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SPECIAL ARTICLE

Galactose Clearance as an Estimate of Effective Hepatic Blood Flow: Validation and Limitations

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INTRODUCTION

Central to understanding the pathophysiology of organ dysfunction is awareness of organ perfusion. This flow–function relationship is well appreciated in diseases of the heart, brain, lungs, kidneys, viscera, and extremities and is no less important in the liver. The position of the liver interposed between the splanchnic mass and central circulation is crucial for many of its vital physiologic functions. Detoxification of drugs and endogenous products of metabolism, metabolism of exogenous carbohydrates, fats, and proteins, and the enterohepatic circulation rely on this strategic location and upon maintenance of portal blood flow.

Studies of portal hypertension and metabolism have been the traditional focus of hepatic blood flow determinations [7, 15, 16, 53, 73, 98]. The etiology of hepatic dysfunction in sepsis and other disease states is poorly understood with recent evidence suggesting that in sepsis, it may lie in inadequate liver perfusion [24, 33, 80]. The desire to have available a reliable technique for estimating hepatic blood flow for both investigational and clinical purposes is obvious. However, the relatively inaccessible dual blood supply from the hepatic artery and portal vein as well as the location of the hepatic veins deep in the hepatic parenchyma has proven relatively problematic in devising methods for determining hepatic blood flow (HBF).

TECHNIQUES FOR MEASURING HEPATIC BLOOD FLOW

The currently available techniques for estimating HBF can be divided into two general categories: (1) direct methods and (2) indirect methods [38, 42, 63]. Direct methods, which include timed hepatic vein output, plethysmography, and electronic or ultrasonic flow probes, are invasive requiring visualization and manipulation of the liver and/or its vessels and are therefore limited to animal investigation or selected surgical procedures. Indirect methods have varying degrees of invasiveness and thus a wider range of clinical and laboratory applications. Among these methods are the clearance techniques, inert gas washout and microsphere injection. Each technique has its inherent strengths and drawbacks and the method employed should be tailored to the purpose at hand.

For studies in our laboratory we were interested in utilizing a method for determining HBF in both small animals and man in order to study the effects of sepsis and other disease states on visceral blood flow. This required a quantitative, relatively noninvasive, and preferably simple and inexpensive technique. The clearance methods and inert gas washout are

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the only available methods that fit this description.

Inert gas washout (e.g., ^{133}Xe or ^{85}Kr) relies on an instantaneous distribution equilibrium of the gas between the blood and tissue after injection or inhalation according to a specific partition coefficient (λ) assumed to be 0.72 for liver and blood [42, 64, 66]. The rate of disappearance of tracer from the liver as measured by external counting devices is dependent on HBF. Disadvantages of this technique include (1) assuming a partition coefficient which may be influenced by hepatic fat (where $\lambda = 0.07$), drugs, or liver disease [42, 66]; (2) requirement of expensive counting devices and computers for isotopic measurements; (3) demonstration that the results were only accurate after intaportal injection of tracer as opposed to peripheral injection or inhalation [64].

CLEARANCE METHODS

Clearance methods in hepatology can be used to estimate either hepatic blood flow or function [44, 45, 55, 58, 61, 65, 68, 89, 95, 99]. The extent to which flow or function determines the rate of clearance depends on the biochemical efficiency of the liver for removal of the substrate relative to flow [45, 68, 99]. Substances efficiently removed relative to flow are used for HBF estimation whereas substances removed with low efficiency relative to flow are useful for estimating hepatic function. Clearance of flow-dependent substances estimates "nutrient" or "effective" hepatic blood flow (EHBF) as opposed to total hepatic blood flow as it measures flow to metabolically active tissue. Anatomical shunts have not been demonstrated in the liver making clearance methodology particularly suited to the liver [10, 52]. Perfused scar, as in cirrhosis, will not actively clear the substrate, therefore that portion of the total HBF will not be measured [62]. EHBF determination is the most useful measure when attempting to correlate flow with function.

