1% glucose in Krebs-Henseleit electrolyte solution (Mihaly *et al.*, 1982), was deoxygenated initially by pre-equilibration with 100% nitrogen over 30 min and warmed to 37°C

ABBREVIATION: IPRL, isolated perfused rat liver.

before the commencement of the experiments. During normal oxygenation (control and recovery phases) perfusate was equilibrated with 100% O_2 in the perfusate oxygenator. Reduction of perfusate oxygen content from this "normal" composition was achieved by replacing oxygen with varying mixes of nitrogen and oxygen in the perfusate oxygenator. In order to maintain bile flow, sodium taurocholate (30 μmol) was added to each 1100 ml of perfusate.

Experimental design. A 5.5-mg bolus dose of omeprazole (total volume, 5.5 ml) was added to the perfusate (1100 ml) to produce a final omeprazole concentration of 5 μg/ml. In three pilot experiments it was found that, at this omeprazole concentration, steady-state hepatic extraction, as indicated by a constant hepatic venous concentration of omeprazole, was reached within 15 to 20 min of liver perfusion during both normal oxygenation (equilibration of perfusate with 100% O_2) and severe hypoxia (equilibration with 100% N_2).

Each experiment (n = 18) was divided into three phases. The first or "control" phase lasted for 30 min and during this initial period perfusate was equilibrated with 100% oxygen. At 25 and 30 min perfusate samples (volume 2 ml) were taken from hepatic inflow and outflow for measurement of omeprazole, omeprazole sulfide and omeprazole sulfone concentrations. At 30 min oxygen content in hepatic inflow and outflow perfusate was also measured.

The aim of the second experimental phase (30–60 min) was to examine omeprazole clearance and metabolite formation clearance over a range of oxygen delivery rates, by producing a different rate of oxygen delivery in each experiment. This was obtained by arranging two fine needle gas flow valves in parallel, one of which was connected to 100% nitrogen and the other to 100% oxygen. Immediately after samples were taken at the end of the control phase (30 min), oxygen partial pressure in the membrane oxygenator was reduced by setting the oxygen flow rate to a value in the range of 0 to 4 liters/min and making up the total gas flow to 4 liters/min with nitrogen. Each preparation received only one level of oxygen delivery during the hypoxia phase. The liver was perfused at this new level of oxygen delivery for a further 30 min to allow for the establishment of new steady-state rates of omeprazole extraction and metabolite formation. At t = 55 min and t = 60 min, inflow and outflow perfusate were again sampled for measurement of omeprazole concentrations, omeprazole metabolite levels and oxygen content.

In all experiments in which oxygen delivery had been reduced during the second phase, control levels of oxygenation were then restored (at 60 min) by re-equilibrating perfusate with 100% oxygen and perfusion was continued for a further 15 min (the third or recovery phase). At 70- and 75-min samples of inflow and outflow perfusate were again taken for measurement of the concentrations of omeprazole and its sulfide and sulfone metabolites and oxygen content, to determine if drug clearance, metabolite formation clearance and oxygen consumption had recovered from the hypoxic episode. The rate of bile flow was measured in each experimental phase by collecting bile in preweighed vials.

In order to assess whether the oxygen dependence of omeprazole elimination was related to drug concentration, a second group of experiments (n = 17) was performed which were of identical design to the first group, except that the concentration of omeprazole in hepatic inflow was reduced from 5 to 1 μg/ml. At this lower inflow drug level, the concentrations of metabolites in hepatic outflow were too low to be measured accurately and therefore samples obtained in these experiments were assayed for omeprazole alone.

Assays. Omeprazole, omeprazole sulfone and omeprazole sulfide concentrations were measured using a selective and sensitive high-pressure liquid chromatography technique (Mihaly *et al.*, 1983). Oxygen and carbon dioxide concentrations and pH were measured immediately after sampling of perfusate using an ILS pH/blood gas analyser (Instrument Laboratory, Lexington, MA) and hemoglobin saturation was calculated automatically. Oxygen consumption was calculated by multiplying the difference between inflow and outflow perfusate oxygen concentrations by the perfusate flow rate.

