Title

An Explanation of Why Dose-Corrected Area Under the Curve for Alternate Administration Routes Can Be Greater than for Intravenous Dosing

Journal

The AAPS Journal, 26(1)

ISSN

1522-1059

Authors

Wakuda, Hirokazu
Xiang, Yue
Sodhi, Jasleen K
et al.

Publication Date

2024

DOI

10.1208/s12248-024-00887-w

The Explanation of Why Dose Corrected Area Under the Curve for Alternate Administration Routes Can Be Greater than For Intravenous Dosing[a]

Hirokazu Wakuda[1,2], Yue Xiang[1], Jasleen K. Sodhi[1,3], Naoto Uemura[2], Leslie Z. Benet[1,*]

[a]Dedicated to Professor David E. Smith, a former Benet Group member, on the occasion of his retirement from the University of Michigan and in recognition of his outstanding accomplishments in over 40 years of groundbreaking research.

*Correspondence: Leslie Z. Benet (Leslie.Benet@ucsf.edu)

[1]Department of Bioengineering and Therapeutic Sciences, Schools of Pharmacy and Medicine, University of California San Francisco, San Francisco, California 94143-0912, USA

[2]Department of Clinical Pharmacology and Therapeutics, School of Medicine, Oita University, 1-1 Idai gaoka, Hasama-machi, Yufu City, Oita 879-5593, Japan

[3]Department of Drug Metabolism and Pharmacokinetics, Septerna, South San Francisco, CA, 94080, USA

ABSTRACT

Purpose It is generally believed that bioavailability (F) calculated based on systemic concentration area under the curve (AUC) measurements cannot exceed 1.0, yet many published studies report this inconsistency. We teach and believe, based on differential equation derivations, that rate of absorption has no influence on measured systemic clearance following an oral dose, i.e., determined as available dose divided by AUC. Previously, it was thought that any difference in calculating F from urine data versus that from systemic concentration AUC data was due to the inability to accurately measure urine data.

Methods A PubMed literature search for drugs exhibiting F>1.0 and studies for which F was measured using both AUC and urinary excretion dose corrected analyses yielded data for 35 drugs.

Results We show and explain, using Kirchhoff's Laws, that these universally held concepts concerning bioavailability may not be valid. Bioavailability, determined using systemic concentration measurements, for many drugs may be overestimated since AUC reflects not only systemic elimination but also absorption rate characteristics, which is most easily seen for renal clearance measures. Clearance of drug from the absorption site must be significantly greater than clearance following an iv bolus dose for F(AUC) to correctly correspond with F(urine).

Conclusions The primary purpose of this paper is to demonstrate that studies resulting in F>1.0 and/or greater systemic vs urine bioavailability predictions may be accurate. Importantly, these explications have no significant impact on current regulatory guidance for bioequivalence testing, nor on the use of exposure (AUC)

measures in making drug dosing decisions.

KEY WORDS bioavailability, Kirchhoff's Laws, systemic concentrations, urinary excretion

ABBREVIATIONS

$A_{systemic\ circulation}$	Amount of drug in the systemic fluids
ACE	Angiotensin converting enzyme
AUC	Area under the systemic concentration time-curve
BCS	Biopharmaceutics classification system
BDDCS	Biopharmaceutics drug disposition classification system
C_{max}	Maximum systemic drug concentration
$C_{systemic\ circulation}$	Concentration of drug in the systemic circulation
CL	Clearance
CL_{gut}	Clearance of drug from the gut following oral dosing
CL_H	Hepatic blood clearance
CL_R	Renal blood clearance
F	Bioavailability
F(AUC)	Bioavailability calculated from systemic concentrations
F(urine)	Bioavailability calculated from urinary excretion amounts
IM	Intramuscular
iv	Intravenous
k	Rate constant
k_a	Absorption rate constant
k_d	Elimination rate constant for a one compartment body model
Q_H	Hepatic blood flow
SubQ	Subcutaneous
U_∞	Amount of drug in the urine at infinite time
V	Systemic volume of distribution

x Subscript reflecting parameter for either oral, intramuscular or subcutaneous dosing

INTRODUCTION

In Wagner's 1981 comprehensive review of the "History of Pharmacokinetics" (1), it appears that analysis of concentration-time curves following oral dosing and considerations of bioavailability began in the 1940's. Those early analyses and all subsequent analyses until today have derived the concepts in terms of amounts and rate constants utilizing differential equations, which is appropriate in the field of Chemistry. But since following human dosing we measure drug concentrations and define elimination processes in terms of clearance values, the differential equation derivations and their solutions are divided by a volume of distribution. Then it is possible to define the clearance by measuring the amount eliminated divided by the exposure driving that elimination, as we recently reviewed (2). Our laboratory has emphasized the possibility that the approach followed in chemistry, in terms of rate constants and differential equations for disposition processes in a fixed volume of fluid, may not be consistent with the pharmacokinetic, clearance and varying volumes of distribution, approach (3, 4). Frequent peer reviewed published manuscripts report drug bioavailabilities for differing routes of drug dosing compared to intravenous bolus doses that exceed 100%. In Table I we cite thirty-five such outcomes following crossover studies in which humans and various animal species received a drug both by intravenous dosing and via either oral, subcutaneous (SubQ) or intramuscular (IM) dosing, where the reported ratio of dose corrected areas under the systemic concentration-time curves (AUC_x/AUC_{iv}) exceeded 1.02. Seventeen of these thirty-five studies were in humans.

Another series of unexplained data pertains to the differences observed in dose corrected oral bioavailability when comparing measures of AUC to measures of amount of drug excreted unchanged in the urine. Eleven such peer reviewed human studies are referenced in Table II, including three of the studies in Table I. Since the measured outcomes in Tables I and II are not consistent with the universally accepted or FDA guidance methods for calculating bioavailability, these data were generally ignored and believed to result from experimental errors. We will revisit these data in the Discussion section.

METHODS AND RESULTS

Literature search

The literature search for the data listed in Tables I and II was a very laborious task as we were searching for published results that are contrary to generally accepted pharmacokinetic theory, as bioavailability measurements greater than unity have typically been attributed to some form of experimental error. Information as to this outcome is rarely, if ever, included in article titles or abstracts. In essence, we searched for any peer reviewed bioavailability study in humans and animals. Specifically, we focused on studies where oral, SubQ or IM dosing was reported alongside iv dosing to determine if the published data had values of dose corrected *AUC* greater than 1.0 and/or where *F* calculated using urinary measurements was lower than that obtained through systemic concentration measurements. We put no period of time restriction on studies to evaluate and as can be seen, the cited references range from 1976 to 2022. The only relevant search term was "bioavailability", but as can be seen in the 32 citations in Tables I and II, one-third were not found using this search term. It should not be concluded that since we found so few studies meeting our criteria that this means the phenomena occurs to a negligible extent, since investigators both in academia and the industry are reluctant to publish results for bioavailability studies that are contrary to accepted theory and thus will be believed by editors, journal reviewers and readers, and even the investigators themselves, to be scientifically flawed.