Fick, in 1872 [23], outlined a method for determining organ blood flow by clearance determinations, a principle most commonly applied to studies of renal blood flow and glomerular filtration rate using *para*-aminohippurate and inulin, respectively, as test substances [8, 75]. The first requirement a substance must fulfill for use as a test substance in indirect clearance measurements as an estimate of EHBF is that the liver is the sole site for removal of the substrate, or if there are other routes, that these can be easily quantitated.

Clearance (Cl) is defined as the smallest volume of blood completely cleared of a substance per unit time. At steady-state blood levels the rate of infusion (I) equals the rate of removal (R). Clearance is then,

$$\mathrm{Cl} = R/c_i = I/c_i, \tag{1}$$

where c_i = inflow concentration (at steady state). Liver flow (F) can be calculated provided the inflow and outflow concentration (c_o) and removal rate are known:

$$F = R/(c_i - c_o). \tag{2}$$

Solving for R in Eqs. (1) and (2) and rearranging, flow and clearance can be related by

$$\mathrm{Cl} = F[(c_i - c_o)/c_i]. \tag{3}$$

The quotient $[(c_i - c_o)/c_o]$ is known as the extraction fraction or extraction ratio (ER). Thus,

$$\mathrm{Cl} = F \cdot \mathrm{ER} \tag{4a}$$

or

$$F = \mathrm{Cl}/\mathrm{ER} \tag{4b}$$

The ER determines the degree to which clearance approximates flow. If 100% of a substance is cleared in a single pass through the liver the ER = 1.0 and F = Cl. When ER is less than 1.0, flow can be calculated after determining c_o.

Bradley *et al.* [14] proposed the use of bromosulphalein (BSP) clearance as an estimate of EHBF in 1945. Both indocyanine green (ICG) and BSP are primarily removed by the liver with ICG having a slightly smaller extrahepatic uptake [30, 38, 92, 93, 97, 100]. BSP has been shown, also, to recirculate in the enterohepatic circulation making ICG the pre-

ferred substance between the two [57, 92]. ICG fulfills other important criteria in that it has a relatively high ER, low toxicity, and is easily measured [93]. Both substances have been extensively utilized for EHBF estimations [30, 92, 93]. However, the ER is not sufficiently high or constant with either ICG or BSP that it can be assumed [30, 42, 92, 93]. Hepatic vein catheterization is mandatory to determine c_o and ER. Catheterization of the hepatic veins is not without its faults [69]. Besides being invasive and somewhat technician dependent it assumes that the blood collected from the cannulated vein is representative of the venous drainage of the entire liver. Bradley demonstrated a variation of up to 20% in BSP concentration in blood successively sampled from different hepatic veins in the same liver [13]. If the catheter is inserted too deeply into the hepatic vein it may inhibit flow and affect clearance from that lobe. If not deep enough, blood may be aspirated retrograde from the vena cava. Also, when $c_i - c_o$ differences are small, analytical errors in the assay may unacceptably influence calculations [38, 93].

NONCATHETERIZATION METHODS

Substances so efficiently removed from circulation through the liver on a single pass that the $c_o = 0$ and thus ER = 1.0 can be used to estimate EHBF without resort to hepatic vein catheterization. Indeed, if hepatic vein concentration is so low that the available assay is insensitive, attempts to determine an ER may be more fraught with error than assuming an ER. Galactose and radiolabeled colloids are two substances that are completely or nearly completely cleared on single pass through the liver and their clearance, without hepatic vein catheterization, can be used to estimate liver blood flow [20, 21, 35, 36, 47, 87, 91, 92, 95].

Radiolabeled colloids given by bolus injection are efficiently taken up by the reticuloendothelial system (RES) primarily in the liver, spleen, and bone marrow [21, 47]. A slightly more complicated expression relates flow to the rate of change of concentration after bolus injection as opposed to steady-state determinations, but the principle is the same. The disadvantages of using radiolabeled colloid clearance to estimate HBF include (1) ER that may not always be greater than 0.90 owing to variations in particulate size [40]; (2) difficulty quantitating extrahepatic uptake [91]; (3) excessive radiation exposure especially locally to the RES cells [42]; and (4) requirement for expensive isotopic counting equipment.

