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UPTAKE AND METABOLISM OF DIPHENYLHYDANTOIN IN THE ISOLATED PERFUSED RAT LIVER*

TADANOBU INABA, TERUHISA UMEDA,† LASZLO ENDRENYI, WILLIAM A. MAHON and WERNER KALOW

Department of Pharmacology, Faculty of Medicine, University of Toronto, Toronto M5S 1A8, Canada

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Abstract—The elimination of diphenylhydantoin (DPH) was studied in isolated perfused rat livers and the dose-dependent kinetics was confirmed. Kinetic analysis using a saturable two-compartmental analogue indicated that, with increasing doses, the V_{max} remained constant but the elimination half-life (2.8–13 min) for the metabolic component and with it the Michaelis constant (2.9–37 μg/ml) increased progressively. Thus, at first sight, the data appeared to be consistent with the hypothesis of product inhibition. However, the rapid elimination of the metabolic product as well as quantitative results of computer simulations render inhibition unlikely as an explanation for the observed features of DPH elimination. The subcellular distribution of DPH and its metabolites in the liver indicated that DPH and its hydroxylated metabolite were localized mainly in the 600 *g* and microsomal fractions; the glucuronide was mainly in the cytosol. The results were compatible with the possibility of capacity-limiting DPH access to the drug-oxidizing enzyme system.

The dose-dependent elimination of diphenylhydantoin (DPH) has been demonstrated in rats using whole animals [1], isolated perfused livers [1] and isolated hepatocytes [2]. The mechanism has been postulated to involve simple Michaelis–Menten-type kinetics [3] and, possibly, product inhibition by the metabolite, 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (HPPH) [4].

Recently, the time-course study of biliary excretion of DPH revealed that the excretion of HPPH glucuronide is saturable with an increasing dose of DPH [5]. The data from an intravenous (i.v.) administration of HPPH suggested that the conjugation of HPPH and transfer of conjugated HPPH from liver cells into bile were not saturable [5]. Therefore, the saturable process may be the transformation step of DPH to HPPH by the microsomal enzyme system or possibly the transfer of DPH to the enzyme system.

The present work was conducted to investigate a possible capacity-limiting step of the dose-dependent elimination of DPH using (a) isolated perfused livers to study the hepatic extraction of DPH, and (b) stepwise centrifugation to examine subcellular distribution of DPH in the liver.

MATERIALS AND METHODS

Materials

Animals. Male Wistar rats (Canadian Breeding Lab, Montreal) weighing 300–330 g were used as liver donors and blood donors.

Chemicals. DPH was supplied by Sigma Chemical Co., St. Louis, MO. [2-^{14}C]DPH (sp. act. 4.60 mCi/m-mole) was obtained from New England Nuclear Corp., Boston, MA. The radiochemical purity of [^{14}C]DPH when checked by thin-layer chromatography (t.l.c.) was found to be greater than 98 per cent.

Drug solutions. An injectable solution of DPH was prepared with propylene glycol–ethanol–0.1 N NaOH (v/v, 4:1:5), as described previously [5]. The final concentrations of DPH solutions were 1 mg/ml and 10 mg/ml (6.7 μCi/ml).

Isolated liver perfusion

The liver was prepared as described by Miller *et al.* [6]. The perfusate was passed through a new type of oxygenator described by Hamilton *et al.* [7] employing 95% O_2 and 5% CO_2.

To prepare 100 ml of the perfusate, 50 ml of blood collected from rats by aortic puncture was mixed with 50 ml of Krebs bicarbonate buffer solution containing 500 I.U. heparin and 5 mg gentamicin. The common bile duct was cannulated with polyethylene tubing for bile collection. The liver blood flow was adjusted to about 14–16 ml/min at portal pressure of 130 mm H_2O.

Uptake, metabolism and biliary excretion in the isolated perfused liver

After equilibration of the liver and perfusate for 30 min, [^{14}C]DPH solution was added to the reservoir with doses of 1, 10, 18 and 30 mg/kg body weight of the liver donor; four livers were used (Table 1).

To determine the hepatic uptake of DPH, an 0.5-ml aliquot of perfusate was taken from inflow and outflow sampling sites. The concentration of DPH was determined by modifying the method of Gerber

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† Present address: Department of Internal Med. III, Kumamoto University Medical School, Kumamoto, Japan.