Application of Kirchhoff's Laws to eliminate the need for solving differential equations.

We recently demonstrated (4), using Kirchhoff's Laws from physics, that overall rate constants for a linear kinetic process or overall clearance for that process can be directly derived without the need to use differential equations. As we first reported (3), the application of Kirchhoff's Laws to clearance can be summarized in Eq. 1 for parallel processes and Eq. 2 for processes in series.

$$CL_{total} = CL_{rate\ defining \| process\ 1} + CL_{rate\ defining \| process\ 2} + \ldots \quad (1)$$

$$\frac{1}{CL_{total}} = \frac{1}{CL_{rate\ defining \in series\ process\ 1}} + \frac{1}{CL_{rate\ defining \in series\ process\ 2}} + \ldots \quad (2)$$

Kirchhoff's Laws may also be applied to rate constants and can be derived via Eqs. 3 and 4, independent of solving differential equations for first order processes.

$$k_{total} = k_{rate\ defining \| process\ 1} + k_{rate\ defining \| process\ 2} + \ldots \quad (3)$$

$$\frac{1}{k_{total}} = \frac{1}{k_{rate\ defining \in series\ process\ 1}} + \frac{1}{k_{rate\ defining \in series\ process\ 2}} + \ldots \quad (4)$$

A rate defining process is defined by a parameter that describes an elimination or movement process for which it is possible under certain conditions that the total

clearance or total rate constant may be equal to this parameter. For example, a rate defining clearance process for hepatic elimination could be hepatic blood flow, i.e., the rate at which drug arrives to the liver is the maximum value that hepatic elimination can be. Thus, for a very high hepatic clearance (CL_H) drug, total CL_H could equal hepatic blood flow (Q_H). To exemplify a rate defining rate constant process, for a series of chemical reactions occurring in a beaker, the elimination rate constant for the parent drug could be the minimum value rate defining process for all subsequent metabolic steps. For example, if the first step in a metabolic elimination process is very slow, the observed rate constant for the subsequent metabolic steps will be that initial rate constant for the metabolism of the parent drug. Understanding this definition is essential in applying Kirchhoff's Laws. The critical aspect of this approach is that only rate defining processes can be combined to determine the overall rate constant for elimination or clearance following Kirchhoff's Laws. Passive permeability, no matter how slow, cannot be a rate defining process for elimination because passive permeability is reversible, i.e., clearance and elimination rate will never be equal to passive permeability. When hepatic basolateral transporters affect permeability and active influx is greater than active efflux, this can be a rate defining process. But not when active efflux is greater than active influx. That is, clearance can never be defined singly as active large efflux minus smaller active influx since the value is negative.

Examples of parallel rate defining processes in the kidney are glomerular filtration and secretion/reabsorption, and in the liver are metabolism and biliary excretion (3). Examples of in series rate defining processes in the kidney and liver are organ blood flow limiting the rate of elimination, so that the actually eliminating mechanism has no effect on the measured rate (4). Of particular relevance in this publication are the in series processes of absorption and elimination, where absorption rate or absorption clearance from the gut (as well as from an injection site whether it is IM or SubQ) can have an effect on the overall elimination process.

Kirchhoff's Laws derivations of overall clearance and overall rate of elimination following oral dosing

Clearance derivation: As we recently presented (4), in series absorption clearance processes can be derived in terms of clearance entering into the systemic circulation and clearance leaving from the systemic circulation.

$$\frac{1}{CL_{after\ oral\ dosing}} = \frac{1}{CL_{entering}} + \frac{1}{CL_{leaving}} = \frac{1}{CL_{gut}} + \frac{1}{CL_{iv\ dosing}}$$

(5)

where the $CL_{entering}$ is the clearance of drug from gut, a parameter previously unmeasured in pharmacokinetics, but can be simply envisioned as the absorption rate constant, k_a, multiplied by the volume of distribution of the gut compartment, again a parameter previously unmeasured in pharmacokinetics. Since overall clearance is defined as the amount eliminated from the systemic fluids

(bioavailability, F, multiplied by the administered dose, $Dose_{oral}$), divided by the area under the systemic concentration time curve over all time, ($AUC_{0\rightarrow\infty}$), solving Eq. 5 yields

$$CL_{after\ oral\ dosing} = \frac{F \cdot Dose_{oral}}{AUC_{0\rightarrow\infty}} = \frac{CL_{gut} \cdot CL_{iv\,dose}}{CL_{gut} + CL_{iv\,dose}} = \frac{CL_{iv\,dose}}{1 + \frac{CL_{iv\,dose}}{CL_{gut}}}$$

(6)

There are important implications to Eq. 6. First, Eq. 6 demonstrates that the clearance measured after oral dosing will not be the clearance after intravenous dosing unless $CL_{gut} \gg CL_{iv\,dose}$, which may often be true but just as likely not true. Certainly, for flip-flop models (38), where the rate of absorption is slower than the overall rate of elimination following an iv dose, we would expect CL_{gut} to be less than $CL_{iv\ dose}$. However, even when the rate constant for absorption is greater than the overall rate constant for elimination, we are comparing clearances, not rate constants in Eq. 6, and one would expect the volume of distribution of drug in the gut to be less than the systemic volume of distribution, so that CL_{gut} may not be markedly greater than $CL_{iv\ dose}$. Thus, the measured clearance ($\frac{F \cdot Dose_{oral}}{AUC_{0\rightarrow\infty}}$) following an oral dose may not be the clearance following an iv dose as we have universally believed until now, by increasing the measured AUC beyond what results from an equivalent iv dose.