Calculations and data analysis. In each experimental phase, the mean of each pair of hepatic inflow omeprazole concentrations (C_{in}) and each pair of outflow concentrations (C_{out}) were used to calculate hepatic clearance, as Q_H (C_{in} − C_{out})/C_{in}, where Q_H is the hepatic perfusate flow rate. Metabolite formation clearance was calculated as ($Q_H \cdot M_{out}/C_{in}$) where M_{out} is the metabolite concentration in the hepatic venous outflow.

Data are presented as mean ± S.D. Statistical comparison of data was made using the paired Student's t test for paired observations (Schefler, 1969).

Results

Oxygen delivery and consumption. During the initial control period of the experiments, when perfusate was equilibrated with 100% oxygen, mean oxygen delivery was 3.33 ± 0.36 μmol/min/g of liver (table 1). Mean oxygen consumption at this rate of oxygen supply was 2.89 ± 0.33 μmol/min/g of liver. A wide range of rates of oxygen delivery was produced during the second experimental phase. The minimum level of oxygen delivery (0.68 μmol/min/g of liver) was achieved in an experiment where perfusate was equilibrated with 100% nitrogen. The maximum rate (3.69 μmol/min/g of liver), was produced when perfusate was equilibrated with 100% O_2 (*i.e.*, as in the control phase). As oxygen delivery was decreased below control rates there was a linear decline in oxygen consumption (r = 0.99, $P < .001$) so that at any level of oxygen delivery approximately 90% of available oxygen was consumed.

In all experiments the return to control rates of oxygen delivery in the final 15 min of the experiment (recovery phase) resulted in recovery of oxygen consumption to within 5% of the rate during the initial control phase, indicating that cellular respiration had recovered from the episode of reduced oxygen delivery (table 1).

The pH of perfusate entering the liver was virtually identical during normoxia and hypoxia (geometric mean, 7.34 [normoxia] *vs.* 7.35 [hypoxial]), as was the pH in hepatic venous effluent (7.18 *vs.* 7.20).

Bile flow and biliary elimination of omeprazole. Bile flow was monitored in all experimental phases. The effect of changes in hepatic oxygen delivery on bile flow during the second phase was measured by calculating the ratio of bile flow during this phase to bile flow during the control phase. Figure 1 shows the relationship between bile flow ratio and oxygen delivery during the graded hypoxia phase, and from this figure it can be seen that bile flow during the second phase was independent of oxygen delivery. In preliminary experiments only very low rates of omeprazole excretion into bile were detected under both control and hypoxic conditions and the sulfone and sulfide metabolites were never detected in bile.

Omeprazole clearance. Mean omeprazole clearance during the initial control phase was 12.5 ± 0.9 ml/min (table 1). In each experiment the impact of the change in oxygen supply on omeprazole clearance during the second (graded hypoxia) phase was quantified by calculating the ratio of clearance during the

TABLE 1

Oxygen delivery, oxygen consumption and omeprazole clearance expressed as mean ± S.D. in the control and recovery phases of high dose studies

Phase	Oxygen Delivery	Oxygen Consumption	Omeprazole Clearance
	μmol/min/g liver	μmol/min/g liver	ml/min
Control, 0–30 min	3.33 ± 0.36	2.89 ± 0.33	12.5 ± 0.9
Recovery, 60–75 min	3.29 ± 0.39	2.72 ± 0.35	12.2 ± 0.7