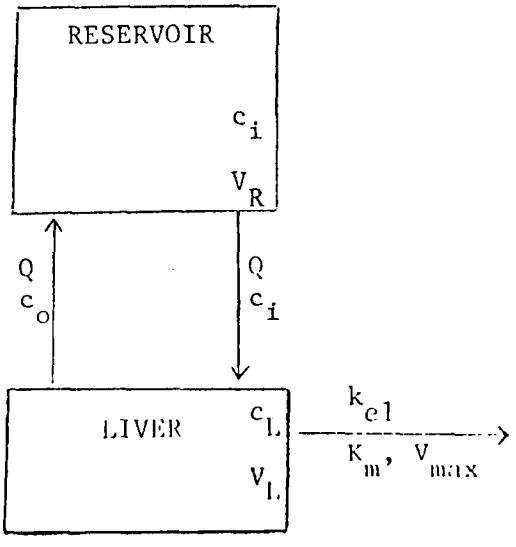
et al. [1]. A 0.4-ml aliquot of each perfusate was diluted with 1.6 ml of 0.1 M phosphate buffer, pH 6.8, and extracted with 10 ml of 1-chlorobutane. The solvent was removed and washed with 2 ml of the same buffer solution. Radioactivity of 5 ml chlorobutane was determined by a liquid scintillation counter. The extraction efficiency of DPH was about 80 per cent and 2 per cent of HPPH was extracted by this procedure. While perfusing, bile was collected during the 15-min period after drug administration. Liver wt was obtained at the end of each experiment.

Aliquots of 50μl of bile were counted in 10 ml Aquasol (New England Nuclear Corp., Boston, MA) to determine the total radioactivity.

Kinetic model of DPH elimination in vitro

Rowland *et al.* [8] described an isolated perfused organ system by two compartments (Scheme 1), one for the eliminating organ, in our case the liver, and another for the reservoir. Here c_i and c_o are drug concentrations in the blood flowing at the rate of Q in and out of the liver, c_L is the concentration in the liver, V_L and V_R are apparent distribution volumes of the liver and reservoir, respectively; V_{max} and K_m are the asymptotic high-concentration velocity and the Michaelis constant, respectively, characterizing the rate of drug elimination by the liver.

Scheme I



By assuming saturable and perfusion rate-limited elimination (when, in a steady state, c_L is proportional to c_o, $c_L = Kc_o$, K being an average apparent partition coefficient), the mass balances for the two compartments are (in similarity to Ref. 8):

$$-V_R\,(dc_i/dt) = Q(c_i - c_o) \quad (1)$$

$$KV_L\,(dc_o/dt) = Q(c_i - c_o) - KV_L\,V_{max}c_o/(K_m + Kc_o). \quad (2)$$

At low concentrations, when $c_o \ll K_m/K$, the second expression is reduced to:

$$KV_L\,(dc_o/dt) = Q(c_i - c_o) - k_{el}\,KV_L\,c_o \quad (3)$$

where $k_{el} = V_{max}/K_m$ is a first-order elimination rate constant.

With increasing initial concentrations, the relative importance of the left-hand side term in Equation 2 diminishes quite substantially. Then also $c_i \approx c_o$ (i.e. the hepatic extraction ratio is reduced under these conditions), and Equations 1 and 2 can be combined to:

$$-V_R(dc_i/dt) = KV_L\,V_{max}\,c_i/(K_m + Kc_i). \quad (4)$$

This expression was helpful at our highest dose, 30 mg/kg, for which the outflow could not be properly assessed. At the intermediate doses, 18 and 10 mg/kg, Equations 1 and 2 were applied, while for the lowest dose Equations 1 and 3 were used.

From these equations, because of the partitioning of the drug in the liver with the perfusate the following parameters can be evaluated: V_R, KV_L, V_{max}/K and K_m/K.

Statistical evaluation

The kinetic model parameters were estimated by using the NONLIN computer program of Metzler *et al.* [9]. All calculations were performed by assuming, in sequence, two limiting behaviors of observational errors [10]. First, on the hypothesis of approximately constant absolute errors, unweighted non-linear regression was performed. Subsequently, constant relative or percentage errors (i.e. constant coefficient of variation) were assumed by applying weights inversely proportional to the squares of the observed concentrations. The presented results are based on the first hypothesis (the constancy of errors) which, as experience with the experimental system and the analysis of residual plots indicates, reasonably characterizes the observations. At any rate, the two error assumption yielded, in all cases, identical conclusions.

The computations were performed on the IBM 370/165II computer of the University of Toronto.

Simulation of the effect of product inhibition on DPH elimination

Extending the study of Perrier *et al.* [11], the rate of metabolic drug elimination is, when inhibited by the reaction product,

$$v = -\frac{dc_o}{dt} = \frac{V_{max}c_o - V_p c_p}{c_o + K_m(1 + c_p/K_p)}.$$

Here c_o and c_p are the concentration of drug and product, respectively, in the effluent, K_p the product inhibition constant, and V_p the corresponding asymptotic velocity. The four constants contain also the liver/effluent partition coefficients for the drug and product, while V_{max} and V_p include also distribution volume terms.

