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ENTEROHEPATIC CIRCULATION OF INDOMETHACIN AND ITS ROLE IN INTESTINAL IRRITATION

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Abstract—The accumulative biliary secretion of indomethacin and its conjugate(s), based upon biliary clearance constants and areas under portal and peripheral plasma profiles, has been determined for five laboratory species and estimated for man. This parameter, termed $\sum_{bile}^{\infty}$, ranges approximately 30-fold from 13 per cent of dosage in the rabbit to 362 per cent in the dog, and provides a quantitative correlate to the wide species variation in sensitivity to indomethacin-related intestinal lesions. Extrapolation of the $\sum_{bile}^{\infty}$ vs. intestinal toxicity relationship to man predicts a therapeutic ratio of *ca.* 20:1 with respect to intestinal irritation. The pharmacokinetic modeling derived for the present purpose should be of general utility in the quantitation of enterohepatic recycling of drugs or metabolites whose loci of intended or untoward effects include the liver or gut.

The sensitivity to indomethacin-induced intestinal lesions varies two orders of magnitude among the several laboratory species examined, minimal toxic dosage for ulcers ranging from *ca.* 0·5 mg/kg/day in the dog to 20 mg/kg/day in the rabbit on a subacute or chronic basis [1]. Extrapolation of these data to man is meaningless without an understanding of species differences in those aspects of the disposition of drug and/or its metabolites which relate to intestinal irritation.

All available evidence suggests that the biliary secretion of unchanged drug and its conjugates is the causative factor for lesions: (1) Acute ED_{50} for lesions in the rat is independent of route of administration, but is markedly influenced by factors influencing biliary secretion of unchanged indomethacin (starvation, bile ligation, phenobarbital induction of metabolizing enzymes) [2]. (2) The known metabolites of indomethacin, desmethyl- and desbenzoylindomethacin, are innocuous in the rat at dosages many times the acute or subacute ulcerogenic dosages of the parent drug (unpublished). (3) Those species (rat and dog) in which the ratio of total biliary to renal excretion of drug is high [3, 4] are most susceptible to lesions.

For a more meaningful correlation of lesions with biliary secretion, total exposure of the intestinal mucosa to the ulcerogenic agent must be calculated, taking into account the extent and duration of enterohepatic recirculation in the intact animal. This, in effect, should be equal to the product of biliary clearance and areas under both portal and systemic plasma profiles weighted according to their respective contributions of blood flow to the bile-forming system.

The present report describes the determination of such total exposure values in five laboratory species, and correlation thereof with sensitivity to intestinal lesions.

* See Appendix for rationale and derivation of biokinetic expressions.

MATERIALS AND METHODS

The experimental plan* includes estimates of plasma concentrations of indomethacin in the portal and peripheral circulation and of plasma and renal clearances in the intact animal. Biliary clearances are determined separately in animals whose bile ducts have been cannulated to permit complete collection.

Indomethacin-2-^{14}C (28·5 μCi/mg) was diluted with carrier to a specific activity of 1×10^7 dis/min/mg. A single stock solution of 10 mg/ml in alcohol, stored at −20°, was used throughout these studies; appropriate aliquots were evaporated to dryness and reconstituted in 0·1 M sodium phosphate buffer, pH 7·5, for intravenous administration. Except where noted, all dosages were at 1·0 mg/kg.

Indomethacin, its desbenzoyl and desmethyl metabolites, and their respective conjugates were measured by the isotope dilution assay procedure described elsewhere [5]. For purposes of clearance calculations, the sum of free and conjugated forms of the drug in bile and urine was used.

In rats and guinea pigs, separate groups of animals were used for determinations of plasma, renal and biliary clearances respectively. Mean plasma clearances were calculated from composite profiles for 4–5 conscious animals bled alternatively via the ocular sinus through 8 hr after dosage. Renal clearances were calculated from the products of plasma clearance and fractions of dose recovered in the urine unchanged (integral method). Biliary clearances were determined in each of 3–4 anesthetized animals with bile collected at 45-min intervals and plasma samples at the midpoint of each collection period (the incremental method).