For our purposes, galactose clearance at low concentrations (GCLC) has proven to be as near ideal a method for estimating EHBF as is available. To appreciate this a thorough understanding of the kinetics of galactose elimination is required. The kinetic analysis defines the limits within which one must operate for GCLC to be valid in both health and disease.

GALACTOSE ELIMINATION: LIVER AS SOLE SITE

Galactose is a simple sugar, a monosaccharide along with glucose of the disaccharide, sucrose. Enzymatic conversion of galactose to glucose proceeds in four steps with the first,

$$\text{Galactose} + \text{ATP} \xrightarrow{\text{GALACTOKINASE}} \text{Galactose 1-P} + \text{ADP}, \quad (5)$$

conversion of galactose and ATP to galactose 1-phosphate and ADP in the presence of galactokinase being rate limiting [27, 28, 50]. Galactokinase has been demonstrated in brain, kidney, gut, RBCs, and muscle but in far less concentration and with less activity than in the liver [11, 18]. RBC galactokinase activity is second to the liver with *in vitro* studies suggesting that galactose elimination by this route is on the order of 1% of the total elimination [34].

The primary site of extrahepatic galactose elimination is the urine [22, 84, 87]. Tygstrup demonstrated that urinary galactose elimination increased linearly with concentration [84]. As blood concentration increased the percentage of galactose elimination accounted for in the urine progressively increased because the liver has a maximal elimination capacity while the kidney does not. Studies on the uti-

lization of galactose after complete hepatectomy demonstrate that urinary galactose elimination accounted for the majority of galactose elimination with the sum of all other pathways on the order of 50% of urinary elimination [12]. In the concentration range required for EHBF determinations in the rat, urinary elimination is <1% of the total galactose elimination [70]. Therefore the sum of all extrahepatic sites for galactose elimination in the appropriate concentration range is roughly 2% fulfilling Fick's first criterion for the use of galactose clearance to determine EHBF.

Bauer, in 1906, suggested the use of galactose elimination as a test of the glycogenic function of the liver [6]. Numerous investigators described what can generally be considered the "galactose elimination capacity" (GEC) of the liver [5, 9, 17, 22, 41, 78, 79, 82, 83, 88, 96]. After a very large bolus injection of galactose the rate of change of galactose concentration in blood was determined by serial blood sampling. The concentrations of galactose achieved in this way were sufficient to saturate the hepatic elimination capacity, and the rate of change in concentration on time was constant. Hepatic vein catheterization at high concentrations revealed a constant hepatic arterial–hepatic venous concentration difference [87, 88]. The GEC became an accepted test of liver function and its value taken to reflect the functional hepatic mass [17, 82, 83, 88, 96]. Normal values were established and abnormal values were used as diagnostic and prognostic indicators [5, 9, 41, 78, 79]. When it was appreciated that the liver was the sole site for galactose removal, galactose was proposed as a test substance for EHBF estimation by the Fick principle using hepatic vein catheterization in the fashion employed with ICG and BSP [32, 87].

A more thorough analysis of the nature of galactose elimination as the concentration fell revealed that at lower concentrations the rate of decline in concentration was not linear, but exponential [88]. Hepatic arterial–venous concentration differences progressively narrowed until at concentrations less than 200 mcg/ml the hepatic vein concentration was virtually zero. Realizing that hepatic vein concentrations of zero meant complete extraction across the liver, Waldenstein and Arcilla, in 1956, recommended examination of this portion of the curve after bolus injection to estimate EHBF without the need for hepatic vein catheterization because the ER could be assumed to be 1.0 [95].