Rate constant derivation: Kirchhoff's Laws may also be used to calculate the overall rate of elimination for these in series processes.

$$\frac{1}{k_{overall\,measured\,rate}} = \frac{1}{k_{entering}} + \frac{1}{k_{leaving}} = \frac{1}{k_a} + \frac{1}{k_{iv\,dose}}$$

(7)

where $k_{iv\ dose}$ would be equal to the rate constant for elimination for a 1-compartment body model (k_d) or for a multi-compartment body model equal to the inverse of the mean residence time following an iv bolus dose, which we can designate as k_{ss}. Solving Eq. 7 for a 1-compartment body model gives

$$k_{overall\,measured\,rate} = \frac{k_a \cdot k_d}{k_a + k_d} = \frac{k_d}{1 + \frac{k_d}{k_a}}$$

(8)

As we reported (4), applying Kirchhoff's Laws to rate constants related to oral absorption is a process that our field has been following for the past 40 years since Yamaoka et al. (39) recognized that the absorption-disposition model could be described by mean residence time concepts. This is true, since mean residence times for each of the processes are the inverse of a rate constant describing that process, i.e., Eq. 7. Thus, it is difficult to argue against the assertion that Kirchhoff's

Laws will correctly describe linear in series pharmacokinetic processes. Mean residence time concepts, well accepted in pharmacokinetics, are the scientific justification for using Kirchhoff's Laws. Our advance is showing that Kirchhoff's Laws can be applied to clearance parameters, just as they are applied to rate constants (3, 4). And it is easy to see that converting Eqs. 7 and 8 to clearance relationships, Eqs. 5 and 6, requires multiplying k_d and k_a by two different volume terms, the systemic volume of distribution for k_d and gut volume of distribution for k_a. This further justifies why the differential equation derivation for oral (IM and SubQ) absorption following measurements of systemic concentration is not valid, although we have taught and believe it to be true for as long as our field has analyzed concentration-time data following oral absorption since only one volume term can be inserted into the rate constant-amount differential equations to convert the rate of change of amount to the rate of change of concentration.

The error in the use of a differential equation derivation to determine systemic clearance following oral absorption

Since this is such an important aspect in understanding the data in Tables I and II, we repeat the common derivation followed for the past 50 years (4). The amount of drug in the systemic circulation as a function of time in terms of the rate constants utilized above is given by Eq. 9

$$A_{systemic\,circulation} = \frac{k_a \cdot F \cdot Dose_{oral}}{k_a - k_d} \cdot \left(e^{-k_d \cdot t} - e^{-k_a \cdot t} \right) \quad (9)$$

Dividing by the systemic volume of distribution (V) gives the concentration-time relationship

$$C_{systemic\,circulation} = \frac{k_a \cdot F \cdot Dose_{oral}}{(k_a - k_d) \cdot V} \cdot \left(e^{-k_d \cdot t} - e^{-k_a \cdot t} \right) \quad (10)$$

Then integrating over all time allows determination of $AUC_{0\to\infty}$

$$AUC_{0\to\infty} = \frac{\frac{k_a \cdot F \cdot Dose_{oral}}{(k_a - k_d) \cdot V}}{k_d} - \frac{\frac{k_a \cdot F \cdot Dose_{oral}}{(k_a - k_d) \cdot V}}{k_a} = \frac{\frac{k_a \cdot F \cdot Dose_{oral}}{V}}{k_a \cdot k_d} = \frac{F \cdot Dose_{oral}}{k_d \cdot V} \quad (11)$$

Therefore, $$CL_{after\,oral\,dosing\,(differential\,equations)} = \frac{F \cdot Dose_{oral}}{AUC_{0\to\infty}} = k_d \cdot V = CL_{iv\,bolus} \quad (12)$$

where our field implicitly believes, slow absorption rate can markedly affect the overall rate of elimination (Eq. 8), but it does not affect the clearance of elimination (Eq. 12). We do recognize that prior to examination of the Kirchhoff's Laws derivations for clearance and rate constants, only differential equation derivations

were possible, thus ignoring any potential difference in volumes of distribution that influence differing processes.

Measurements of renal clearance following oral and intravenous dosing

It is generally believed today that renal clearance will be unchanged following oral and intravenous dosing, just as it is presently believed that input rate following oral, IM and SubQ dosing will have no effect on the area under the systemic concentration-time curve. The advantage of examining this belief with respect to renal clearance is that the calculation of CL_R is not based on any assumptions, just that renal clearance is equal to the measured amount of drug eliminated unchanged in the urine divided by the measured systemic exposure driving that elimination ($AUC_{0\rightarrow\infty}$). In contrast, F in Eqs. 9-12 is not a measured value, but rather a calculated value. Thus, if input does increase the systemic concentration time-curve, then the renal clearance following oral, IM and SubQ dosing should be less than the renal clearance following iv dosing.

Such analyses, not previously considered, are possible for the 1-deamino-8-arginine vasopressin (8), sodium fluoride (18) and cimetidine (21) studies. For each of the 16 cimetidine oral dosings in 9 subjects (21), it is possible to compare these renal clearances, which average 21.6 ± 10.6 L/hr following oral dosing and 35.6 ± 10.0 L/hr following iv dosing (paired t-test for the oral – iv difference yielded a p value of 0.0018). When renal clearances of the 10 paired dosings of sodium fluoride in the 6 subjects (18) were analyzed the renal clearance iv averaged 70.2 ± 16.6 ml/min and the renal clearance oral averaged 53.8 ± 16.3 (paired t—test for the oral-iv difference yielded a p value of 0.007). For 1-deamino-8-arginine vasopressin the authors try to explain the high AUC ratio based on adsorption of the drug to the syringe following iv dosing, but CL_R following SubQ is 76% of CL_R following iv (the same percentage decrease found in the sodium fluoride study), which is independent of adsorption. Statistical comparison was not possible.

Justification for dose corrected $\mathbf{AUC_x/AUC_{iv} > 1.0}$ and $\mathbf{AUC_x/AUC_{iv} > U_{\infty x}/U_{\infty iv}}$

Unless clearance from an absorption site (oral, IM, SubQ) is significantly greater than clearance following an intravenous dose of drug (Eq. 6 for oral dosing), the AUC following absorption dosing may be greater than the AUC for an intravenous comparable available dose. When bioavailability is calculated based on systemic concentration measurements, this explains why experimental AUC_x/AUC_{iv} values can often be greater than 1.0 as shown in Table I**.** On the other hand, when bioavailability is calculated using measures of unchanged drug in the urine there are no assumptions being made relating absorption processes to elimination processes, therefore if absorption affects systemic concentrations, AUC_x/AUC_{iv} will be greater than $U_{\infty x}/U_{\infty iv}$ independent of the F value calculated. A potential challenge to accurately calculating bioavailability using urinary data is the fact that

the major route of elimination for Biopharmaceutics Classification System (BCS) and Biopharmaceutical Drug Disposition Classification System (BDDCS) Class 1 and 2 drugs is metabolic elimination. Since measures of unchanged drug will be small, inherent variability will make these comparisons difficult to interpret. As we have reported (40), Class 1 and 2 drugs comprise approximately 70% of all marketed small molecules, making bioavailability measurements using unchanged drug in the urine questionable.

Calculation of CL_{gut} when both systemic concentrations and urinary elimination of unchanged drug measures for oral and iv dosing are available.