The rate of appearance of the product is

$$dc_p/dt = v - k_{ep}\,c_p$$

where k_{ep} is the rate constant for first-order product elimination.

The time courses of drug and product concentrations were simulated using the CSMP computer program (IBM System/360 Continuous System Modeling Program, No. 360A-CX-16X). During their progress, the semilogarithmic drug concentration vs time curves exhibited diminishing curvatures. Consequently, they were judged to reach their

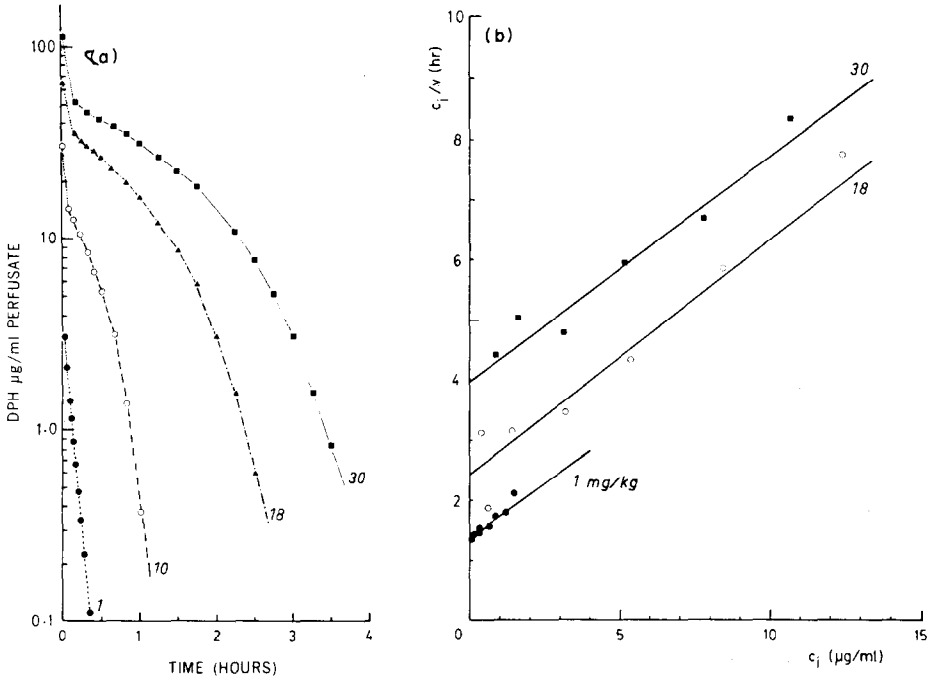


Fig. 1. Panel A: logarithmic concentrations of DPH in the perfusate vs time in isolated perfused livers after 1, 10, 18 and 30 mg DPH/kg of liver donor. Panel B: Hanes plot [12] for hepatic removal rates of DPH vs inflow concentrations.

apparently terminal, linear segments when their slopes (and thereby their apparent biological half-lives) ceased to change more than 5 per cent during a period of an average half-life.

Subcellular distribution of DPH in the liver

In vivo. Rats were anesthetized with ether. Three rats received 10 mg DPH/kg body weight, and three rats 1 mg/kg intravenously. Ten min after the injection, animals were decapitated, and their livers removed and placed immediately in 1.15% KCl solution at 4°. The livers were homogenized in a motor-driven Potter-Elvehjem homogenizer with a plastic pestle in an equal volume of 1.15% KCl with 0.05 M Tris buffer, pH 7.4 [12]. The homogenate was subjected to differential centrifugation at 600 *g* for 10 min (nuclei, cell debris), at 10,000 *g* for 10 min (mitochondria) and at 100,000 *g* for 60 min (microsomes) [13]. The 600 and 10,000 *g* fractions were washed twice with 0.05 M Tris buffer and the 10,000 *g* pellet washed once. The final supernatants were combined to form the cytosol fraction. Each pellet was resuspended with 15 ml of 0.1 M phosphate buffer, pH 6.8, and subjected to a DPH assay described later.

In vitro. Four rats were sacrificed by decapitation, and their livers were removed and placed in 1.15% KCl solution at 4°. Sixty ml of 50% homogenate was transferred into six polypropylene centrifuging tubes. [^{14}C]DPH (0.15 mg) was added to each of three tubes containing 10 ml homogenate, and 1.50 mg ^{14}C DPH to each of the other three tubes. After shaking for 10 min at 4° each homogenate was immediately subjected to differential centrifugation as described above.