In dogs and spider monkeys, plasma and renal clearances were determined in each of 3 animals, intact, and subsequently with enterohepatic recirculation interrupted by total collection of bile via the common bile duct with gallbladder isolated. In all experiments, the animals were anesthetized with phenobarbital and infused with mannitol-phos-

Table 1. Overall recoveries of indomethacin and metabolites in six species.*

Species		Total ^{14}C†	Indo	DMI	DBI	DMBI
Rat	Urine	44·9	1·6	24·2	4·4	6·7
	Feces	47·7	6·2	41·0	0·5	1·0
	Bile‡ (5)	(100)	73·0	24·0	2·7	1·3
Guinea pig	Urine	57·7	13·0	17·0	30·2	2·0
	Feces	37·3	NA	NA	NA	NA
	Bile (4)	(100)	56·5	31·5	7·6	4·4
Rabbit	Urine	79·1	30·7	18·6	29·1	nil
	Feces	4·8	NA	NA	NA	NA
	Bile (4)	(100)	74·0	24·0	2·0	
Beagle dog	Urine	7·2	0·4	0·5	3·0	
	Feces	89·2	73·0	1·5	7·0	
	Bile (3)	(100)	64·0	25·0	4·0	7·0
Spider monkey	Urine	63·8	46·0	4·1	15·3	nil
	Feces	18·8	NA	NA	NA	NA
	Bile (3)	(100)	63·5	32·5	4·0	
Man	Urine	54·3	31·7	15·9	12·3	1·9
	Feces	24·3	0·8	14·1	1·1	15·5
	Bile	NA	NA	NA	NA	

* Abbreviations are as follows: DMI, desmethylindomethacin; DBI, desbenzoylindomethacin; DMBI, desmethyl-desbenzoyl indomethacin (5-hydroxy-2-methyl-3-indoleacetic acid); NA, not available.

† Values for total ^{14}C according to Hucker *et al.* [3].

‡ Recovery values for individual metabolites in urine are expressed as per cent of dosage and include free plus conjugated forms; bile values are relative abundances, representing means for the numbers of animals indicated in the parentheses.

phate [6] at 0·2 ml/min/kg. Renal and biliary clearances were calculated by both the incremental and integral methods (see Appendix).

In rabbits, individual anesthetized (Urethan–allobarbital) animals were used for determinations of plasma clearance, bile clearance (incremental method) and renal clearance (integral method) based upon extrapolation of 0–48 hr urinary recoveries.

In all species except dogs, which were prepared with chronic portal fistulas enabling determination of complete portal plasma profiles, the portal: peripheral drug gradients were calculated from a limited number of samples withdrawn at intervals through 24 hr so as to obtain a representative average value.

RESULTS

An overall summary of the excretion of intravenous indomethacin and its metabolites in five laboratory species and man is presented in Table 1. Examination of the respective contributions of the renal and fecal routes to irreversible elimination of drug reveals a marked variation among species. For example, the ratio of recoveries of total radioactivity in urine : feces ranges from 0·08 in the dog to 16·5 in the rabbit. To the extent that fecal elimination following i.v. dosage can be considered a rough index of biliary secretion of the drug, only a very qualitative correlation between this value and intestinal irritation can be made; for example, the rat, which is most sensitive to lesions, has a urine : feces ratio not greatly different from that of the guinea pig.

A direct comparison of biliary excretion of drug over arbitrary intervals following dosage [3, 4], or as extrapolated to infinity, provides a better correlation, but not a definitive one, these values varying at most over a 5-fold range. Furthermore, the experimental design employed in such measurements, unless provision is made for return of all but negligible aliquots of bile (as by a stream-splitting device), precludes relevance to normal physiological conditions.

The alternative undertaken in the present study is based on biokinetic inference following the separate determinations of disposition kinetics in the intact animal and of biliary clearance rates. Estimates of areas under the plasma concentration curve were obtained by extrapolation of log-linear regression lines on residuals. Plasma clearances, $\dot{V}_{cl,p}$ were calculated by $D/\int_0^\infty Cp\,dt$. Renal and biliary clearances were determined either by the integral method ($f_u \cdot \dot{V}_{cl,p}$ and $f_b \cdot \dot{V}_{cl,p}$) or by the incremental method ($C_u \cdot \dot{V}_u/C_{p,m}$ and $C_b \cdot \dot{V}_b/C_{p,m}$) where subscripts u and b refer to urine and bile; C, $\dot{V}$ and f are concentration, flow rate and fraction* of dose respectively; and $C_{p,m}$ is the plasma concentration at the midpoint of the collection period.