As indicated in Eq. 6, $CL_{after\ oral\ dosing}$ will be equal to CL_{iv}, as is now universally believed and taught, only if CL_{gut} is markedly greater than CL_{iv}. Thus, for the data in Table II it is possible to estimate CL_{gut} and the ratio CL_{iv}/CL_{gut} if CL_{iv} is available in the publication. Such calculations could be made for all of the studies listed in Table II, except for cilazapril and cilazaprilat. These results are presented in Table III. We demonstrate here this methodology for the cimetidine study (21), where the dose corrected *AUC* ratio exceeds 1.0 and the urinary and systemic concentration measurements of bioavailability are statistically different. From the published study the mean values of $U_{\infty oral}/U_{\infty iv}$, $\frac{Dose_{oral}}{AUC_{0\rightarrow\infty\ oral}}$ and CL_{iv} were available for the 16 dosings in 9 heathy volunteers as given in columns 2, 3 and 5, respectively, of Table III. Column 4, $CL_{after\ oral\ dosing}$ is the product of the model independent urinary measure of bioavailability, column 2, and $\frac{Dose_{oral}}{AUC_{0\rightarrow\infty\ oral}}$, column 3. Then CL_{gut}, column 6, is calculated from rearrangement of Eq. 6

$$CL_{gut} = \frac{CL_{iv}}{\frac{CL_{iv}}{CL_{after\ oral\ dosing}} - 1} \quad \text{(6a)}$$

The last column of Table III then gives the ratio of CL_{iv}/CL_{gut}, which for cimetidine is 1.6. It is unfortunate that this analysis could not be conducted for cilazapril, which showed the greatest statistical difference between the bioavailability measurements using systemic concentrations and urinary bioavailability measurements in Table II, but the reported bioavailability comparisons only used measurements out to 24 hr, without providing the potential extrapolated areas (35).

DISCUSSION

The general approach of our field to the many values presented in Tables I and II is to assume that the measurements are a function of experimental errors, or when

the values are divergent but so close to an expected outcome that the divergence is just due to inherent variability. And in many cases, this could be true. In fact, it is often very hard to justify that experimental errors have been made, with the major published justification being that these reported outcomes are not consistent with present universally accepted pharmacokinetic theory. Examination of the studies for sodium fluoride (18) and cimetidine (21) listed in both Tables I and II reflect the variance found in the literature in discussing the reported results. The data in the crossover studies reported in Table II offer a unique perspective, i.e., the ability to compare paired statistical analyses for two different measurements for the same study. The authors of neither study conducted this analysis, but since individual data for the study subjects were presented in the papers, we were able to conduct this analysis. In the cimetidine study, the paired p statistic between AUC_{oral}/AUC_{iv} and $U_{\infty oral}/U_{\infty iv}$ was less than 0.001, while the sodium fluoride study was close, but not statistically different ($p = 0.055$). Both sodium fluoride and cimetidine are BCS/BDDCS class 3 drugs (40) so sufficient amounts of drug in the urine were measurable. The authors of the sodium fluoride study (18) summarized their results in the abstract as: “There were large day-to-day variances in renal clearance of fluoride. This was shown to be due to differences in the urinary flow, an increase in flow causing an increase in renal clearance… When apparent bioavailability was calculated from plasma and from urinary data, there was great intra- and intersubject variation, as well as poor agreement between the two methods of calculations. This was found to be due to the day-day variation in renal clearance, which, in turn varied with urinary flow. By use of equations that corrected for these variations, it was found that the bioavailability of sodium fluoride tablets is approximately 100%.” In the sodium fluoride study, there were two overall results that were not consistent with pharmacokinetic theory at the time of the study (and still today). First, mean bioavailability was greater than 100%, although not statistically significant ($p = 0.055$) unless the large difference in one of two studies in subject L.K. was eliminated ($p = 0.011$). Second, $CL_{R,oral}$ was statistically less than $CL_{R,iv}$ ($p = 0.007$ paired t-test) even when the outlier subject L.K. data were included. The authors therefore needed to explain the reasons that these values cannot be accepted. They proposed that since renal clearance was dependent on urine flow rate, which it is, and renal flow was highly variable, they could ignore the measured oral renal clearances (which are highly significantly different than the measured iv renal clearances) and substitute the iv renal clearances for these values in each subject. Thus, by ignoring the real difference in measured renal clearance following oral dosing, they could show that bioavailability was close to unity. However, renal clearance is renal clearance whether it is highly variable or not. If the authors ignore these measured differences, the reason that F exceeds 1.0 due to absorption affecting systemic concentrations when CL_{gut} is not significantly greater than $CL_{iv\ dose}$, the measured systemic bioavailability inconsistency will disappear. There is no scientific justification for the conclusion of Ekstrant et al. (18) but recognizing that the derivations presented in this present manuscript were unknown until now. –

The authors of the cimetidine study (21) did not try to propose an error analysis for their AUC_{oral}/AUC_{iv} equal to 1.106 and $U_{\infty oral}/U_{\infty iv}$ equal to 0.595 writing " The results clearly demonstrate that bioavailability studies using AUC-measurements are misleading for several drugs including cimetidine." However, they did not report the statistical analysis of this comparison ($p < 0.001$ paired test) nor did they calculate or compare CL_R oral vs iv for their study ($p = 0.0018$ paired t-test). _

The third significantly different comparison of AUC_{oral}/AUC_{iv} and $U_{\infty oral}/U_{\infty iv}$ in Table II ($p < 0.0001$) is for active cilazaprilat measurements following cilazapril dosing (35). An important aspect of these data is the recognition that AUC_{oral}/AUC_{iv} measurements may not reflect accurate bioavailability measurements, even when the ratio is less than 1.0. Thus, *AUC* ratio measurements may potentially overestimate actual bioavailability if CL_{gut} is not much greater than $CL_{iv\ dose}$ (Eq. 6) for drugs of any bioavailability. In therapeutic practice and drug approval of ACE inhibitors, the prodrug is dosed to increase solubility and bioavailability before hydrolysis to its active molecule in vivo. The actual amount of active drug that reaches the systemic circulation may be lower than the results predicted using AUC_{oral}/AUC_{iv} **.** This is reflected in the results for the parallel study in the same 12 subjects where cilazaprilat was dosed orally and intravenously (35). Here, following cilazaprat dosing, the ratio of $U_{\infty oral}/U_{\infty iv}$ to AUC_{oral}/AUC_{iv} for active drug was 0.64, even lower than the ratio of 0.74 following cilazapril dosing, yet the difference was not significant ($p = 0.055$). This is due to the increased intersubject variability that is observed as measures of bioavailability are decreased as documented by Hellriegel et al. (41)