DPH assay of subcellular fractions. The suspension of any given pellet was sonicated for 6 min to yield a clear solution and adjusted to pH 6.8. One ml of each fraction was subjected to fractional extraction of DPH and its metabolites as described earlier [5]. One 0.1-ml portion of each fraction was counted in 15 ml Aquasol to determine the total radioactivity.

RESULTS

Figure 1a shows the time-course of DPH disappearance from the perfusate of isolated livers. Four separate experiments were carried out with varying initial concentrations which correspond to doses of 1, 10, 18 and 30 mg DPH/kg body weight of the liver donor. The terminal apparent half-life was dose-dependent in this system and ranged from 2.8 to 13 min. The linearized representation (Fig. 1b) illustrates that the observations are described well by the Michaelis-Menten relationship [14]. The parallel lines in the Hanes plot indicate that the asymptotic velocity is independent of the DPH dose but that the Michaelis constant increases with larger doses. The rapid initial decline is due to the hepatic uptake of DPH.

The initial hepatic uptake was determined from inflow and outflow concentrations of DPH in the perfusate. Figure 2 shows the initial variation of DPH concentrations from both sides; the initial uptake appeared to be complete in 4 min. During this period, about a quarter of the dose was taken up independently of the initial concentration (Table 1).

The hepatic extraction ratios (HER) were calculated from each pair of inflow (c_i) and outflow (c_o) concentrations as $(c_i - c_o)/c_i$. After the initial uptake phase which showed decreasing HER, HER was less than 0.05 at the high dose (18 mg/kg) and ranged from 0.4 to 0.7 at the low dose (1 mg/kg).

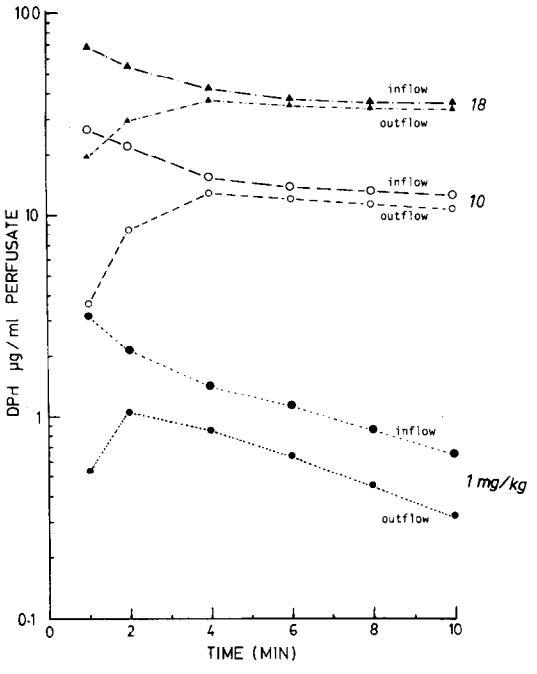


Fig. 2. Inflow and outflow concentrations of DPH in the perfusate during the first 10 min after 1, 10 and 18 mg DPH/kg of liver donor.

Least-squares analysis of the kinetics of the liver perfusion experiments was performed first by assuming that a given parameter was changing with the differing doses of DPH, and then by supposing the independence of the parameter value from the dose. The minimum sums of squares calculated in the two analyses were compared. Substantial (statistically significant) difference indicated the distinctness of the investigated parameters.

The asymptotic velocity (V_{max}) and the estimated volumes in the liver (V_L) and reservoir (V_R) were found to be approximately independent of the DPH dose. In contrast, increasing doses brought about a reduction of the estimated elimination rate constant (k_{el}) and, consequently, an increase of the Michaelis constant (K_m) and elimination half-life (Table 2). The relationship between the latter and the DPH dose is illustrated in Fig. 3. An upward curvature is exhibited.

As a contrast, the corresponding relationships between the elimination half-life and DPH dose obtained in computer simulations are shown in Fig. 4. These simulations assumed that the metabolic product inhibits the elimination of the drug. The resulting curves were either approximately linear or exhibited a downward curvature. These trends were consistently observed over a wide range of the conditions and parameters. The DPH dose was varied between 0.1 and 200 arbitrary units, the product inhibition constant (K_p) between 1 and 50, and the product elimination constant (k_{ep}) between 10^{-4} and 10^{-2}. These doses and K_p values were generally higher than those applied by Perrier *et al.* [11] in their simulations. For the sake of overall compatibility with their study, the values of V_{max} and K_m were fixed at 0.2 and 10 respectively. These values determined the magnitudes and arbitrary units of the primary variables: time and drug concentration. Figure 5 illustrates that appropriate combinations of the parameters and doses yielded time profiles of the drug concentration which are quite similar to those