Rat. The composite plasma profile for a group of animals, each sampled at several intervals on an alternating schedule, is depicted in Fig. 1. Slopes for the initial and terminal regression lines were 0·056 and 0·0014 min^{-1} respectively. Correlation coefficients, r, for both lines were of a magnitude insuring that each individual was representative of the group. Thus, the

* Calculated by extrapolation to infinity of a double-reciprocal plot of accumulative recovery vs. time.

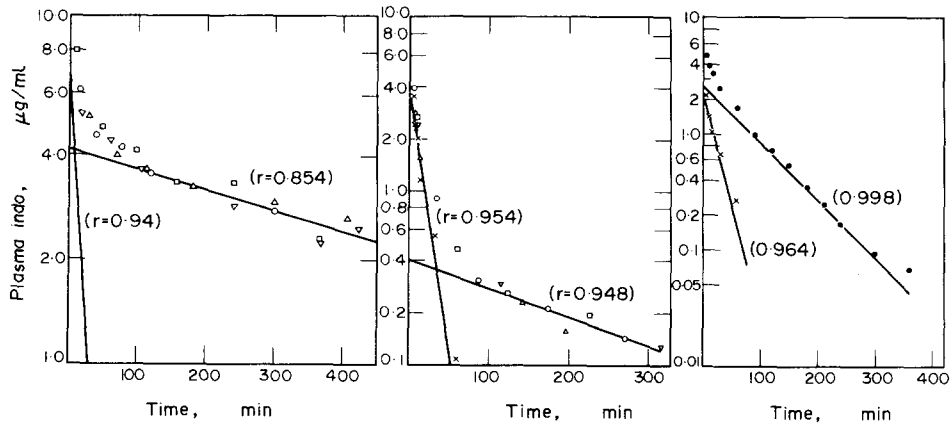

Fig. 1. Plamsa profiles for unchanged indomethacin after 1 mg/kg i.v. Left panel, group of 4 male Sprague–Dawley rats (180–200 g) were sampled alternatively via the ocular sinus; center panel, 4 guinea pigs (400–500 g); right panel male New Zealand rabbit (2·5 kg) was sampled via femoral catheter.

validity of using separate groups for determinations of plasma, bile and renal clearances is indicated.

The area under the plasma profile was uniquely high in the rat (3074 $\mu g \cdot min \cdot ml^{-1}$), and plasma clearance, the sum of all irreversible processes leading to loss, was concomitantly small at 0·32 $ml \cdot min^{-1} \cdot kg^{-1}$. The contribution of renal clearance to this total is negligible (0·008 $ml \cdot min^{-1} \cdot kg^{-1}$). The mean biliary clearance for 6 rats was $0{\cdot}39 \pm 0{\cdot}10$ $ml \cdot min^{-1} \cdot kg^{-1}$, a value which, by itself, exceeds plasma clearance. In view of the very substantial contribution of biotransformation to irreversible loss, only 7·8 per cent of dosage being excreted unchanged (Table 1), net biliary clearance in the intact animal must be far lower than that calculated in the bile-diverted animals; i.e. a major portion of secreted drug is reabsorbed.

The mean portal : cardiac plasma gradient for indomethacin in 8 rats sacrificed at 8 and 16 hr post drug was only $1{\cdot}15 \pm 0{\cdot}10$ (Table 2). This was not unexpected in light of the relatively low absolute value for $\dot{V}_{cl,b}$, and the lack of a gall bladder in this species, precluding large "pulses" of drug being delivered to the gut. Substitution of this value in equation 7 (see Appendix) and assuming $C_p^{hep} = C_p^{venous}$, for a dosage of 1·0 mg/kg, $\sum_{bile} = 1343$ $\mu g/kg$, or 134 per cent.