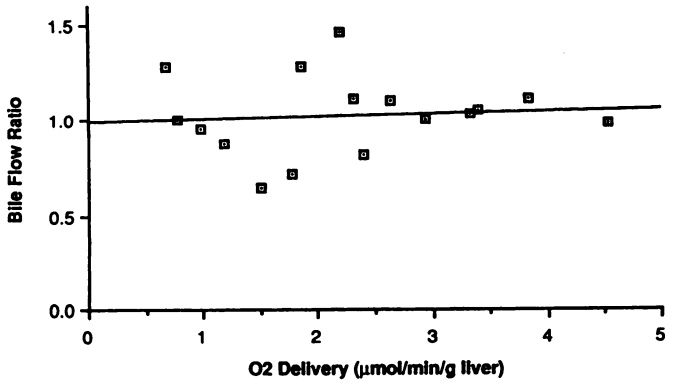

Fig. 1. Ratio of bile flow during the control phase (0–30 min) to bile flow during the graded hypoxia phase of the high dose studies. The slope of the regression line was not significantly different from zero ($P > .25$). Each experimental point refers to a different liver preparation.

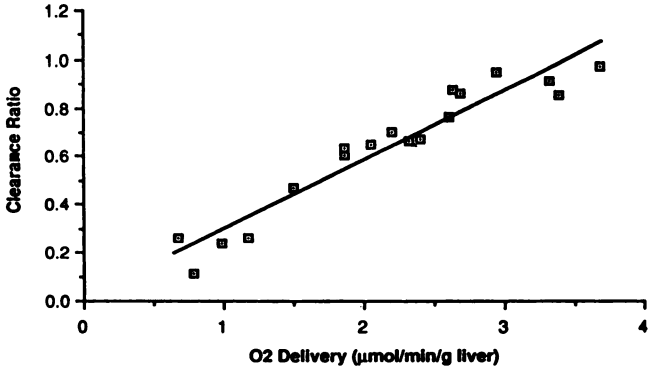

Fig. 2. Ratio of omeprazole clearance during the graded hypoxia phase (30–60 min) to omeprazole clearance during the control phase (0–30 min) *vs.* oxygen delivery during the graded hypoxia phase of the high dose studies. There was a linear decline in omeprazole clearance as oxygen delivery was reduced below control levels ($y = 0.02 + 0.27\ x$; $r = 0.96$, $P < .01$).

second phase to clearance during the control phase. In this way, each experiment acted as its own control and the influence of interanimal variation in the efficiency of drug metabolism was minimized.

Figure 2 shows omeprazole clearance ratio plotted against the rate of oxygen delivery during the second phase. From figure 2 it can be seen that there was a linear decline in omeprazole clearance ratio as oxygen delivery was reduced progressively below the mean control value of 3.33 μmol/min/g of liver. This suggests that the processes involved in omeprazole clearance may not be fully oxygen saturated even at rates of oxygen delivery achieved during the control phase of experiments.

Consistent with the linear correlation seen between oxygen delivery and consumption, there was a linear relationship between the omeprazole clearance ratio and the rate of oxygen consumption during the second phase ($r = 0.96$, $P < .001$). Thus, omeprazole clearance declined as oxygen consumption fell below control levels. Importantly, in all experiments in which oxygen delivery was decreased during the second phase, restoration of normal oxygenation in the final (recovery) phase resulted in rapid return of omeprazole clearance to control values (table 1).

Metabolites. The mean formation clearance rate of the sulfone metabolite under control conditions was 0.23 ± 0.04 ml/min, indicating that only 2% of omeprazole clearance was mediated *via* sulfone metabolism. The effect of changing oxygen supply to the liver in the second phase on the concentration of this metabolite in hepatic outflow was quantified by calculating the ratio of the formation clearance during this phase to control formation clearance. Figure 3 indicates that there was a steady decline in formation clearance of the sulfone as oxygen delivery was reduced below a threshold of 3.0 μmol/min/g of liver. In all experiments, after restoration of control rates of oxygen delivery in the final phase, formation clearance rates recovered to within 10% of control values.