Table 1. Initial uptake of DPH by the isolated perfused liver

Dose (mg/kg)	Initial concn (mg/100 ml*)	Blood flow (ml/min/g liver)	Liver wt (g)	Uptake (μg/4 min)	% of Dose
1	0.30	1.20	10.0	75.7	25.2
10	3.00	1.70	8.3	771.6	25.7
18	6.12	1.23	12.6	1806.8	29.5
30	11.10	1.18	13.6	†	†

* Reservoir volume.
† Not determined.

Table 2. Kinetic parameters estimated for the elimination of DPH by isolated perfused livers*,†

Dose (mg/kg liver donor)	c_{i_0} (μg/ml)	k_{el} (hr^{-1})	K_m/K (μg/ml)
1	4.35 ± 0.72‡	19.1 ± 8.6	2.91 ± 1.34
10	37.01 ± 0.78	9.8 ± 1.2	5.69 ± 0.83
18	97.6 ± 1.8	4.61 ± 0.47	12.1 ± 1.6
30	65.3 ± 2.1	1.50 ± 0.08	37.1 ± 3.7

* Definitions: c_{i_0}, extrapolated initial drug concentration in the inflow; k_{el}, terminal, apparently first-order elimination rate constant; K_m, Michaelis constant; V_{max}, asymptotic, zero-order elimination rate approached at high concentrations; V_L, apparent distribution volume of perfusate in liver; V_R, apparent distribution volume of reservoir; and K, average partition coefficient between the perfusate in the liver and the effluent.

† In addition the value for $V_{max}/K = 55.7 \pm 4.6$ μg. ml^{-1} hr^{-1}, for $V_L K = 4.48 \pm 0.05$ ml, and for $V_R = 2.90 \pm 0.11$ ml.

‡ Parameters and standard errors were estimated by nonlinear regression.

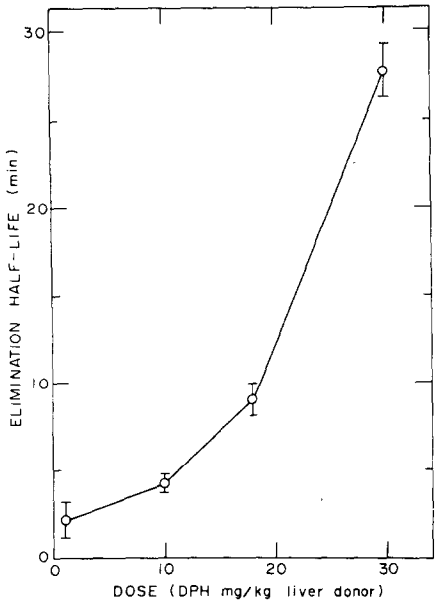


Fig. 3. Relationship between experimental elimination half-lives and DPH doses. The half-lives are estimated (Table 2) from the observed time–courses of DPH concentrations in isolated perfused rat livers.

observed experimentally (Fig. 1). Increasing V_p values introduced complications to the relationships but did not change the conclusions reached from the inspection of Figs. 4 and 5.

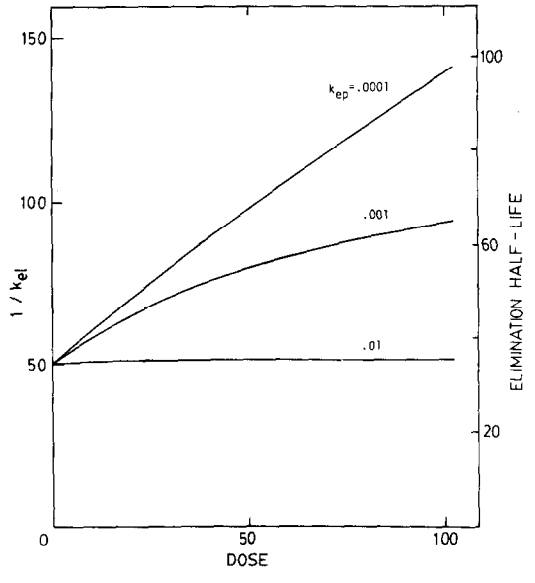


Fig. 4. Relationships between drug doses and apparently first-order elimination half-lives which were computer simulated by assuming drug elimination inhibited by the metabolic product. In the simulations, $V_{max} = 0.2$ and $K_m = 10$ were assumed for the product-yielding reactions, and therefore, a true elimination rate constant of $k_{el} = V_{max}/K_m = 0.02$. To characterize product inhibition, $V_p = 0$ and $K_p = 50$ were used here. All model constants and experimental conditions have arbitrary units. The relationship between elimination half-life and drug dose is either approximately linear or exhibits a downward curvature. This is in contrast with the shape of the experimentally observed relationship (Fig. 3).