There are many disadvantages to studying the elimination kinetics of a substance after bolus injection [29, 32, 38, 74, 89, 94]. The blood galactose concentration depends on the amount administered, blood and tissue space volumes, and rate of equilibration between the two, and on the sum of all elimination pathways. To assume that the fall in concentration over time is due entirely to hepatic elimination it is necessary to assume that the volume of distribution is constant over a wide range of concentrations and that the equilibrium between the plasma and other compartments is rapid. Continuous infusion to steady-state concentrations (*i.e.*, $dc/dt = 0$) eliminates the necessity for volume of distribution determination because at steady state the removal rate equals the infusion rate [29, 94]. Volume of distribution differences produced by disease is negated in the calculations [38, 70].

Using the continuous infusion method and assuming the ER = 1.0 (as proposed by Waldenstein *et al.* [95]), we see by Eqs. (1)–(4) that EHBF can be determined by measuring low, steady-state galactose concentration (c_{ss}) at a known infusion rate (I) by

$$\text{EHBF} = I/c_{ss}. \tag{6}$$

Although theoretically sound, the practical application of this method awaited development of a galactose assay sufficiently sensitive in the concentration range required. Not until 1980 when Henderson and Fales developed a fluorometric method sensitive to galactose concentrations down to 10 mcg/ml [34] could GCLC as an estimate of EHBF without hepatic vein catheterization be confidently applied. Henderson *et al.* confirmed the near complete extraction of galactose across the liver (ER = 0.94) in subjects without hepatic disease by performing hepatic vein catheterization [35].

To apply GCLC as an estimate of EHBF in the face of disease or hepatic dysfunction it is important that one work within the limitations of the test. It is possible that a disease process could alter the metabolism of galactose in such a way or to such a degree that its clearance, even at low concentrations, was no longer flow dependent. Assuming an ER of 1.0 when, in fact, it was 0.80, would lead to a 20% underestimation of flow. The most extensive work on galactose elimination kinetics was done by Keiding and co-workers [43–45, 48, 50, 85, 99]. The "sinusoidal perfusion model" they developed and empirically validated provides a sound theoretical justification for using galactose clearance at low concentrations to estimate EHBF [44, 45, 48, 50, 99]. It also provides a means of defining the limitations of this method in health and disease based on awareness of the galactose elimination constants, V_{max} (or GEC) and K_m (the half-saturation concentration).

GALACTOSE ELIMINATION KINETICS

A general equation for hepatic galactose elimination is

$$GE = I - (dc/dt)\,DW - UE, \qquad (7)$$

where GE = hepatic galactose elimination, I = infusion rate, dc/dt = rate of change of galactose concentration, D = volume of distribution, W = animal weight, and UE = urinary galactose elimination, which takes into account a number of the previously discussed variables. Varying the infusion rate to achieve a range of blood concentrations allows plotting of GE as a function of concentration. Work in our laboratory as well as numerous others reveals that hepatic galactose elimination follows Michaelis–Menten saturation kinetics [43, 50, 70]. Its hallmarks are an early, concentration-dependent (first-order) elimination phase followed by a definable clearance maximum (V_{max} or GEC) at higher concentrations (zero-order phase).

Michaelis–Menten kinetics are classically used to describe enzyme reactions *in vitro* and have been "borrowed" from this arena to explain many of the physiologic phenomena observed *in vivo* [60]. In enzymology the constants, V_{max} and K_m, under defined reaction conditions are the "fingerprint" of that enzyme–substrate reaction. The K_m is actually the dissociation constant of enzyme and substrate. Suppose, for example, the simplest enzyme–substrate reaction

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad (8)$$

where E = free enzyme, S = substrate, ES = enzyme–substrate complex, P = product, and k = the rate constant in the direction of the arrow. Then,

$$K_m = (k_{-1} + k_2)/k_1 = [E][S]/[ES]. \qquad (9)$$

The K_m has units of concentration. The Michaelis–Menten equation,

$$v = (V_{max} \cdot c)/(K_m + c), \qquad (10)$$

where v = reaction velocity and c = substrate concentration, reveals that when $K_m = c$, then $v = \frac{1}{2}V_{max}$. Thus K_m is termed the half-saturation concentration. For each enzyme–substrate system K_m has a characteristic value that is independent of the quantity of enzyme but is dependent on the reaction conditions such as pH, temperature, and reaction milieu. Alterations in its value imply a disturbed enzyme–substrate reaction.