When experimental studies yield systemic bioavailability measures greater than 1.0, investigators, particularly for human studies, try to explain why the results are either inconsequential or due to a confounding experimental error that can be corrected, suspecting that otherwise the study may not be accepted for publication. Since in this manuscript we justify why systemic $F > 1.0$ and $CL_{R,oral} > CL_{R,iv}$ results may be obtained and that such results are not necessarily in error, here we summarize explanations of such results for 7 human studies. a) For 1-deamino-8-arginine vasopressin the authors (8) try to explain the 1.66 *AUC* ratio based on adsorption of the drug to the syringe following iv dosing, but urinary excretion following equivalent iv and SubQ doses is only 20% less for the iv administration. Furthermore, if absorption has no effect on AUC, CL_R should be the same for the iv and SubQ doses independent of adsorption. However, the SubQ CL_R is 76% of that following iv dosing. b) The supposed corrected bioequivalence values for sodium fluoride (18) were obtained by replacing the measured $CL_{R,oral}$ values in each subject following oral dosing with measured $CL_{R,iv}$ values believing that CL_R should be the same following iv and oral dosing. c) The teicoplanin analysis shows that the 90% confidence interval is 108-116% around the 112% mean, which indicates that the systemic bioavailability greater than 1.0 is statistically significant. In the paper, the authors (22) write: "Since the 90% confidence interval for the ratio of areas under the serum concentration-time curve falls within the range of 80–120%, the extent of

systemic absorption of teicoplanin following IM administration is equivalent to that following iv administration." The authors misinterpreted the bioequivalence guidelines by equating clinically significant differences with actual measures of statistical difference. d) A paired t-test analysis for cimetidine, not previously carried out by the authors (21), shows that the dose corrected areas following oral dosing is greater than iv with $p<0.001$. As noted by the authors, a study showing comparable bioavailability was published the previous year by US investigators (43). However, the oral dosage forms were not the same, with a 300 mg tablet manufactured in the US vs a 200 mg tablet manufactured in Sweden. e) The levetiracetam analysis (23) shows that the 90% confidence interval 105-113% around the 109% mean indicates that the systemic bioavailability greater than 1.0 is statistically significant. f) Since the mean systemic F value for the ofloxacin study (26) was 1.05, one might suspect that this result being so close to 1.0 may just be due to the normal variance found in human studies. However, the authors report "Because of a small intrasubject variability (coefficient of variation, 4.5%) in the *AUC* values, the difference in the plasma *AUC* values between the p.o. and i.v. doses (4.7%) was found to be statistically significant ($P < 0.05$), with the p.o. dosage form having the larger *AUC* ." Thus, at least for these seven human studies we believe there is compelling evidence supporting that systemic bioavailability can exceed 100% and these results are not attributable to experimental errors.

The common response we receive when documenting the systemic F >1.0 studies is "How do you know that the results are not just the function of saturation of elimination processes for the oral, IM or SubQ doses?" Saturation phenomena related to elimination could be a possible explanation when the oral, IM or SubQ dose is greater than the iv dose, however, we could not identify any studies where experimental data supported this explanation in the studies listed in Tables 1 and 2. However, there are a number of studies that show that saturation is not the explanation when one examines renal clearance data. For example, in the cimetidine study (21) 9 subjects received a 100 mg iv dose with mean $CL_{R,iv}$ = 35.6 ± 10.0 L/hr, 3 of the 9 received a 100 mg oral dose with mean $CL_{R,oral}$ = 27.0 ± 10.6 L/hr, all 9 received a 400 mg oral dose with mean $CL_{R,oral}$ = 21.6 ± 10.6 L/hr, and 4 received an 800 mg oral dose with mean $CL_{R,oral}$ = 27.2 ± 15.2 L/hr. For the sodium fluoride study (18), all 6 subjects received an iv dose of 3 mg with a mean $CL_{R,iv}$ = 70.2 ± 16.7.6 ml/min. Those 6 subjects received a low oral dose (2.82mg 4 subjects or 4 mg 2 subjects) with a mean $CL_{R,oral}$ = 55.1 ± 13.0 ml/min. Four of the subjects received a higher oral dose (9.4 mg 3 subjects or 5.5 mg 1 subject) with a mean $CL_{R,oral}$ = 51.8 ± 22.5 ml/min. None of the results for cimetidine or sodium fluoride support a saturation effect.

For the remaining 15 human studies listed in Table I, all but desmin were carried out investigating the same dose iv and oral, IM or SubQ and no indication of any saturation effects is presented in any of the studies. Thus, there are no data or

essentially even the possibility that saturation can explain the *F* >1 values in Table I.

The major objective of this report was to demonstrate that studies resulting in F>1.0 and/or marked differences in systemic vs urine predictions in bioavailability may be accurate and should not be considered experimentally flawed. And further, that it is incorrect to assume that following oral, IM and SubQ dosing, that the rate of absorption from the dosing site has no effect on the measured area under the curve, as is presently universally believed. However, we recognize that this belief has been the result of having no way to determine the correct relationship between clearance and the rate of absorption, because prior to our introduction of Kirchhoff's Laws to determine clearance for in series processes (3, 4) that are inherent in absorption studies, the only derivation of clearance possible was to define the relationship in terms of rate constants and then divide by the systemic volume of distribution.

The important question to now address is what effect will these new understandings have with respect to regulatory issues related to bioavailability? Foremost, it must be recognized that the measured *AUCs* following oral, IM and SubQ dosing are not affected by the analyses reported here. Regulatory guidances for assessing bioequivalence and food effect study data are only based on the measured *AUC* and characteristics of *AUC* related to absorption rate criteria, i.e., C_{max} or *AUC* up to peak time. Similarly, pharmacodynamic outcomes, such as selecting the appropriate dose and dosing interval for a new drug or adjustments in drug dosing due to disease states, drug interactions or pharmacogenomic and physiologic differences, are only based on *AUC* measurements. So, what will change? First, reported bioavailability values may be an overestimate unless dose corrected systemic concentration and urinary excretion ratios are similar. Second, since food effect studies often result in changes in bioavailability, can the food effect be a change in gut volume of distribution as well as a change in gut rate of absorption? That is, is there discontinuities between rate constant changes and gut clearance changes that have not been addressed previously? Third, bioavailability studies with F>1.0 or studies with significant differences in ratio for *AUC* and unchanged drug in the urine, are no more likely to be experimentally flawed than any other study. Fourth, although the changes described here should have no effect on regulatory issues related to bioavailability and drug dosing decisions, present attempts to predict drug bioavailability, bioequivalence and food effects using PBPK models may not be considering all relevant aspects of drug absorption.

