(*S*)-(−)-propranolol is entirely cleared by the liver. The value of $CL_{u,int}$ for each model was estimated from Eqs. 1 and 2 using the (*S*)-(−)-propranolol clearance of 6.61 mL/min/100 g observed following intravenous administration 1 d after catheter insertion. Q was calculated using this value for clearance and an extraction ratio of 0.959. Table I lists the predictions and compares the ratio of predicted or observed values at the time of increased binding to the original observation. As expected, significant differences between the models were observed with the venous equilibrium equations predicting 2.17- and 2.28-fold increases in F and AUC, respectively, given the observed binding change, while the sinusoidal model predicted 6.00- and 7.63-fold increases in these parameters. Clearly, the predictions of the venous equilibrium model more closely approximate the values observed by Terao and Shen (11). In fact, there is remarkably good agreement with only 14.1 and 13.4% error in the predictions of F and AUC, respectively, using this model.

Although this data is limited to observations in one species with a single substrate, it does provide *in vivo* evidence in support of the venous equilibrium model for hepatic clearance. This is important information since there are many clinical situations in which liver blood flow, protein binding, or intrinsic drug clearance may be altered. Knowledge of the appropriate physical model for hepatic clearance allows one to predict with reasonable accuracy the effect of changes in these factors on important pharmacokinetic parameters such as F and AUC. This is particularly crucial when highly extracted compounds are administered orally. Prospective studies examining this issue further with other highly extracted drugs are needed in order to confirm that the venous equilibrium model is indeed the preferred physical model for hepatic drug clearance.

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Method for Determination of First-Pass Metabolism in Human Skin

Keyphrases □ Metabolism, first-pass—transdermal formulations, human skin □ Transdermal formulations—percutaneous first-pass metabolism, human skin □ Viprostol—transdermal formulations, first-pass metabolism

To the Editor:

The technological advances in drug-delivery systems have allowed increased utilization of transdermal formulations for therapeutic purposes. This route of administration is most suitable for potent drugs possessing short biological half-lives, extensive first-pass metabolism after oral administration, and narrow therapeutic indices. By controlling the rate of delivery and absorption through the skin, it is possible to maintain therapeutic drug concentrations in the blood and/or at the site of action. The systemic blood concentrations of such compounds after transdermal administration in humans is very low and often below the sensitivity levels of specific assay procedures. Nitroglycerin, which undergoes extensive first-pass metabolism when given orally, is one of the most widely used drugs in transdermal systems. To circumvent the assay sensitivity problem and allow for a comparison of dosage forms, Karim (1) has proposed, and effectively implemented, blood sampling from the ipsilateral antecubital forearm veins after application of the topical formulation on the volar surface of the wrist. The purpose of this communication is to describe a similar procedure that can also estimate the percutaneous first-pass metabolism of topically applied compounds, even when extensive biotransformation takes place in the skin.

Drug and/or drug and metabolite (*i.e.*, total) concentrations in samples from the contralateral antecubital forearm will be low and represent the systemic blood concentrations (C_c). The ipsilateral samples will contain higher blood concentrations (C_i), and represent the concentration of the drug absorbed plus C_c at any given time. After correction for C_c, the ipsilateral concentrations will directly reflect the flux of the drug (C_j) through the skin; *i.e.*,

$$C_j = C_i - C_c \qquad \text{(Eq. 1)}$$

where $C_i = C_{p,j} + C_{m,j} + C_{p,c} + C_{m,c}$, $C_c = C_{p,c} + C_{m,c}$, and $C_j = C_{p,j} + C_{m,j}$. In this case C_p is the concentration of the unchanged drug and C_m is the total concentration of metabolites.

In the absence of any metabolism and/or degradation of the drug during the percutaneous absorption process, C_j will consist of the intact drug only, *i.e.*, $C_j = C_{p,j}$. If during the absorption processes the drug undergoes metabolism, C_j will consist of the concentrations of the drug ($C_{p,j}$) and metabolite(s) ($C_{m,j}$) in the ipsilateral samples at any time. In such a situation the magnitude of the first-pass metabolism can be estimated by calculating the fractions of unchanged drug and metabolite(s). Biotransformation and/or binding in the vein from the absorption site (the skin) up to the point of blood sampling would be accounted for but not differentiated from metabolism in the skin. The same is true if degradation processes other than biotransformation were involved.

When the rate of absorption through the skin is constant, and C_j reaches steady-state level, the fraction metabolized in the skin (f_s) can be estimated by:

$$f_s = \frac{C_{m,j}}{C_{p,j} + C_{m,j}} \qquad \text{(Eq. 2)}$$

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Table I—Concentrations of II ($C_{p,i}$) and Total Radioactivity in the Ipsilateral (C_i) and Contralateral (C_c) Plasma Samples After Application of a 1.5-mg Dose on the Forearm of Two Male Subjects