Rearranging Eq. (10), we obtain,

$$1/v = (K_m/V_{max})(1/c) + 1/V_{max}, \qquad (11)$$

which is the Lineweaver–Burk equation [37, 56]. It describes a line when $1/v$ is plotted against $1/c$ with a y intercept of $1/V_{max}$ and slope of K_m/V_{max}. Plotting of $1/v$ vs $1/c$ allows determination of these constants by extrapolation of $c \to \infty$.

When applied to *in vivo* elimination processes the constants are usually referred to as "apparent" K_m and V_{max} as the *in vivo* values may not necessarily reflect the *in vitro* values. *In vivo* reactions have membrane bound proteins, metabolite production, several rate-limiting steps, varying metabolic conditions, and directional sinusoidal perfusion, to name a few of the conditions, that make it distinctly dif-

ferent than controlled enzyme reactions *in vitro* [45].

In enzymology V_{max} has standardized units relative to a known quantity of enzyme. Its value is so characteristic that the purity of an enzyme preparation can be tested by how close it approximates the ideal value. When extended to explain complex *in vivo* reaction processes the denominator must be clearly defined. We will define units of velocity as amount of galactose removed per unit time per 100 g of body weight (as opposed to rate per individual man [82, 96] or rate per g of liver weight [43, 50] as has been used elsewhere).

Figure 1 displays the GE vs concentration of 22 rats after determination of galactose concentrations, dc/dt, D, and UE at known infusion rates. Figure 2 plots 1/GE vs $1/c$ from the same data points with linear regression to determine the elimination constants. Superimposed on Fig. 1 is the line generated from the elimination constants by Eq. (10). Having determined that galactose elimination obeys Michaelis–Menten saturation kinetics we can now define the limits within which galactose clearance is flow dependent through awareness of V_{max} and K_m.

SINUSOIDAL PERFUSION MODEL

The basic Michaelis–Menten equation (Eq. (10)) is independent of flow and is therefore limited when applied to *in vivo* processes. It is obvious by Eqs. (2) and (3) that both c_i and c_o are flow dependent. The hepatocytes are not bathed in a solution with a constant concentration of substrate as is the case in test tubes. Blood flows unidirectionally through sinusoids of a definable length and diameter at a prescribed rate [4, 26, 27]. The inflow concentration of galactose exceeds the outflow concentration with a gradient through the liver. The hepatocytes in the proximal portion of the sinusoid see a higher galactose concentration than those downstream. The "sinusoidal perfusion model" takes into account this falling concentration of substrate along the length of the sinusoid based on the Michaelis–Menten elimination nature at each cross section of the sinusoid [44, 45, 99]. The assumptions of the model are that blood flows unidirectionally through sinusoidal units, each functionally identical, with instantaneous diffusion equilibrium at each transverse section and that the rate-limiting step in removal is irreversible obeying Michaelis–Menten kinetics [45]. The "new" Michaelis–Menten equation which applies to clearance determinations in hepatology is

$$v = (V_{max} \cdot \hat{c})/(K_m + \hat{c}), \qquad (12)$$

where $\hat{c}$ = the logrithmic average concentration,

$$\hat{c} = (c_i - c_o)/\ln(c_i/c_o). \qquad (13)$$

Considering the fall in concentration along the sinusoids becomes especially important

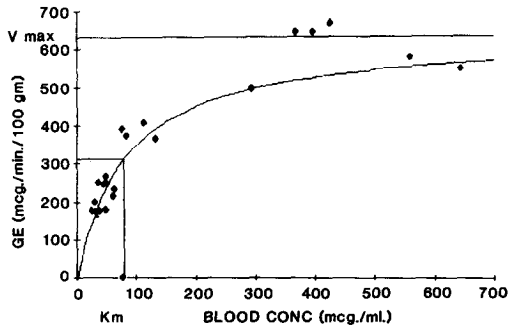

FIG. 1. Galactose elimination kinetics. Points represent individual animals. Superimposed line as determined by the Michaelis–Menten equation using the elimination constants, V_{max} and K_m, from Fig. 2.