The rat, which has by far the lowest plasma clearance rate of the species examined, would be the most likely species to show a significant accumulation in plasma upon repeated dosage. Since the dosage required for intestinal lesions decreases greatly upon repeated daily dosage (12 mg/kg acutely; 2·5 mg/kg/day × 21 days), and since $\sum_{bile}^{\infty}$ is, in effect, a product of intensity and duration, it is vital to establish which of these components of total exposure is the more critical in determining toxicity. Accordingly, an attempt was made through computer simulations to determine the degree of drug accumulation on repeated daily dosage, assuming complete absorption (Fig. 2). It is apparent that a steady state is established within 3 days, with the incremental profile for any subsequent 24-hr period only 15 per cent higher than that following the first dosage. This is experimentally verified by comparisons of plasma and tissue levels after a first and third daily dosage (Fig. 2).

Guinea pig. The composite plasma profile for a group of 4 animals is presented in Fig. 1. Slopes for initial and terminal segments were 0·069 and 0·0038

Table 2. Species difference in total exposure resulting from biliary secretion of indomethacin*

Species	Clearance ($ml \cdot min^{-1} \cdot kg^{-1}$)			Area ($\mu g \cdot min \cdot ml^{-1}$)		Plasma gradient	Total exposure	Minimum toxic dosage (mg/kg/day)
	Plasma	Urine	Bile	Venous	Portal			
	$V_{cl,p}$	$V_{cl,r}$	$V_{cl,b}$	$\int_0^t C_p^{ven} \cdot dt$	$\int_0^t C_p^{port} \cdot dt$	C_p^{port}/C_p^{ven}	$\sum_{bile}^{\infty}$	
Dog	8·2	<0·1	13·3	122	310†	2·54†	362	0·5
Rat	0·32	0·01	0·39	3074	3535	1·15	134	0·75
Monkey	8·3	3·0	2·2	121	121	1·0	26	1·0
Guinea pig	6·25	1·85	1·20	158	181	1·15	21	6·0
Rabbit	3·62	1·09	0·40	278	334	1·20	13	20·0
Man	1·79	0·22	0·16‡	592	592	1·0§	9·5	

* All disposition data for single intravenous dosage of 1·0 mg/kg except man [5], for which 25 mg total dosage normalized to 1·0 mg/kg.

† Based upon complete 0–24 hr portal and systemic plasma profiles; for all other species, mean of > five measurements at interval specified in text.

‡ Calculated from $f_{bile} = 0{\cdot}09$ (H. B. Hucker, unpublished).

§ Assumed.

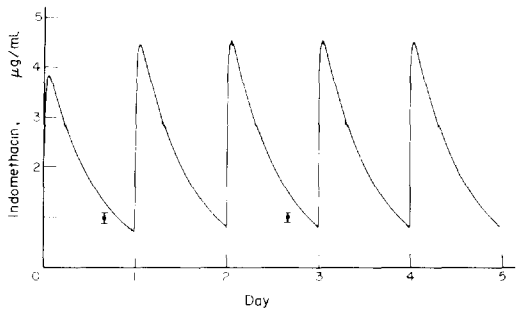

Fig. 2. Indomethacin content in the central (plasma) compartment of the rat on repeated oral administration. Line is a computer simulation; symbols are experimental values for systemic plasma.

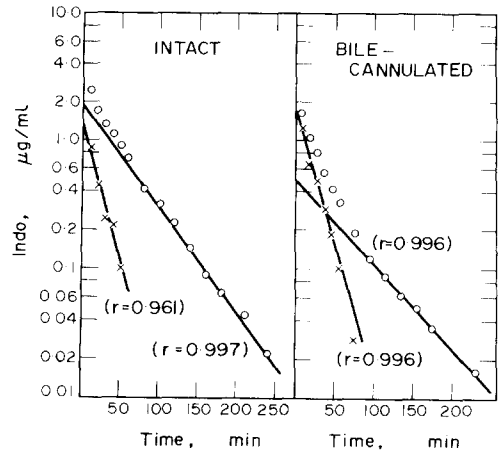

Fig. 3. Plasma profile for indomethacin in intact and bile-cannulated dog. A male beagle (8·5 kg) received indomethacin-^{14}C at 1·0 mg/kg i.v. and blood was sampled from the jugular vein at the intervals indicated (left panel). After a 10-day interval, the same animal, provided with a bile-duct cannula, received the same dosage and bile was collected through 4 hr.

