Commentary

Kirchhoff's Laws and Hepatic Clearance, Well-Stirred Model –
Is There Common Ground?

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Running Title: Kirchhoff’s Laws and the well-stirred model

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ABSTRACT

Clearance concepts are extensively applied in drug development and drug therapy. The well-stirred model (WSM) of hepatic elimination is the most widely adopted physiologic model in pharmacokinetics owing to its simplicity. A common feature of this organ model is its use to relate hepatic clearance of a compound to the physiological variables: organ blood flow rate, binding within blood, and hepatocellular metabolic and excretory activities. Recently, Kirchhoff's laws of electrical network have been applied to organ clearance (Pachter et al., 2022; Benet and Sodhi, 2023) with the claim that they yield the same equation for hepatic clearance as the WSM, and that the equation is independent of a mechanistic model. This commentary analyzes this claim and shows that, implicit in the application of Kirchhoff's approaches are the same assumptions as those of the WSM. Concern is also expressed in the interpretation of permeability or transport parameters and related equations, as well as the inappropriateness of the corresponding equation defining hepatic clearance. There is no value, and some dangers, in applying Kirchhoff's electrical laws to organ clearance.

SIGNIFICANCE STATEMENT

This commentary refutes this claim by Pachter et al 2022, and Benet and Sodhi, 2023, who suggest that the well-stirred model (WSM) of hepatic elimination, the most widely applied physiologic model of hepatic clearance, provides the same equation as Kirchhoff's laws of electrical network that is independent of a physiologic model. A careful review shows that the claim is groundless and fraught with errors. We conclude that there is no place for the application of Kirchhoff's laws to organ clearance models.
Introduction

Clearance ($CL$) equations pertaining to drug removal in organs, particularly the liver, have surfaced during the past fifty years to describe substrate removal and metabolite formation (Rowland et al. 1973; Winkler 1973; Wilkinson and Shand, 1975, Pang and Rowland, 1977; Roberts and Rowland, 1986; Gray and Tam, 1987; Pang et al., 2019). Discrete differences in hepatic drug clearance ($CL_H$) models have been linked to the pattern of liver microcirculation (bulk, plug or dispersive flow), giving rise to the well-stirred (WSM), parallel tube (PTM) and dispersion (DM) models, resulting in different degrees of mixing and unbound substrate concentration in the liver cell ($Cu_{cell}$) on which the metabolic enzymes and canalicular transporters act (Pang et al., 2019). Of note is that these models can yield differing estimates of in vivo intrinsic clearances ($CL_{int}$ or $V_{max}/(K_m+[S])$) for a given observed value of $CL_H$ (Rowland and Pang, 2018; 2022; Pang et al., 2019; Li and Jusko, 2022), an important and relevant parameter in the development of IVIVE (in vitro-in vivo extrapolation), and in the associated prediction of in vivo pharmacokinetics under various conditions (Kusuhara and Sugiyama, 2009; Tess et al., 2022).

The early account of $CL_H$ by Rowland et al. (1973) describes the liver as a single compartment and a blood or reservoir compartment as a second compartment, in a perfused model akin to a two-compartment model for unbound, flow-limited distribution compounds. Here, they use the elimination rate constant ($k_m$) and an effective volume of distribution for the liver, given by liver volume, $V_E$, x $K_P$, the liver tissue to plasma concentration ratio (Dedrick and Bischoff, 1968 or Dedrick et al., 1972). Concomitantly, Gillette (1971) ascribes this clearance of drug by the liver as $V_{max}/K_m$ and Wilkinson and Shand (1975) rename the product ($k_m·V_E·K_P$ or $V_{max}/K_m$ as the hepatic intrinsic clearance. Finally, to ensure mass balance, which is independent of any model, the amount eliminated across the liver per unit time [e.g. $Q_H(C_{in} - C_{out})$] must equal the amount eliminated within the liver per unit time (e.g. $f_{ub}·CL_{int}·C_{out}$ for the WSM). Collectively, these assumptions and conditions lead to the familiar equation for hepatic clearance for the WSM for substrates that are flow-limited in distribution, with rapid permeation across the cell membranes (Wilkinson and Shand, 1975)

$$CL_H = Q_H \left[ \frac{f_{ub} · CL_{int}}{Q_H + f_{ub} · CL_{int}} \right]$$

(1)
that considers the unbound fraction in blood, \( f_{\text{ub}} \), hepatic blood flow, \( Q_H \), and the hepatic intrinsic clearance, \( CL_{\text{int}} \).