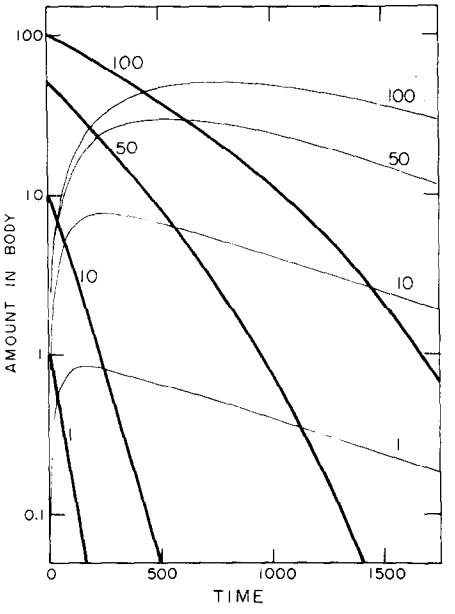


Fig. 5. Time–courses of drug and metabolic product concentrations. The curves were computer-simulated on the assumption of drug elimination inhibited by the product. The simulations assumed, with arbitrary units, $V_{max} = 0.2$, $K_m = 10$, $V_p = 0$ and $K_p = 10$. The drug doses, introduced as a bolus, are indicated in the diagram. Thick curves show the time courses of drug concentrations (or amounts), thin curves those of the metabolic product. The simulated curves are similar to those observed experimentally. Increasing curvatures and larger apparent half-lives are noted at higher drug doses.

Bile was collected throughout the perfusion experiment; more than ¾ of the dose was excreted by this route. Figure 6 shows the excretion rate of radioactivity in bile. The rate approached a common maximum level of 2.4 to 3.4 μg DPH equiv/min/g of liver, or 0.6 to 0.9 μmole/hr/g of liver.

Table 3 shows the distribution of radioactivity in various components of the liver 10 min after an i.v.

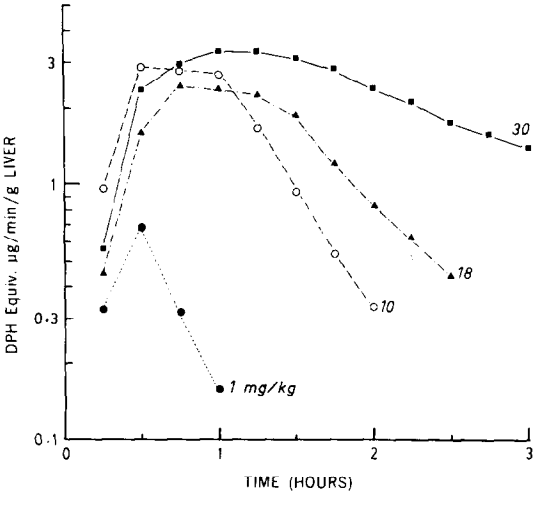


Fig. 6. Rates of excretion of radioactivity in bile after 1, 10, 18 and 30 mg [^{14}C]DPH/kg of liver donor in isolated perfused livers.

Table 3. Subcellular distribution of radioactivity in rat liver after addition of [^{14}C]DPH *in vivo* and *in vitro*

Subcellular fraction	*In vivo**				*In vitro*†			
	1 mg/kg		10 mg/kg		15 μg/ml		150 μg/ml	
	%‡	R.S.A.§	%	R.S.A.	%	R.S.A.	%	R.S.A.
Homogenate		1.00		1.00		1.00		1.00
600 *g*	35.1	1.07	42.4	1.34	31.4	1.19	31.1	1.04
10,000 *g*	2.4	0.04	2.3	0.28	2.6	0.24	2.5	0.22
100,000 *g* (microsome)	26.0	1.62	30.4	1.73	33.9	1.92	28.2	1.67
Supernatant (cytosol)	26.5	0.72	24.8	0.65	32.1	0.71	28.2	0.91

* Rats were sacrificed 10 min after [^{14}C]DPH, i.v.
† Liver homogenate was incubated with [^{14}C]DPH for 10 min at 4°.
‡ % = Percent radioactivity in each fraction.
§ Relative specific activity: per cent radioactivity/per cent dry wt of each fraction.

injection of [^{14}C]DPH (*in vivo*) and also after an addition of [^{14}C]DPH *in vitro* to the liver homogenate at 4°. Radioactivity was highly localized in two fractions, namely the 600 *g* (nuclei) and 100,000 *g* (microsomes). Radioactivity in subcellular fractions of the liver was characterized by a differential extraction [5]. The relative amounts of DPH, HPPH and conjugated HPPH in the liver after 10 min of perfusion are shown in Fig. 7. DPH and HPPH were predominant with concentrations of 84 and 85 μg/g dry wt of liver homogenate, respectively; only small amounts of conjugates were present. DPH and HPPH were found mostly in the 600 and 100,000 *g* fractions, whereas conjugated HPPH was found in the cytosol.