ACKNOWLEDGEMENTS AND DISCLOSURES. This study was supported by the UCSF Benet Fund for Excellence, generated from individual contributions and Dr. Benet's consultation, expert witness and board of director fees that are made payable to the Regents of the University of California. Dr. Wakuda's stay in the Benet Laboratory

was supported by grants from the Japanese Society of Clinical Pharmacology and Therapeutics and by the study abroad program of Oita University, Oita, Japan. Dr. Benet is a member of the UCSF Liver Center supported by NIH grant P30 DK026743.
We wish to make an additional disclosure and apology. In reference 3, we made the statement “We believe that this is the first application of Kirchhoff's Laws to pharmacological processes but anticipate that many more uses will be identified in the future.” This not true and we apologize to the authors for our mis-statement. A number of papers have utilized Kirchhoff’s Laws to generate simulations of various processes (e.g., references 43-45). However, these applications did not utilize Kirchhoff’s Laws to derive relationships that are not consistent with present pharmacokinetic principles based on differential equation derivations, which is the object of each of our papers.

Data availability statement All data generated or analyzed during this study are included in this article or in the references provided.

AUTHOR CONTRIBUTIONS

H.W. and L.Z.B. wrote the first draft of the manuscript and all authors contributed to revisions. L.Z.B., J.K.S and N.U. designed the research. H.W. and Y.X. performed the research and literature searches. All authors analyzed the data.

DECLARATIONS

Conflict of interest statement The authors declare that they have no conflict of interest.

REFERENCES

1. Wagner JG. History of pharmacokinetics. Pharmacol Ther. 1981;12:537–62.
2. Benet LZ, Sodhi JK, Makrygiorgos G, Mesbah A. There is only one valid definition of clearance: Critical examination of clearance concepts reveals the potential for errors in clinical drug dosing decisions. AAPS J. 2021;23:67.
3. Patcher JA, Dill KA, Sodhi JK, Benet LZ. Review of the application of Kirchhoff's Laws of series and parallel flows to pharmacology: Defining organ clearance. Pharmacol Ther. 2022;239:108278.
4. Benet LZ, Sodhi JK. The uses and advantages of Kirchhoff's Laws vs differential equations in pharmacology, pharmacokinetics and (even) chemistry. AAPS J. 2023;25:38. https://doi.org/10.1208/s12248-023-00801-w
5. Abu-Basha EA, Bani Ismail Z, Ababneh MM, Hamzeh E, Gehring R. Pharmacokinetics and bioavailability of tildipirosin following intravenous and subcutaneous administration in horses. J Vet Pharmacol Ther. 2021;44:544–51.
6. Miglioli M, Pironi L, Ruggeri E, Serra C, Zamboni V, Barbanti M, Canova N, Calanni F, Milani MR, Palazzini E. Bioavailability of desmin, a low molecular weight dermatan sulfate, after subcutaneous administration to healthy volunteers. Int J Clin Lab Res. 1997;27:195–8.
7. Corum O, Corum DD, Er A, Yildiz R, Uney K. Pharmacokinetics and bioavailability of tolfenamic acid in sheep. J Vet Pharmacol Ther. 2018;41:871–7.
8. Fjellestad-Paulsen A, Höglund P, Lundin S, Paulsen O. Pharmacokinetics of 1-deamino-8-D-arginine vasopressin after various routes of administration in healthy volunteers. Clin Endocrinol (Oxf). 1993;38:177–82.
9. Champoux N, Du Souich P, Ravaoarinoro M, Phaneuf D, Latour J, Cusson JR. Single-dose pharmacokinetics of ampicillin and tobramycin administered by hypodermoclysis in young and older healthy volunteers. Br J Clin Pharmacol. 1996;42:325–31.
10. Nagilla R, Deshmukh DD, Copedge KJ, Miller S, Martin B, Bell EC, Duran SH, Ravis WR. Enantiomeric disposition of ketorolac in goats following administration of a single intravenous and oral dose. J Vet Pharmacol Ther. 2009;32:49–55.
11. Corum O, Durna Corum D, Atik O, Eser Faki H, Altan F, Uney K. Pharmacokinetics and bioavailability of danofloxacin in chukar partridge (Alectoris chukar) following intravenous, intramuscular, subcutaneous, and oral administrations. J Vet Pharmacol Ther. 2019;42:207–13.
12. Monfrinotti A, Ambros L, Prados AP, Kreil V, Rebuelto M. Pharmacokinetics of ceftazidime after intravenous, intramuscular and subcutaneous administration to dogs. J Vet Pharmacol Ther. 2010;33:204–7.
13. Kaartinen L, Salonen M, Alli L, Pyörälä S. Pharmacokinetics of enrofloxacin after single intravenous, intramuscular and subcutaneous injections in lactating cows. J Vet Pharmacol Ther. 1995;18:357–62.
14. Rubio-Langre S, De Lucas JJ, Litterio NJ, Aguilar S, Boggio JC, San Andrès MI. Pharmacokinetic behavior of marbofloxacin after intravenous, subcutaneous and intramuscular administrations in llamas (Lama glama). Small Ruminant Research. 2012;106:64–9.