A recent paper by Pachter et al (2022), much repeated by Benet and Sodhi (2023), claims that the hepatic clearance equation defined by the WSM (Rowland et al., 1973, Wilkinson and Shand, 1975; Pang and Rowland, 1977) may simply be obtained by applying Kirchhoff’s laws of electrical network (Kirchhoff, 1847), independent of any mechanistic model, i.e., without any assumption concerning the structure of, and processing by, the liver. We examine this claim and find it wanting. We mention on passing that this is not the first application of Kirchhoff's Laws to pharmacological processes (e.g., Mikulecky, 2001; Thakker, 1984). In making this claim to connect elimination with electrical processes, these authors considered the following basic equation, which deals with a time averaged, steady-state, linear relationship of the general form

\[
J = K \cdot f = \left(\frac{1}{R}\right) \cdot V
\]

where \( J \) is flux, \( f \) is the driving force, and \( K \) is a proportionality constant. When applied to electrical circuits, \( J \) is current (\( I \)), \( f \) is voltage (\( V \)) and \( K \) is conductance (\( 1/R \) or \( 1/\text{resistance} \), the conductance). When applied to organ elimination in pharmacokinetics, \( J \) is rate of elimination, \( f \) is concentration, and \( K \) is clearance (\( CL \)) (Eq. 2).

**Analysis and Discussion**

The properties and assumptions underlying the WSM are first considered before linking these to the application of Kirchhoff’s laws. The WSM assumes that each of the extracellular and intracellular water space of the liver is well-stirred, that is, any change in concentration of a drug that is highly permeable in any one of these spaces is instantaneously reflected in the rest of that space. And, in the simplest and original form of the WSM, distribution across these spaces by passive diffusion of unbound drug occurs so rapidly that effectively, distribution equilibrium is maintained instantaneously between drug in blood (vascular space) and the rest of the organ, with the same unbound concentration throughout these spaces, and also that in the blood leaving the organ, \( C_{\text{out}} \) (Dedrick et al., 1972). Elimination is assumed to occur intracellularly, by metabolism and biliary excretion, operating on
unbound drug in cell water under linear conditions. With respect to distribution, it is a steady-state model in that the only reason for a fall in the concentration of drug within the liver is elimination. A rearranged form of Eq. 1 gives

\[
\frac{1}{CL_H} = \frac{1}{Q_H} + \frac{1}{fu_b \cdot CL_{int}}
\]  

(3)

Turning to hepatic clearance, in the application of Kirchhoff’s laws, Pachter et al. (2022) first set \( Q_H \) and \( fu_b \cdot CL_{int} \) as the upper and lower rate limiting processes driving clearance [which they subsequently redefined as rate determining processes, which can become rate limiting under certain conditions (Benet and Sodhi 2023)]. They then arranged them in series, arguing that blood carrying drug entering the liver is one process which precedes the second process, elimination of drug within the liver. Now for two resistors (\( R_1 \) and \( R_2 \)) arranged in series in an electrical circuit, \( I/R_T = I/R_1 + I/R_2 \), where \( R_T \) is the total resistance of the system. And, as the current \( I \) is the same throughout such a circuit, it follows that \( 1/R_T = 1/R_1 + 1/R_2 \). Substituting \( CL_H \) for \( R_T \), \( Q_H \) for \( R_1 \), and \( fu_b \cdot CL_{int} \) for \( R_2 \) gives Eq. 3. These manipulations look benign, but several hidden assumptions have been made.

First, modeling of the elimination processes in the liver by applying Kirchhoff’s Laws to the simplest network model with only two elements in series (i.e., one node), already contains the assumption that the compartment, the liver in this instance, is well-mixed. This means that the network topology (microvascular network) underlying the implementation of Kirchhoff’s Laws needs to be greatly simplified in order to obtain an explicit solution. In other words, a complex flow network with many mixing nodes that could more realistically account for intrahepatic mixing has been reduced to a well-mixed system, the same as assumed in the WSM.

Second. Why was \( fu_b \cdot CL_{int} \) chosen and not simply \( CL_{int} \), which operates on unbound drug? The reason is that the constant \( fu_b \) links the unbound concentration of compound in the intracellular site, where elimination occurs, to the whole blood concentration within the liver (see Eq. 4 below). And, being a fixed ratio implies equilibration of unbound drug between the two locations, another assumption of the WSM.

Third. For a flow-limited substrate, the vascular compartment within the liver, being well-stirred, implies that the same unbound concentration exists throughout this space, including
that at the exit, thereby linking this exit concentration to that intracellularly at the elimination site, i.e., $C_{cell} = C_{out}$. Again, this is an assumption of the WSM.