Omeprazole sulfide did not reach measurable concentrations in hepatic outflow during the initial control phase in any of the experiments. It was therefore not possible to compare formation clearance of the sulfide during the second phase with control values. However, a plot of formation clearance for omeprazole sulfide during the second phase against oxygen delivery, shows that detectable levels of the sulfide appeared in perfusate in all experiments in which oxygen supply was reduced below 2.3 μmol/min/g of liver and that the rate of formation of this metabolite was raised with more severe degrees of hypoxia (fig. 4). After reoxygenation formation clearance of omeprazole sulfide returned to low or undetectable levels.

Low dose studies. The preparations used in this second group of experiments were comparable to those used in the higher dose experiments. As can be seen by comparing tables 1 and 2, the mean rates of oxyen delivery, oxygen consumption and omeprazole clearance during the control phase of the two

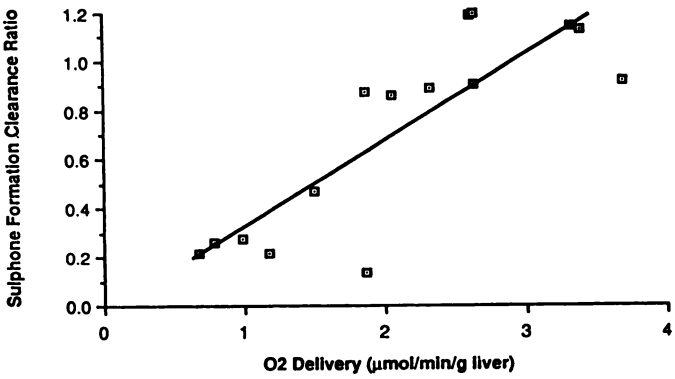

Fig. 3. Omeprazole sulfone formation clearance ratio (graded hypoxia phase/control phase) *vs.* oxygen delivery during the graded hypoxia phase. There was a linear decline in formation of the sulfone as oxygen delivery was reduced below control levels ($y = 0.02 + 0.35\ x$; $r = 0.84$, $P < .001$).

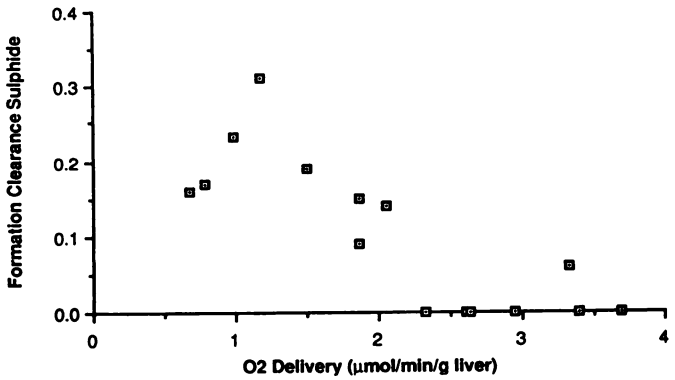

Fig. 4. Omeprazole sulfide formation clearance (milliliters per minute) during the graded hypoxia phase *vs.* oxygen delivery during this phase. Formation clearance was greater at lower rates of oxygen delivery.

TABLE 2

Oxygen delivery, oxygen consumption and omeprazole clearance expressed as mean ± S.D. in the control and recovery phases of low dose studies

Phase	Oxygen Delivery	Oxygen Consumption	Omeprazole Clearance
	μmol/min/g liver	μmol/min/g liver	ml/min
Control, 0–30 min	3.49 ± 0.31	2.95 ± 0.42	13.6 ± 0.4
Recovery, 60–75 min	3.40 ± 0.40	2.78 ± 0.32	12.9 ± 0.6

groups of experiments were similar. The range of oxygen deliveries produced during the second phase of the low dose experiments was 0.77 to 3.96 μmol/min/g of liver and the relationship between oxygen delivery and consumption was similar to that seen in the high dose experiments.