min^{-1}, respectively, with excellent correlation coefficients for both regression lines. The area under the plasma curve, 158 $\mu g \cdot min \cdot ml^{-1}$, was only 1/20th that for the rat, and the plasma clearance was concomitantly higher at 6·3 $ml \cdot min^{-1} \cdot kg^{-1}$. Of this total, approximately 15 per cent is attributable to renal clearance (0·96 $ml \cdot min^{-1} \cdot kg^{-1}$), and an unknown but substantial portion is attributable to biotransformation, approximately 50 per cent of the dosage being excreted as metabolites in urine alone (Table 1) [3].

Biliary clearance of unchanged indomethacin plus conjugate, which together comprised *ca.* 55 per cent of total ^{14}C in bile, averaged $1{\cdot}2 \pm 0{\cdot}25$ $ml \cdot min^{-1} \cdot kg^{-1}$. The mean value for portal : peripheral ratio for indomethacin (I) in 6 animals was 1·15. Assuming that portal blood comprises 80 per cent of total blood supply to the liver, the product of biliary clearance and weighted mean for areas under the portal and systemic plasma profiles yields a value for $\sum_{bile}$ corresponding to 22 per cent of dosage.

Rabbit. While the area under the plasma profile for the rabbit (268 $\mu g \cdot min \cdot ml^{-1}$; Fig. 1) is small, biliary clearance (0·40 $ml \cdot min^{-1} \cdot kg^{-1}$) is lowest among the five species examined, and comparable to that in the rat. Unlike the rat, however, in which $\dot{V}_{cl,b}$ actually exceeds plasma clearance while metabolic clearance is high (Table 2), thus demanding an appreciable enterohepatic recirculation, bile clearance in the rabbit is only 11 per cent of plasma clearance. Combined f_u values for drug and metabolites at infinity are clearly in excess of 0·8 (extrapolation of total ^{14}C values; Table 1), accounting for almost all of the difference between plasma and biliary clearances. A very limited recirculation of drug in the rabbit is thus suggested on this basis alone. Based upon the product of biliary clearance and area under the plasma profile, adjusted for an observed portal : peripheral gradient of 1·20, a total exposure value of 13·4 per cent of dosage is obtained.

Dog. Paired plasma profiles for a single dog, intact and after 10 days, with bile collected, are depicted in Fig. 3, and are typical of three such experiments. The irreversible removal of bile has the effect of reducing the area under the plasma curve from a mean of 122 to 72 $\mu g \cdot min \cdot ml^{-1}$ and increasing plasma clearance from 8·2 to 13·6 $ml \cdot min^{-1} \cdot kg^{-1}$.

Biliary clearance is 13·3 $ml \cdot min^{-1} \cdot kg^{-1}$, which, when compared with a $\dot{V}_{cl,p}$ of 13·6, confirms the fact that renal clearance and metabolic clearance outside the hepatic portal system are negligibly small in the dog (Table 1). The substantial difference between biliary clearance in the bile-diverted dogs and plasma clearance in the intact animals (13·3 vs. 8·2) is indicative of extensive enterohepatic recycling in the intact animal. This is confirmed by plasma measurements in 3 dogs prepared with chronic portal cannulas, and ligation of the gallbladder, but with the biliary system otherwise intact. Areas under the portal plasma profile ranged from 241 to 344, with a mean of 310 ± 46 $\mu g \cdot min \cdot ml^{-1}$, i.e. a portal : peripheral gradient of 2·54. Again, assuming portal blood to constitute 80 per cent of total hepatic blood supply, the total exposure is calculated to be 362 per cent of dosage.