DISCUSSION

Dose-dependent elimination of DPH in an isolated perfused rat liver was reported by Gerber *et al.* [1]. With initial concentrations of 10 and 92 μg DPH/ml of perfusate, the half-lives of DPH were found to be 15 and 64 min respectively. However, at the higher initial concentration, they monitored DPH concentrations only for 80 min and, therefore, it could not be ascertained whether the decline of the DPH concentration was exponential or linear.

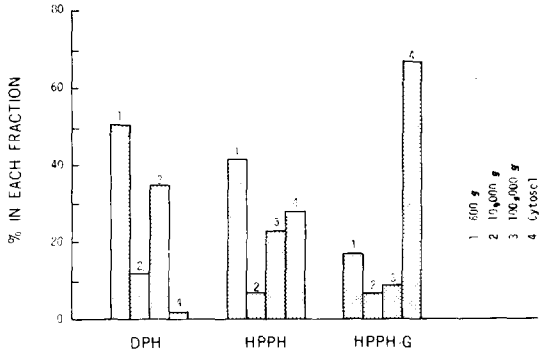


Fig. 7. Subcellular distribution of DPH and its metabolites in the liver after a 10-min perfusion with 10 mg DPH/kg of liver donor. Concentrations of DPH, HPPH and HPPH-G (glucuronide) in the whole homogenate were 84, 85 and 18 μg DPH equiv./g dry wt.

The time-course of DPH disappearance, as presented in Fig. 1a, showed an apparent saturable phase, especially when the concentration was above 5 μg/ml (0.2 mM). The perfusion data reported by Gerber *et al.* [1] could be fitted to limited portions of our data, namely the initial parts following the 10 and 30 mg/kg doses. The terminal apparent half-life was dose-dependent and ranged from 2.8 to 13 min in the present system.

Although it might not be clear from Fig. 1, there was another phase of rapid decline of DPH concentration within the first 10 min. This was the initial uptake which could be described as the distribution of DPH within the liver. Figure 2 shows that this initial uptake was complete in about 4 min and the amount of drug taken up ranged from 25 to 30 per cent (Table 1). Therefore, the initial uptake of DPH or the distribution within the liver was independent of the dose and not a saturable process at the doses studied. This is consistent with the studies with isolated hepatocytes which showed that DPH was extensively bound to hepatocytes and that the concentration ratio (hepatocyte/suspension medium) was about 20-fold [2]. The data reported by Gerber *et al.* [1] was indicative of extensive initial uptake of DPH within 10 min by the liver. The work reported by Shand *et al.* [15] showed that the initial uptake of propranolol was complete in 10–12 min and that the high-affinity binding site became saturated with 400 μg propranolol/g of liver. The difference between the uptake of propranolol and DPH might be due to the large differences in the hepatic extraction ratios of these two drugs.

Drugs with high hepatic extraction ratios, such as propranolol [15] and lidocaine [16, 17], exhibit interesting blood flow-dependent kinetics. The large fluctuation of HER for DPH was very striking. The instantaneous clearance of DPH fluctuated in proportion to the HER (instantaneous clearance = hepatic blood flow × instantaneous HER) [8], since constant blood flow was maintained throughout the experiment. At a dose of 18 mg/kg, the initial rapid decline in HER was followed by steady, low HER of less than 0.05, while at an inflow concentration of less than 10 μg/ml, HER gradually rose to 0.2. After the lowest dose, 1 mg/kg, while

HER changed very markedly with decreasing inflow concentrations, it remained at relatively high levels between 0.4 and 0.7. These values are compatible with those observed by Shand *et al.* [18]. It appears that HER is a sensitive indicator of dose-dependent elimination of a drug. The detailed observation of the varying HER will be described elsewhere.