The relationship between the ratio of clearance during the graded hypoxia phase to clearance during the control period and the oxygen delivery during the graded hypoxia phase was similar to that seen in the high dose studies ($y = 0.19 + 0.24x$, $r = 0.93$, $P < .001$). As in the higher dose studies, there was a linear decline in omeprazole clearance ratio as oxygen delivery was reduced below control levels. The slope of this line was very similar to that in the higher dose studies (0.24 in low dose *vs.* 0.27 in high dose). The relationship between oxygen consumption and omeprazole clearance ratio in these low dose studies ($r = 0.92$, $P < .001$) was consistent with a simple linear relationship similar to that found in the high dose experiments.

Restoration of normal oxygenation to the preparations at the beginning of the recovery phase resulted in return to control rates of oxygen consumption and omeprazole clearance (table 2).

Discussion

The isolated perfused rat liver has been shown to be a useful model for studying the effects of hypoxia on hepatic drug disposition (Jones *et al.*, 1984; Webster *et al.*, 1985; Miller and Oliver, 1986). Previous studies in the isolated liver have, in general, examined the impact of large reductions in hepatic oxygen supply. However, in the current study, drug elimination and metabolite formation were examined over a wide range of oxygen delivery rates, enabling the relationships between the efficiency of these processes and the rate of hepatic oxygen supply to be defined more precisely. In contrast to previous work, a nonrecycling liver perfusion system was used in these experiments. This design has the advantage of allowing direct measurement of drug clearance and metabolite formation clearance. Furthermore, in the nonrecycling system, the liver is always perfused with fresh perfusate. One can therfore ensure that the pH of perfusate entering the liver in constant, and that the effects of hypoxia are not partially due to pH effects or to the accumulation of potentially toxic compounds in perfusate.

Hypoxia might be expected to affect the efficiency of hepatic drug elimination primarily by inhibiting drug metabolism (Jones, 1981). However, it is possible that it may also inhibit other important processes involved in hepatic drug disposition, such as energy-dependent drug uptake and excretion. The results of the current study are unlikely to be due to changes in drug uptake as the experiments were conducted under steady-state conditions. Moreover, previous work (Webster *et al.*, 1985; Hoffman *et al.*, 1986) and our own preliminary studies demonstrated that biliary eliminaiton of unmetabolized omeprazole is minimal. Thus, the changes in steady-state omeprazole clearance at different levels of hepatic oxygen supply are likely to reflect changes in the overall rate of omeprazole metabolism.

Omeprazole clearance was measured in the initial phase under similar conditions in all preparations, using a standard 10% red cell containing perfusate equilibrated with 100% oxygen, so that for each experiment clearance was determined under control conditions. The rates of oxygen consumption and bile flow in the IPRL under these conditions were consistent with reports by other investigators (Riedel *et al.*, 1983; Jones *et al.*, 1984) and similar to values reported in the rat *in vivo* (Mitzkat and Meyer, 1973). Omeprazole was eliminated rapidly under these conditions (mean hepatic clearance, 12.5 ± 0.9 ml/min). However, its rate of elimination was very sensitive to reductions in oxygen supply during the second phase. Clearance fell in a linear fashion as oxygen supply was reduced below control levels, indicating that the critical threshold below which oxygen delivery becomes rate-limiting for omeprazole metabolism is at or above the mean control rate of oxygenation in the system of 3.33 μmol/min/g of liver.

Under control conditions there was little change in the overall rate of omeprazole clearance when the concentration of omeprazole in perfusate was reduced from 5 μg in the high dose studies to 1 μg/ml in the low dose studies. Thus, it would appear that the omeprazole elimination was first order within this dose range. The relationship between omeprazole clearance ratio and oxygen delivery in the low dose experiments was very similar to that observed in the high dose studies, indicating that at these drug levels, the sensitivity of omeprazole elimination to hypoxia is independent of the drug's concentration in perfusate. The relationship between omeprazole clearance ratio and oxygen consumption was also comparable in the two groups.