Monkey. Paired plasma profiles for unchanged I in a representative spider monkey, intact and with bile diverted, are presented in Fig. 4. In each of 3 animals, diversion of bile did not result in a significant change in plasma clearances, as was the case in dogs. Biliary clearance was 2·2 $ml \cdot min^{-1} \cdot kg^{-1}$

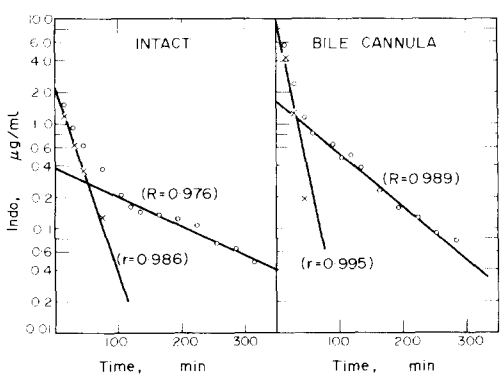

Fig. 4. Plasma profile for indomethacin in intact and bile-cannulated spider monkey. Experimental design and notation are as per Fig. 3, except dosage in the bile collection experiment was 3·0 mg/kg.

(about 25 per cent of plasma clearance in the intact animal; see Table 2).

These results, taken at face value, would indicate either: (1) a very limited enterohepatic recirculation, or (2) a recirculation of indefinite magnitude with a virtually complete confinement of reabsorbed drug to the portal circulation, which was not sampled in these animals.* The latter would appear to be precluded in view of the *ca.* 50 per cent bioavailability of orally administered indomethacin relative to i.v. dosage (see Table 8 in Yesair *et al.* [14]). Rather, the lack of effect of bile diversion upon plasma clearance is consistent with the low value of $V_{cl,b}$ relative to $V_{cl,p}$. The monkey, then, more closely resembles the guinea pig and rabbit, in which metabolic clearance (Table 1) and renal clearance, rather than biliary clearance, account for the major part of total irreversible loss, and is qualitatively different from the rat and dog, in both of which biliary clearance exceeds total irreversible loss.

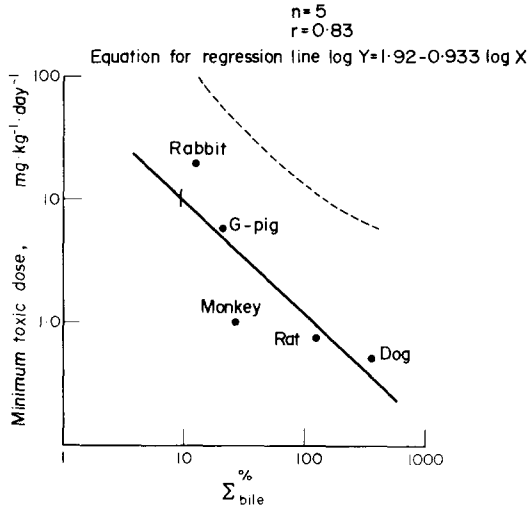

Fig. 5. Correlation of total biliary secretion of indomethacin with sensitivity to intestinal lesions. Dotted line indicates 95 per cent confidence limit.

DISCUSSION

Available evidence is consistent with the assumption, inherent in the present study, that unchanged indomethacin and/or its conjugate(s) as secreted in bile is the primary causative factor for observed intestinal lesions. Thus, intestinal sensitivity to orally administered drug is virtually abolished by bile duct ligation and greatly reduced upon fasting, which decreases bile flow [2]. Pretreatment with phenobarbital or spironolactone, both of which induce *O*-demethylase activity and thus decrease the amount of drug secreted unchanged [7], markedly reduces gastrointestinal toxicity of indomethacin in the rat [2, 8]. The desmethyl and desbenzoyl metabolites of indomethacin are innocuous at dosages 10 times the ED_{50} for intestinal lesions of indomethacin (H. Jacoby, unpublished). Finally, those species in which biliary excretion, as measured acutely, is high relative to renal excretion of drug [3, 4] are the most sensitive to lesions.