Hours of Application	Subject 1 Concentration, ng/mL					Subject 2 Concentration, ng/mL				
	C_c	C_i	$C_{p,i}$	$C_{m,i}$	f_s[a]	C_c	C_i	$C_{p,i}$	$C_{m,i}$	f_s[a]
2	—	18.2	5.8	12.4	0.68	—	2.8	1.2	1.6	0.57
4	0.48	14.7	4.6	10.1	0.69	0.43	4.8	1.4	3.4	0.71
6	0.65[b]	19.3	8.0	11.3	0.59	0.52[b]	11.7	3.7	8.0	0.68
8	0.85	31.5	10.0	21.5	0.68	0.62	11.1	3.2	7.9	0.71
12	0.52	27.8	9.8	18.0	0.65	0.57	14.8	3.2	11.6	0.78
16	—	6.7	2.7	4.0	0.60	—	10.8	2.7	8.1	0.75
Mean					0.65					0.70
CV					6.2%					10%

[a] $f_s = C_{m,j}/C_j$, where $C_j = C_i - C_c$ and $C_{m,j} = C_j - C_{p,j}$. Because $C_{p,i} = C_{p,j} + C_{p,c}$ and the values of $C_{p,c}$ are below the detectable level of 0.05 ng/mL, $C_{p,j}$ was assumed to be essentially the same as $C_{p,i}$ as presented in this table. [b] Interpolated values.

where $C_{p,j}$ and $C_{m,j}$ are concentrations of the drug absorbed either unchanged or as a metabolite(s), respectively. Assuming that (*a*) skin metabolism is much higher than the metabolism in other tissue up to the point of ipsilateral sampling and (*b*) there is no preferential tissue binding of the drug or metabolites, the areas under the plasma concentrations *versus* time profiles of the drug ($AUC_{p,j}$) and metabolite ($AUC_{m,j}$) will be independent of the distribution volume, and the summation of the two values will represent the area under the C_j *versus* time profile (AUC_j). Therefore, f_s can also be calculated by:

$$f_s = \frac{AUC_{m,j}}{AUC_{p,j} + AUC_{m,j}} \quad \text{(Eq. 3)}$$

or:

$$f_s = \frac{AUC_{m,j}}{AUC_j} \quad \text{(Eq. 4)}$$

as by definition $AUC_j = AUC_{p,j} + AUC_{m,j}$.

Methyl (±)-(11α,5*Z*(and 5*E*),13*E*,16*R*(and 16*S*)-16-ethenyl-11,16-dihydroxy-9-oxoprosta-5,13-dien-1-oate (viprostol; I), a prostaglandin analogue, is a topically effective antihypertensive agent (2). In the blood and body it apparently undergoes instantaneous and complete ester hydrolysis to give an equally effective compound, (±)-(11α,5*Z*(and 5*E*), 13*E*,16*R*(and 16*S*))-16-ethenyl-11,16-dihydroxy-9-oxoprosta-5,13-dien-1-oic acid (II). The transdermal administration of [^{14}C]I has shown good absorption in various animal models, as measured by total concentrations of carbon-14 (3). The low systemic concentrations (contralateral) of I or II, require a sophisticated GC–MS technique for quantitation (4). In humans, effective doses of I result in low concentrations of II (not I) in the systemic circulation, often below the sensitivity limit of the GC–MS procedure (76 pg/mL). To study the transdermal absorption, 1.5 mg (specific activity 18.99 μCi/mg) of [^{14}C]I in petrolatum bandages[1] (10 cm^2) was applied to the volar surface of the forearm of normal male subjects, and serial ipsilateral and contralateral blood samples were collected into heparinized tubes. Following centrifugation, the plasma was removed and then stored at −40°C until assay. Total radioactivity in each sample was determined by scintillation counting. The concentration of the unchanged drug (II) in the ipsilateral plasma samples were determined by the specific GC–MS assay procedure (4). Because of the rapid hydrolysis of I to II, it was only possible to measure the concentrations of II by GC–MS, and II is considered to be the unchanged active drug ($C_{p,i}$). Therefore, all calculations were done for II.

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Table I summarizes the concentrations of II ($C_{p,i}$) and total radioactivity in the ipsilateral ($C_i = C_{p,i} + C_{m,i}$) and contralateral (C_c) plasma samples after application of a 1.5-mg dose on the forearm of two subjects, randomly selected for this report. In general, C_c was relatively low, <1 ng/mL of which, on the average, <10% (~0.05 ng/mL) was in the form of unchanged II and would not be readily measurable by the GC–MS procedure. C_i, of which 35 and 30% was in the form of unchanged II in subjects 1 and 2, respectively, was quite high. These data indicate that about two-thirds of the dose underwent metabolism during absorption from the topical application site and/or in transit to the point of blood sampling in the same arm. As part of a pharmacokinetic study of topical and intravenous [^{14}C]nitroglycerin, Wester *et al.* were able to measure the absolute bioavailability (57%) and estimate the percutaneous first-pass metabolism (16–21%) in monkeys (5). Basically, the procedure is the same as that used for determination of first-pass metabolism after oral administration, *i.e.*, utilizing the difference between the total radioactivity and unchanged nitroglycerin which reached the systemic circulation. The ipsilateral–contralateral method offers the possibility of determining the percutaneous metabolism after one application in the same subject at the same time.

In summary, the results of this study suggests that this simple procedure may be used for the estimation of percutaneous first-pass metabolism of drugs which are administered transdermally and where the skin is a significant site of biotransformation. It would also be of interest to examine this procedure for percutaneous metabolism of other potent drugs which undergo metabolism in the skin.

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