Fourth. Central to hepatic elimination is mass balance, restated here. At steady state, the mass of compound eliminated measured across the liver must equal the mass eliminated within the liver. In pharmacokinetics, the rate of elimination across the liver is defined as $CL_H \cdot C_{in}$, where $C_{in}$ is the steady-state input blood concentration to the liver. When considering events within the liver, Pachter et al. (2022) state (bottom of page 2) that the total elimination clearance within liver, $CL_{elim} = fu_b \cdot CL_{int} = fu_b \cdot (CL_{int, met} + CL_{int, sec})$, where $CL_{int, met}$ and $CL_{int, sec}$ are the intrinsic clearances associated with metabolism and biliary excretion, respectively, which, according to Kirchhoff’s laws are additive, since the processes operate in parallel. The important question is: what concentration should $fu_b \cdot CL_{int}$ be multiplied by to obtain the rate of elimination within the liver? Clearly, it is $C_{out}$, and not $C_{in}$. This may be seen by considering unbound events.

$$CL_{int} \cdot C_{cell} = CL_{int} \cdot C_{out} = CL_{int} \cdot fu_b C_{out}$$

Rate of elimination

One arrives at the same conclusion (Eq. 1) by noting that the analogy of current in an electrical circuit is mass flow rate when considering events across the liver. For the liver, we have one inflow ($I_1 = Q_H \cdot C_{in}$) and two output rates: blood leaving the liver ($I_2 = Q_H \cdot C_{out}$) and that eliminated within the liver ($I_3 = fu_b \cdot CL_{int} \cdot C_{out}$). According to Kirchhoff’s first law, $I_1 + I_2 + I_3 = 0$, which is equivalent to conservation of mass, or mass balance, across the liver. Solving the equation for $CL_H = Q_H \cdot (C_{in} - C_{out})/C_{in}$, yields Eq. 1. It must be emphasized that this holds if and only if $I_3 = fu_b \cdot CL_{int} \cdot C_{out}$, i.e., when the well-mixed assumption holds and the unbound concentration in the liver (at the site of elimination) is identical with the outflow unbound concentration.

Summarizing the comments above, we see that inherent in the procedure adopted by Pachter et al. (2022) and repeated by Benet and Sodhi (2023), are the same assumptions that were used in the development of the simple WSM. Nothing is gained by applying Kirchhoff’s Laws, and a lot is lost when one wants to become more mechanistic, and include some of the complexities inherent in the liver, and other eliminating organs. In addition, Eq.
1 is the steady-state solution of the WSM for compounds that permeate rapidly (membrane is not a barrier). In its development the WSM starts with the differential rate equation across the liver (Rowland et al., 1973). Specifically, at any time

\[ V_H \frac{dC_H}{dt} = V_E \cdot K_p \frac{dC_{out}}{dt} = Q_H \cdot C_{in} - Q_H \cdot C_{out} - CL_{int} \cdot fU_b \cdot C_{out} \]

where the effective volume of the liver \( V_H \) (or \( V_E \cdot K_p \)) and the total liver tissue concentration \( C_H \) is perfused with blood with entering and leaving concentrations as previously defined. And, with perfusion rate-limited distribution, equilibrium exists at all times between drug in tissue and that in blood leaving the organ, characterized by a partition coefficient, \( K_p = C_H/C_{out} \) (Dedrick et al., 1972). Accordingly, on appropriately substituting for \( C_H \) in Eq. 5 and noting the equality in Eq. 4 and it should be readily apparent from the foregoing that solving Eq. 5 at steady state (\( dC_H/dt = 0 \)) yields Eq. 1.

And, while failing to characterize well events early after a bolus input, it does give some idea of how long it takes to reach steady state of drug within the liver following constant rate input. Moreover, the WSM, in the form of the differential equations, can readily handle numerically transient and nonlinear elimination processes (e.g. Hall et al, 1988) and be made part of the rate equations that constitute whole body PBPK (physiologically-based pharmacokinetic) models, that are now widely applied in drug discovery and development.

Notably, there are other important comments to be made when hepatocellular permeability of the drug is relatively low and transporters are involved, in addition to passive diffusion, and when Pachter et al. (2022) extend their comments to renal elimination. Concerning the latter, these authors formulate the renal clearance of a compound as the sum of the clearances associated with glomerular filtration and secretion minus that associated with tubular reabsorption (their Eq. 4), implying that under the Kirchhoff rules they occur in parallel whereas, anatomically they occur sequentially or in series. Furthermore,
reabsorption operates on the combined filtered and secreted loads and is driven primarily by the renal tubular concentration of drug.