Direct measurements of the recovery of a drug in bile for arbitrary intervals after administration, or kinetic extrapolation of such values to infinity can, at best, provide a qualitative index of the ultimate extent of biliary secretion in the normal physiological state, i.e. where enterohepatic recirculation is not impaired as a consequence of sampling. The degree of recirculation will obviously depend upon the efficiency of intestinal absorption of the chemical species in question, and where its hepatic excretion is in the form of a polar conjugate, upon the efficiency of appropriate hydrolytic systems in the gut to reconvert the conjugate to an absorbable form. In addition, irreversible biotransformation of the drug or its conjugates in either the gut wall or in intestinal contents can decrease the quantity of drug available for reabsorption. While each of these parameters might be estimated directly, their individual effects upon the net reabsorption of drug need not be known. Rather, the resulting portal and peripheral concentrations of drug, the relative contributions of those two sources to the bile-forming system, and bile clearance of the drug are the only values required for calculation of total recirculation through the bile (see Appendix).

This value, termed $\sum^{\%}_{bile}$, was found to vary approximately 30-fold among the five laboratory species examined, and is qualitatively an inverse monotonic function of the minimal toxic dosage of indomethacin. This quantity and $\sum^{\%}_{bile}$ are log-linearly related with a correlation coefficient of 0·83 (Fig. 5). $\sum^{\%}_{bile}$ thus appears to be a reasonable predictive of intestinal toxicity. Extrapolation of the regression line to the best available estimate for $\sum^{\%}_{bile}$ in man (9·5) suggests a minimal toxic dosage level of *ca.* 10 mg/kg/day. This approximation is consistent with clinical experience, in which the frequency of intestinal (as opposed to gastric) irritation is far below that which would be anticipated on the basis of gastrointestinal studies with dog or rat.

In the present context, the concept of total biliary secretion of drug has been determined for the specific purpose of establishing a correlate for an untoward effect upon one segment of the enterohepatic recirculation, viz. the proximal small intestine. The same basic interpretation of disposition kinetics should be of general utility in predicting intensity and/or duration of response to drugs whose major locus of action is the liver or the gut or both. A striking illustration of this is provided in the case of the xanthine oxidase inhibitor, 3-(4-pyrimidinyl)-5-(4-pyridyl)-1,2,4-traizole, whose prolonged duration of inhibitory effect *in vivo* is not consistent with its very short half-life in the peripheral circulation. Examination of bile and of portal blood revealed a massive enterohepatic recirculation, resulting in a highly disproportionate confinement of drug within the major sites of xanthine oxidase activity [9].

* In two intact monkeys sacrificed at 4 and 6 hr after intravenous dosage, the portal : femoral concentration gradient for free indomethacin was 1·0.

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APPENDIX

In general, the incremental biliary secretion of a drug, $\Delta B/\Delta t$, is related to its biliary clearance rate, $\mathring{V}_{cl,b}$, and its mean plasma concentration $\bar{C}_p$. Thus,

$$\mathring{V}_{cl,b} = \frac{\Delta B/\Delta t}{\bar{C}_p} \tag{1}$$

where ΔB is the amount secreted over the time interval Δt. Given due regard to possible discontinuities, according to the Law of the Mean,

$$\bar{C}_p = \frac{\int_{t_1}^{t_2} C_{p.dt}}{\Delta t} \tag{2}$$

Combining equations 1 and 2,

$$\Delta B = \mathring{V}_{cl,b} \int_{t_1}^{t_2} Cp \,.\, dt \tag{3}$$

i.e., the amount secreted over the interval t_1 to t_2, is the product of biliary clearance rate and incremental area under the plasma curve. Thus following a given dosage, D, total cumulative secretion $\sum_{\text{bile}}$, expressed as percentage of dosage, will be

$$\Sigma_{\text{bile}}^{\infty} = \frac{\mathring{V}_{cl,b} \int_0^{\infty} Cp \,.\, dt}{D} \times 100 \tag{4}$$

Since hepatic blood flow includes both hepatic arterial and portal venous blood, the effective plasma concentration of a drug to be cleared by the liver is the mean of its respective concentrations in these two compartments, weighted according to their respective flow rates, i.e.