15. Tse FL, Szeto DW. Theophylline bioavailability in the dog. J Pharm Sci. 1982:71:1301–3.
16. Altan F, Corum O, Corum DD, Atik O, Uney K. Pharmacokinetics and bioavailability of marbofloxacin in lambs following administration of intravenous, intramuscular and subcutaneous. Small Ruminant Research. 2018;159:5–10.
17. ten Voorde G, Broeze J, Hartman EG, van Gogh H. The influence of the injection site on the bioavailability of ampicillin and amoxycillin in beagles. Vet Q. 1990;12:73–9.
18. Ekstrand J, Ehrnebo M, Boréus LO. Fluoride bioavailability after intravenous and oral administration: importance of renal clearance and urine flow. Clin Pharmacol Ther. 1978;23: 329–37.
19. Wade M, Baker FJ, Roscigno R, DellaMaestra W, Hunt TL, Lai AA. Absolute bioavailability and pharmacokinetics of treprostinil sodium administered by acute subcutaneous infusion. J Clin Pharmacol. 2004;44:83–8.
20. Gordon E, Dirikolu L, Liu CC, Ulanov AV, Li Z, Welborn M, Armstrong C, Vallotton D, Keeton S, Camlic S, Scully CM. Pharmacokinetic profiles of three dose rates of morphine sulfate following single intravenous, intramuscular, and subcutaneous administration in the goat. J Vet Pharmacol Ther. 2022;45:107–16.
21. Grahnén A, von Bahr C, Lindström B, Rosén A. Bioavailability and pharmacokinetics of cimetidine. Eur J Clin Pharmacol. 1979;16:335–40.
22. Antony KK, Lewis EW, Kenny MT, Dulworth JK, Brackman MB, Kuzma R, Yuh L, Eller MG, Thompson GA. Pharmacokinetics and bioavailability of a new formulation of teicoplanin following intravenous and intramuscular administration to humans. J Pharm Sci. 1991;80,605–7.
23. Ramael S, de Smedt F, Toublanc N, Otoul C, Boulanger P, Riethuisen JM, Stockis A. Single-dose bioavailability of levetiracetam intravenous infusion relative to oral tablets and multiple-dose pharmacokinetics and tolerability of levetiracetam intravenous infusion compared with placebo in healthy subjects. Clin Ther. 2006;28:734–44.
24. Jung D, Mroszczak E, Bynum L. Pharmacokinetics of ketorolac tromethamine in humans after intravenous, intramuscular and oral administration. Eur J Clin Pharmacol. 1988;35:423–5.
25. Kuo GM, Boumpas DT, Illei GG, Yarboro C, Pucino F, Burstein AH. Fludarabine pharmacokinetics after subcutaneous and intravenous administration in patients with lupus nephritis. Pharmacotherapy. 2001;21:528–33.
26. Yuk JH, Nightingale CH, Quintiliani R, Sweeney KR. Bioavailability and pharmacokinetics of ofloxacin in healthy volunteers. Antimicrob Agents Chemother. 1991;35:384–6.
27. Rodriguez GI, Kuhn JG, Weiss GR, Hilsenbeck SG, Eckardt JR, Thurman A, Rinaldi DA, Hodges S, von Hoff DD, Rowinsky EK. A bioavailability and pharmacokinetic study of oral and intravenous hydroxyurea. Blood. 1998;91:1533–41.
28. Strandgârden K, Höglund P, Nordle O, Polacek J, Wännman H, Gunnarsson PO. Dissolution rate-limited absorption and complete bioavailability of roquinimex in man. Biopharm Drug Dispos. 1999;20:347–54.

29. Martin W, Koselowske G, Töberich H, Kerkmann T, Mangold B, Augustin J. Pharmacokinetics and absolute bioavailability of ibuprofen after oral administration of ibuprofen lysine in man. Biopharm Drug Dispos. 1990;11:265–78.
30. Anttila M, Penttilä J, Helminen A, Vuorilehto L, Scheinin H. Bioavailability of dexmedetomidine after extravascular doses in healthy subjects. Br J Clin Pharmacol. 2003;56:691–3.
31. Tamassia V, Corvi G, Moro E, Tosolini GP Fuccella LM. Pharmacokinetics and bioavailability of indoprofen in man. Eur. J. Clin. Pharmacol. 1976;10:257–62.
32. Siouf A, Gauducheau N, Pineau V, Marfil F, Jaouen A, Cardot JM, Godbillon J, Czendlik C,
Howald H, Pfister C, Vreeland F. Absolute bioavailability of letrozole in healthy postmenopausal women. Biopharm Drug Dispos. 1997;18:779–89.
33. Breithaupt B, Tittel M. Kinetics of allopurinol after single intravenous and oral doses. Noninteraction with benzbromarone and hydrochlorothiazide. Eur J Clin Pharmacol. 1982;22: 77–84.
34. Tett SE, Cutler DJ, Day RO, Brown KF. Bioavailability of hydroxychloroquine tablets in healthy volunteers. Br J Clin Pharmacol. 1989;27:771–9.
35. Williams PE, Brown AN, Rajaguru S, Francis RJ, Walters GE, McEwen J, Durnin C. The pharmacokinetics and bioavailability of cilazapril in normal man. Br J Clin Pharmacol. 1989;27 (Suppl 2):181S–8S.
36. James CA, Mant TG, Rogers HJ. Pharmacokinetics of intravenous and oral sodium 2-mercaptoethane sulphonate (mesna) in normal subjects. Br J Clin Pharmacol. 1987;23:561–8.
37. Garg DC, Weidler DJ, Eshelman FN. Ranitidine bioavailability and kinetics in normal male subjects. Clin. Pharmacol. Ther. 1983;33:445–52.
38. Garrison KL, Sahin S, Benet LZ. Few drugs display flip-flop pharmacokinetics and these are
primarily associated with classes 3 and 4 of the BDDCS. J Pharm Sci. 2015;104:3229–35.
39. Yamaoka K, Nakagawa T, Uno T. Statistical moments in pharmacokinetics. J Pharmacokinet Biopharm. 1979;16:547–58.
40. Bocci G, Oprea TI, Benet LZ. State of the art and uses for the biopharmaceutics drug disposition classification system (BDDCS): New additions, revisions, and citation references. AAPS J. 2022;24:37.
41. Hellriegel ET, Bjornsson TD, Hauck WW. Interpatient variability in bioavailability is related to the extent of absorption: Implications for bioavailability and bioequivalence studies. Clin Pharmacol Ther. 1996;60:601–7.
42. Walkenstein SS, Dubb JW, Randolph WC, Westlake WJ, Stote RM, Intoccia AP. Bioavailability of cimetidine in man. Gastroenterology. 1978;74:360-365.
43. Thakker KM, Wood JH, Mikulecky DC. Dynamic simulation of pharmacokinetic systems using the electrical circuit analysis program SPICE2. Comput Programs Biomed. 1982;15:61-72.
44. Thakker KM. Pharmacokinetic-pharmacodynamic modelling and simulation using the electrical circuit simulation program SPICE2. Biopharm Drug Dispos. 1984;5:315-333.

45. White JC. Use of the circuit simulation program SPICE2 for analysis of the metabolism of anticancer drugs. Bull Math Biol. 1986;48:353-380.

Table I Published Crossover Studies Where Dose Corrected AUC_x/AUC_{iv} Is Greater Than 1.02