In order to consider membrane permeability, Pachter et al. (2022), and Benet and Sodhi (2023), simply added another reciprocal clearance term to Eq. 3. Namely, they added the difference in influx and efflux clearances across the membrane, (or more strictly the basolateral (sinusoidal) membrane permeability-surface area products for influx and efflux, $PS_{influx}$ and $PS_{efflux}$), terms that include both passive diffusion and transporter-mediated processes, specifically as $1/[f_{ub} (PS_{influx} - PS_{efflux})]$, into Eq. 3, which, upon solving for $CL_H$, results in their Eq. 5, repeated here.

$$CL_H = \frac{Q_H \cdot f_{ub} \cdot CL_{int} \cdot (PS_{influx} - PS_{efflux})}{(Q_H + f_{ub} \cdot CL_{int}) (PS_{influx} - PS_{efflux}) + Q_H \cdot CL_{int}}$$

(6)

However, Equation 5 of Pachter et al. (2022), as well as Equation 14 of Benet and Sodhi (2023), as shown above, cannot be rearranged into Eq. 7, the familiar form of the Extended Clearance Equation (Shitara et al., 2006; Yoshikado et al., 2017),

$$CL_H = Q_H \left[ \frac{f_{ub} PS_{influx} CL_{int}}{PS_{efflux} + CL_{int}} \right] = Q_H \left[ \frac{f_{ub} CL_{int,all}}{Q_H + f_{ub} CL_{int,all}} \right], \text{ where } CL_{int,all} = \frac{PS_{influx} CL_{int}}{PS_{efflux} + CL_{int}}$$

(7)

As mentioned above, an important criterion as to the veracity of these two equations, independent of any model, is the need to ensure mass balance when, at steady state, the rate of loss of compound across the liver must equal the rate of elimination within it. Now across the liver, the rate of elimination is always $CL_H \cdot C_{in}$ and within the liver it is always $CL_{int} \cdot C_{cell}$. As shown in the Appendix below, which deals with cell membrane permeability/transporter considerations, one arrives at Eq. 7 when mass balance is met, attained by adopting the usual assumption that $PS_{influx}$ and $PS_{efflux}$ are considered separately, and each is multiplied by the unbound concentration in the extracellular or intracellular milieu, respectively. However, also shown in the Appendix below, not only is Eq. 6 not recapitulated by applying the need for mass balance but also one arrives at a
dubious general equation for $CL_H$ that is devoid of $CL_{int}$ (Eq. A8, Appendix). The reason this arises is that by applying a common term $fu_b$ to both $PS_{influx}$ and $PS_{efflux}$, Pachter et al. (2022) have implicitly assumed that the unbound concentrations on both sides of the membrane are equal, which cannot be true when active transport processes are involved. Korzekwa and Nagar (2023) also criticized the application of Pachter et al. (2022) for applying Kirchhoff’s laws to hepatic clearance by stating that the liver cannot be modeled by resistors ($R_1$ and $R_2$) arranged in series, since this would hold only for the special case of passive diffusion across a series of barriers. They also noted that Eq. 6 yields $CL_H = 0$ when (“$PS_{influx}$” = “$PS_{efflux}$”) and attains a negative $CL_H$ when “$PS_{influx}$” < “$PS_{efflux}$.”. Both results are not physiologically sound for a clearance organ such as the liver.

In summary, to explain our intentions, we quote Popper (1962), “Now if we look upon a theory as a proposed solution to a set of problems, then the theory immediately lends itself to critical discussion - even if it is non-empirical and irrefutable. For we can now ask questions such as: Does it solve the problem? Does it solve it better than other theories? Has it perhaps merely shifted the problem? Is the solution simple? Is it fruitful?” The answer to each of these questions, as they apply to the application of Kirchhoff’s laws of electrical network to organ clearance, is no. Furthermore, such applications are confusing.

Appendix: Membrane Permeability/Transporter Considerations

1. The Full or Extended Clearance Equation

Consider the expanded form of the rate equation within the eliminating cell (hepatocyte) of the early form of the WSM, Eq. A1, when membrane permeability becomes a factor, characterized by an influx and efflux basolateral permeability-surface area product, $PS_{influx}$ and $PS_{efflux}$ (Gillette and Pang, 1977; Sato et al., 1986; de Lannoy and Pang, 1987; Sirianni and Pang, 1997; Pang et al., 2019). Alternate to the PS terms used are $CL_{influx}$ again representing the sum of passive diffusion and basolateral influx transporter clearances by OATPs, OCT1, or OAT2, and $CL_{efflux}$ representing the sum of passive diffusion and basolateral efflux transporter clearances such as MRP3 or MRP4, which may be determined separately in vitro, as part of IVIVE (Hirouchi et al., 2009),