Based on a modification of the isolated perfused organ system model described by Rowland *et al.* [8], various kinetic parameters were computed by least-squares fitting. Although a direct comparison of the results in Table 2 to those found by Gerber and Wagner [3] is difficult, the K_m estimated in the present studies (2.9 to 37 μg DPH/ml of perfusate) is in the same range as their K_m (9.0 to 15.1 μg/ml whole blood) found in whole animal preparation. The computer-estimated V_{max} and K_m of these authors had a large standard deviation [3] and it was not clear whether the V_{max} and K_m were dependent on the dose of DPH. The present study demonstrated clearly that V_{max} remained constant with little variation between doses, whereas K_m increased significantly with increasing doses.

At first sight our observation of the time profile of DPH concentrations (Fig. 1) would appear to be compatible with the hypothesis that the metabolism of the drug is inhibited by its product [4, 11]. Indeed, this assumption was invoked in order to explain the lengthening apparent half-lives observed with increasing DPH doses [4]. By comparing Fig. 1 and 5, our own computer simulations, based on a scheme of product inhibition, also would seem to be consistent with such experimental data.

However, a closer, more quantitative examination of the results of the experiments and simulations raises some questions. An upward curvature was noted when the elimination half-life (or the K_m at constant V_{max}) evaluated from the observations at the various DPH doses was plotted against the dose (Fig. 3). In contrast, similar plots obtained from the computer simulations (Fig. 4) showed either linearity or downward curvatures. This quantitative disagreement raises doubts about the appropriateness of assuming product inhibition as an explanation of the anomalies of DPH elimination.

Furthermore, earlier we demonstrated [5] that the elimination rate of HPPH was considerably greater than that of DPH. This suggests that HPPH is eliminated too rapidly to exhibit product inhibition.

The simulated relationships between elimination half-life and drug dose illustrate also the effect of product elimination rate. When the metabolic product is eliminated fairly rapidly, its concentration remains below the inhibitory level and, therefore, it does not affect the terminal, apparently first-order elimination constant which approximates its true values. (In the present case, the true $k_{el} = V_{max}/K_m = 0.02$ or $1/k_{el} = 50$.) In contrast, slow elimination of the product leads to its build-up and thereby to product concentrations far exceeding those of the unchanged drug. Under these conditions, the apparently first-order elimination half-life is related linearly to the administered dose. At intermediate product elimination rates, the elimination half-life still increases with increasing doses but less than linearly.

The distinction between apparent biological and elimination half-lives should be stressed. The DPH concentrations observed in the isolated perfused livers could be characterized on the basis of the kinetic model illustrated in Scheme I. This is kinetically equivalent to a two-compartmental open model for which the elimination and apparent biological half-lives are known to be different [19]. The former is calculated (as in Table 2); the latter is directly obtained from the observed time–course of the concentrations (Fig. 1). By contrast, computer simulations of the effect of product-inhibition do not require any reference to a perfused organ system, and with this to a two-compartmental kinetic model. The concentration time–course (Fig. 5) characterized by one-compartmental kinetics yields directly the elimination half-life (Fig. 4).

The major route of eliminating DPH in the rat is by the bile [5] in which the drug is excreted mostly as a glucuronide of HPPH [5, 20]. The maximum rate of biliary excretion approached 0.6 to 0.8 μmole/hr/g of liver. This rate was comparable to the maximum rate of metabolism by the microsomal preparation [12] and by isolated hepatocytes [2]. The present data were consistent with the previous data *in vivo* [5], the DPH metabolites once formed being excreted promptly through the bile.

The liver is one of the major organs where DPH accumulates [1, 21, 22]. In fact, as discussed earlier, 25–30 per cent of the administered dose was trapped in the liver within the first 4 min. Therefore, subcellular distribution of radioactivity after addition of [^{14}C]DPH *in vivo* and *in vitro* was investigated. Table 3 shows that, after i.v. injections of 1 and 10 mg/kg, radioactivity was highly localized in the 600 *g* and microsomal fractions and also that the subcellular distribution was independent of the dose [21] and of the route by which the drug reached the liver. The distribution of radioactivity after addition of DPH *in vitro* at 4° was very similar to that of *in vivo* data. Therefore, the subcellular distribution is dependent on the physico-chemical nature of DPH, such as partition and lipophilicity.

Radioactivity in various fractions from a perfusion experiment was characterized and summarized in Fig. 7. It should be noted that DPH was localized in the 600 *g* and microsomal fractions and that HPPH-glucuronide was present in the cytosol. These findings are analogous to the subcellular distribution of benzpyrene and its metabolites reported by Levine and Singer [13].

Since a significant portion of DPH taken up by the liver is localized or bound in other fractions than microsomes, it is possible that the access of DPH to the drug-oxidizing enzyme may be a capacity-limiting step in overall elimination of DPH.

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