Previous studies have suggested that the sulfone is a relatively minor metabolite of omeprazole and that with normal oxygenation the sulfide is produced in very small or undetectable amounts (Webster *et al.*, 1985; Hoffman, 1986). The present study confirmed these findings. During control oxygenation, the mean formation clearance of the sulfone was 0.23 ml/min, *i.e.*, only 2% of omeprazole clearance was accounted for by sulfone production, whereas omeprazole sulfide could not be detected at all.

Although the sulfone and sulfide are minor metabolites of omeprazole, their study provided interesting insights into the effects of changes in hepatic oxygen supply on two specific metabolic pathways. *S*-oxidations are known to be catalyzed by cytochrome P450 (Timbrell, 1982) and therefore alterations in the rate of formation of omeprazole sulfone at different levels of oxygenation are likely to reflect changes in the availability of oxygen for cytochrome P450. The formation of the sulfone metabolite was very sensitive to reduction in oxygen delivery and its relationship to oxygen delivery appeared to be similar to that for overall omeprazole clearance (fig. 2 and 3). Thus, it is likely that in the isolated rat liver, the oxygen dependence of the major metabolic pathways of omeprazole are similar to those of *S*-oxidation.

In marked contrast, production of the reductive sulfide metabolite appeared to be enhanced by reduction in oxygen supply. Indeed, the sulfide could not be detected in outflow perfusate until hepatic oxygen supply had been reduced substantially (fig. 4). Omeprazole sulfide metabolism is likely to be catalyzed by

sulfoxide reductases (Hoffman *et al.*, 1986). The apparent stimulation of sulfide production by hypoxia is consistent with previous work showing that the activity of sulfoxide reductases is inhibited by oxygen (Douch and Buchana, 1979). Although reductions in oxygen supply led to increased sulfide production, it remained a minor metabolite even during marked hypoxia. Thus, in contrast to the effects of hypoxia on misonidazole metabolism (Smith *et al.*, 1983), stimulation of reductive metabolism did not significantly affect the overall inhibitory influence of hypoxia on omeprazole clearance.

In the IPRL, the levels of omeprazole metabolites in outflow perfusate must reflect a balance between their formation and subsequent elimination by further metabolism or by excretion into bile. An alternative explanation to account for the changes in outflow concentrations of sulfone and sulfide metabolites during hypoxia, is that hypoxia predominantly affected the rate of further metabolism of these compounds rather than their rate of formation. This explanation appears less likely because, although these compounds are likely to share the same metabolite fate, one would need to postulate that during hypoxia sulfone metabolism was enhanced whereas sulfide elimination was impaired.

From studies in subcellular fractions Jones (1981) predicted that *in vivo* the hepatic metabolism of many drugs that are substrates for cytochrome P450 may be inhibited by mild hypoxia. The current study has confirmed that in the intact liver drug clearance dependent on oxidative metabolism is sensitive to any reduction in hepatic oxygen supply below that which is commonly considered to be normal in the isolated rat liver. In a previous study, hexobarbital metabolism in the IPRL (also dependent on hepatic mixed function oxidases) was also shown to be very sensitive to reductions in hepatic oxygen supply (Roth and Rubin, 1976). It is clear from these studies that the rate of oxygen supply is a critical determinant of the rate of oxidative drug metabolism in isolated liver systems. Furthermore a comparison of the critical levels of oxygen supply below which omeprazole elimination is impaired in the isolated perfused rat liver with rates of hepatic oxygen supply calculated from studies in intact animals [4–7 μmol/min/g of liver (Preisig *et al.*, 1972; Lutz *et al.*, 1975; Lautt, 1976)] supports the contention that, *in vivo*, reductions in hepatic oxygen supply may significantly affect the hepatic disposition of drugs which are substrates for microsomal oxidation.

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