$$\Box V_{cell} \frac{dC_{cell}}{dt} = PS_{influx} \cdot Cu_{EC} - (PS_{efflux} + CL_{int}) \cdot Cu_{cell}$$ (Eq. A1)
where \( V_{cell} \) is the volume of the liver cell, \( C_{cell} \) is the total concentration of compound in the cell, \( C_{u,EC} \) is the unbound concentration of compound in the extracellular fluid surrounding the eliminating cells and in the blood within and leaving the liver, and \( C_{u,cell} \) is the intracellular unbound concentration of the compound in the eliminating cells bathing the transporters and enzymes. At steady state, \( \frac{dC_{cell}}{dt} = 0 \), therefore

\[
C_{u,cell} = \frac{PS_{influx} \cdot C_{u,EC}}{(PS_{efflux} + CL_{int})}
\]

(Eq. A2)

and, given that \( C_{u,EC} = fu_b \cdot C_{out} \), where \( C_{out} \) is the total drug concentration in emergent blood leaving the liver, it follows that rate of elimination in the liver is,

\[
CL_{int} \cdot C_{u,cell} = \frac{PS_{influx} \cdot fu_b \cdot CL_{int} \cdot C_{out}}{(PS_{efflux} + CL_{int})}
\]

(Eq. A3)

and given that at steady state \( C_{out} = (1 - E_H) \cdot C_{in} \), where \( E_H \) is the extraction ratio of the drug across the liver, it follows that

\[
CL_{int} \cdot C_{u,cell} = \frac{PS_{influx} \cdot fu_b \cdot CL_{int} \cdot (1 - E_H) \cdot C_{in}}{(PS_{efflux} + CL_{int})}
\]

(Eq. A4)

But for mass balance to hold at steady state the rate of elimination across the liver, given by \( CL_H \cdot C_{in} = Q_H \cdot E_H \cdot C_{in} \), must equal the rate of elimination within the liver (Eq. A4), which on rearranging and solving for \( E_H \), yields the relationship for \( CL_H \).

\[
CL_H = Q_H \cdot E_H = Q_H \left[ \frac{fu_b \cdot PS_{influx} \cdot CL_{int}}{(PS_{efflux} + CL_{int})} \right] = Q_H \left[ \frac{fu_b \cdot PS_{influx} \cdot CL_{int}}{Q_H (PS_{efflux} + CL_{int}) + fu_b \cdot PS_{influx} \cdot CL_{int}} \right]
\]

(Eq. A5)

2. Equation 5 of Pachter et al. (2022) and Equation 14 of Benet and Sodhi (2023)

These authors argue that when including cell membrane permeability, one should consider the difference, \([PS_{influx} - PS_{efflux}]\) as the operator rather than the individual parameters separately. Both \( PS_{influx} \) and \( PS_{efflux} \) should be not multiplied to \( C_{u,EC} \), since only \( PS_{influx} \)
should be multiplied to $Cu_{EC}$, but $PS_{\text{efflux}}$, to $Cu_{cell}$. Unfortunately, upon adopting their approach to events occurring within the eliminating cell,

$$V_{\text{cell}} \frac{dC_{\text{cell}}}{dt} = (PS_{\text{influx}} - PS_{\text{efflux}}) \cdot Cu_{EC} - CL_{\text{int}} \cdot Cu_{cell} \tag{Eq. A6}$$

the rate of elimination at steady state within the cells, $CL_{\text{int}} \cdot Cu_{cell}$ is now given by

$$CL_{\text{int}} \cdot Cu_{cell} = (PS_{\text{influx}} - PS_{\text{efflux}}) \cdot Cu_{EC} \tag{Eq. A7}$$

Following Pachter et al.’s logic as adopted above, i.e., substituting $Cu_{EC}$ by $f_{in} \cdot (1 - E_{in}) \cdot C_{in}$ and requiring that at steady state the rate of elimination within the eliminating cells must equal the rate of elimination across the liver, we obtain

$$CL_{H} = Q_{H} \cdot E_{H} = Q_{H} \left[ f_{in} \cdot (PS_{\text{influx}} - PS_{\text{efflux}}) \right] \left[ Q_{H} + f_{in} \cdot (PS_{\text{influx}} - PS_{\text{efflux}}) \right]^{-1} \tag{Eq. A8}$$

Clearly, Eq. A8 shows that Pachter et al.’s Equation 5 or Benet and Sohi's (2023) Equation 14 are incorrect, as it implies that even in the general case $CL_{H}$ (Eq. A8) is independent of $CL_{\text{int}}$. 
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