Drug	Species	Route	AUC_x/AUC_{iv}	Reference
Tildipirosin	Horse	SubQ	4.01	5
Desmin	Healthy Humans	SubQ	1.67	6
Tolfenamic acid	Sheep	IM	1.67	7
1-Deamino-8-arginine[a] vasopressin	Healthy Humans	SubQ	1.66	8
Ampicillin	Healthy Elderly	SubQ	1.61	9
S-Ketorolac	Goat	Oral	1.39	10
R-Ketorolac	Goat	Oral	1.36	10
Danofloxacin	Chukar Partridge	SubQ	1.34	11
Ceftazidime	Dog	IM	1.33	12
Tolfenamic acid	Sheep	SubQ	1.31	7
Enrofloxacin	Lactating Cows	SubQ	1.30	13
Marbofloxacin	Llama	SubQ	1.27	14
Theophylline (Tablet)	Dog	Oral	1.26	15
Marbofloxacin	Lamb	SubQ	1.25	16
Amoxycillin	Dog	IM	1.24	17
Sodium Fluoride[b]	Healthy Humans	Oral	1.23	18
Theophylline (Capsule)	Dog	Oral	1.23	15
Ampicillin	Healthy Young	SubQ	1.19	9
Treprostinil sodium[c]	Healthy Humans	SubQ	1.13	19
Tolfenamic acid	Sheep	IM	1.13	7
Morphine sulfate	Goat	SubQ	1.11	20
Cimetidine[e]	Healthy Humans	Oral	1.11	21
Teicoplanin[d]	Healthy Humans	IM	1.12	22
Levetiracetam[f]	Healthy Humans	Oral	1.09	23
Marbofloxacin	Llama	IM	1.09	14
Ketorolac tromethamine	Healthy Humans	IM	1.08	24
Fludarabine	Lupus Nephritis Patients	SubQ	1.07	25
Ofloxacin[h]	Healthy Humans	Oral	1.05	26
Amoxycillin	Dog	SubQ	1.05	17
Hydroxyurea[g]	Patients with Solid Tumors	Oral	1.08	27
Roquinimex	Healthy Humans	Oral	1.04	28
Morphine sulfate	Goat	IM	1.04	11
Ibuprofen	Healthy Humans	Oral	1.03	29
Dexmedetomidine	Healthy Humans	IM	1.03	30
Indoprofen	Healthy Humans	Oral	1.03	31

[a] The authors attempt to explain the high AUC ratio based on adsorption of the drug to the syringe following iv dosing, but CL_R following SubQ is 76% of CL_R following iv, which is independent of adsorption.

[b] $p = 0.055$ for 10 measurements in 6 subjects. When one of the two measurements in subject LK, the dosing involving the greatest change in AUC is deleted, $p = 0.011$ for 9 measurements in 6 subjects. The authors explain the F>1.0 results to be a function of not considering urine flow rates. However, they don't make adjustments based on urine flow rates, but rather replace all measured renal clearances following oral dosing with the renal clearance following iv dosing. When they do this, the AUC ratio decreases to approximately 1.0.

[c] The authors report the standard deviation for the measurements is 1.13 ± 0.10 but provide no statistical analysis.

[d] The authors report that the 90% confidence interval for the mean 1.12 ratio is 1.08-1.16, which indicates that the mean ratio is statistically significantly greater than 1.0.

[e] $p < 0.001$

[f] The authors report that the 90% confidence interval for the mean 1.09 ratio is 1.05-1.13, which indicates that the mean ratio is statistically significantly greater than 1.0.

[g] The authors reported F in the 22 patients to be 108 ± 19%. No statistics are reported for the bioavailability studies. However, the authors do report a difference in renal clearance oral vs iv "with a moderate inverse relationship between the AUC and renal clearance of hydroxyurea ($r = -.59$, $P < .01$)".

[h] $p < 0.05$. The authors report: "Because of a small intrasubject variability (coefficient of variation, 4.5%) in the AUC values, the difference in the plasma AUC values between the p.o. and i.v. doses (4.7%) was found to be statistically significant ($P < 0.05$), with the p.o. dosage form having the larger AUC."

Table II Published Crossover Studies in Healthy Humans Exhibiting Differences in Oral Bioavailability Using Dose Corrected AUC (AUC_{oral}/AUC_{iv}) Versus Urinary Excretion ($U_{\infty oral}/U_{\infty iv}$) Data

Drug	AUC_{oral}/AUC_{iv}	$U_{\infty oral}/U_{\infty iv}$	Reference
Sodium Fluoride	1.233 ± 0.308	0.886 ± 0.191[a]	18
Cimetidine	1.119 ± 0.399	0.595 ± 0.156[b]	21
Indoprofen (100 mg capsule)	1.031 ± 0.340	0.901 ± 0.248[c]	31
Letrozole	0.991	0.937	32
Allopurinol	0.904	0.814	33
Indoprofen (200 mg tablet)	0.867 ± 0.146	0.809 ± 0.214[d]	31
Hydroxychloroquine	0.79 ± 0.12	0.69 ± 0.15[e]	34
Cilazapril (measure cilazaprilat)	0.775 ± 0.101	0.571 ± 0.103[f]	35
Mesna	0.680 ± 0.413	0.580 ± 0.173[g]	36
Ranitidine	0.52 ± 0.11	0.38	37
Cilazaprilat	0.290 ± 0.148	0.186 ± 0.099[h]	35

[a] $p = 0.055$ 10 paired measurements in 6 subjects; $p = 0.011$ when one of the two paired measurements in subject L.K. not included in the analysis.

[b] $p < 0.001$ 16 paired measurements in 9 subjects

[c] $p = 0.378$ 4 paired measurements

[d] $p = 0.689$ 4 paired measurements

[e] $p = 0.43$ 5 paired measurements

[f] $p < 0.0001$ 12 measurements of active cilazaprilat dosing cilazapril in 12 subjects. Calculated from means and SDs since individual data not given.

[g] $p = 0.69$ 5 paired measurements

[h] $p = 0.055$ 12 measurements of cilazaprilat dosing cilazaprilat in the same 12 subjects who were also dosed cilazapril. Calculated from means and SDs since individual data not given.

Table III Calculation of Clearance Gut (CL_{gut}) and the Ratio of Clearance Gut to Clearance iv ($\frac{CL_{gut}}{CL_{iv}}$) for Drugs in Table II Where Bioavailability Measures Using Systemic Concentrations and Unchanged Amounts in the Urine Are Available.

Drug	$F=\frac{U_{\infty oral}}{U_{\infty iv}}$	$\frac{Dose_{oral}}{AUC_{0\rightarrow\infty oral}}$	$CL_{after\ oral\ dosing}$	CL_{iv}	CL_{gut}	$\frac{CL_{gut}}{CL_{iv}}$
		L/hr	*L/hr*	*L/hr*	*L/hr*	
Sodium Fluoride (18)	0.886	9.81	8.68	10.8	44.3	4.1
Cimetidine (21)	0.595	50.4	30.0	48.5	78.6	1.6
Indoprofen (Capsule) (31)	0.901	3.48	3.14	3.47	33.0	9.5
Letrozole (32)	0.937	2.04	1.91	2.21	14.1	6.4
Allopurinol (33)	0.814	50.7	41.3	46.6	363.	7.8
Indoprofen (Tablet) (31)	0.867	3.39	2.94	2.97	291.	98.
Hydroxychloroquine (34)	0.69	63.9	44.1	50.0	374.	7.5
Mesna (36)	0.58	123.	71.5	77.9	870.	11.
Ranitidine (37)	0.38	84.7	32.2	44.6	116.	2.6