$$C_p^{\text{eff.}} = \left(\frac{p}{h+p} \,.\, C_p^{\text{port}}\right) + \left(\frac{h}{h+p} \,.\, C_p^{\text{hep}}\right) \tag{5}$$

where h and p are fractions of hepatic blood flow deriving from the hepatic artery and the portal vein such that $h + p = 1$. For substances which are eliminated (metabolized, conjugated, excreted) by the liver only, C_p^{hep} will be equal to C_p^{ven} at any peripheral sampling site, and C_p^{port} will exceed C_p^{hep} by an increment related to gastrointestinal absorption. Where elimination is not confined to the liver, the approximation $C_p^{\text{hep}} = C_p^{\text{ven}}$ will be more or less affected by the extent of extrahepatic elimination and whether or not the eliminating organ(s) is interposed between the sampling site and the liver.

The effect of enterohepatic circulation on the relationship between C_p^{hep} and C_p^{port} will be less predictable if biotransformation occurs in the gut wall. Because the absence of enterohepatic circulation cannot be generally assumed, and because p is several times larger than h, C_p^{port} should be experimentally determined. In so doing, potential errors arising from biotransformations occurring in the circulating fluid will be minimized, since they are confined almost exclusively to the estimations of C_p^{hep}, whose product with p is the minor component of C_p^{eff}. Finally, since arterial plasma concentration is the same for all parts of the body [10], C_p^{hep} can be estimated by sampling from any artery. In practice, the more convenient approximation by C_p^{ven} is usually adopted.

The experimental determinations of $\mathring{V}_{cl,b}$, whether by the incremental method ($C_b \cdot \mathring{V}_b\, C_{p,m}$) or the integral method ($f_b \cdot \mathring{V}_b$), are based upon measurements obtained while bile is collected, and enterohepatic recirculation is thus circumvented. Under these special conditions, $C^{\text{port}} = C^{\text{ven}}$ and estimates $\mathring{V}_{cl,b}$ will be subject only to such error as might relate to extrahepatic elimination occurring between the sampling site and the liver.

Products of biotransformation may also be ulcerogenic and each to a different degree relative to the parent drug. Therefore, the total exposure of ulcerogenic substances from a given dose should, in general, be represented by their weighted sum:

$$\Sigma_{\text{bile}}^{\infty} = \frac{\Sigma_i^{Q} \cdot \mathring{V}_{cl,bi} \,.\, (h \,.\, \int_0^{\infty} C_p^{\text{hep}}\, dt + p \,.\, \int_0^{\infty} C_p^{\text{port}}\, dt)_i}{D(h+p)} \times 100 \tag{6}$$

where Q_i is the ulcerogenicity of the "ith" chemical moiety relative to the administered substance, the Q value for which is, by definition, equal to 1·0. It is evident that the application of equation 6 required knowledge of the biliary clearance rate and the weighted areas under the plasma concentration curve for each of the active substances and their relative activities.

In the case of indomethacin (I), the suspected ulcerogenic agents are I and its conjugate(s). Existing data in all animal species indicate that while I conjugates are secreted in bile in large quantities relative to I, they are readily hydrolyzed to I in the intestine [3]. Hence, Q for I conjugates may be taken to be 1·0, equal to that for I. Plasma concentrations of I conjugates are negligibly small in all species, precluding any possibility of a separate determination of $\sum_{\text{bile}}$. However, because of its ulcero-genicity, I conjugate contents in bile must be included in the determination of $\mathring{V}_{cl,b}$, which can be viewed as the net clearance of ulcerogenic substances through the bile resulting from the passage of I across the liver. Experimentally, plasma concentrations are estimated from serial samples taken from the portal and a peripheral vein. Hence, the working equation in the determination of total exposure following intravenous administration of I is:

$$\Sigma_{\text{bile}}^{\infty} = \frac{\mathring{V}_{cl,b}[h \int_0^{\infty} C_p^{\text{ven}}\, dt + p \int_0^{\infty} C_p^{\text{port}}\, dt]}{D(h+p)} \times 100 \tag{7}$$

Values of $h = 0{\cdot}2$ and $p = 0{\cdot}8$ have been reported for the dog [11] and, in the absence of more definitive data, have been assumed to apply for all species, including man.