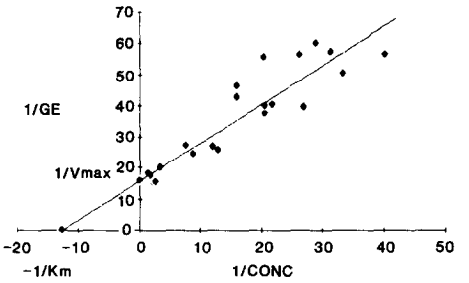

FIG. 2. Lineweaver–Burk plot of 1/GE vs 1/conc. Extrapolation of concentration to infinity allows determination of V_{max} and K_m.

when determining the dissociation constants. K_m and V_{max} are by definition independent of flow. The K_m in Figs. 1 and 2 were determined from values of c_i and cannot be considered flow independent. Other studies report elimination constants based on inflow concentration, outflow concentration [15, 98], average concentration (i.e., $(c_i + c_o)/2$) [51], homogenized liver tissue [18], and finally logrithmic average concentration [48, 50]. Figure 3 shows representative Lineweaver–Burk plots when the elimination constants are determined by c_i, c_o, or $\hat{c}$. The V_{max} is unaffected by the value used (because when $c_i \gg K_m$, $c_i \doteq c_o \doteq \hat{c}$) but the K_m differs greatly. Keiding demonstrated empirically that $\hat{c}$ was independent of flow [48, 50]. As flow decreases, c_i increases. However, because the transit time is increased in the sinusoid, there is more time for clearance, and c_o decreases. The increase in c_i and decrease in c_o produce no change in their logrithmic average.

The "apparent" K_m for galactose elimination in the example sited previously, by the sinusoidal perfusion model, is 30.1 mcg/ml [70] (assuming conservatively a 90% first-pass clearance at this concentration), which is in close agreement with values reported by others [18, 27, 43, 48, 50].

VALIDATION OF GCLC AS AN ESTIMATE OF EHBF

Another way of writing Eq. (12) which contains all observable parameters is

$$v = FK_m \cdot \ln(c_o/c_i) + V_{max}. \quad (14)$$

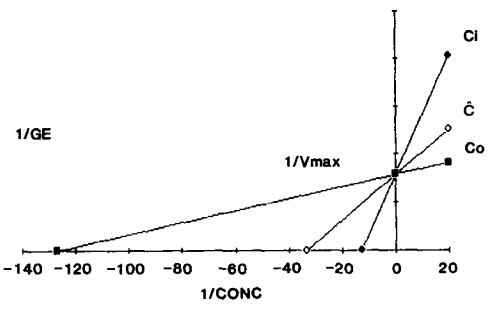

FIG. 3. Kinetic constants as a function of c_i, c_o, and $\hat{c}$. Only $\hat{c}$ is flow independent.

From the Fick principle we know that

$$v = F(c_i - c_o). \quad (15)$$

Solving for c_o and rearranging we have

$$v = F(1 - e^{-(V_{max}-v)/FK_m}), \quad (16)$$

which is the general clearance equation valid for all values of c_i. This relationship is no more than the familiar clearance relationship expressed in Eq. (4a) where v/c_i = clearance, F = flow, and $(1 - e^{-(V_{max}-v)/FK_m})$ = extraction ratio. The extraction ratio determines the degree to which clearance approximates flow. Figure 4 is a graph of extraction ratio as a function of $(V_{max} - v)/FK_m$. Values of $(V_{max} - v)/FK_m > 2.3$ translate into ERs greater than 0.90.

This model allows prediction of the ER based on an awareness of the elimination constants [44]. Certain restriction of the parameters permits classification of substances into categories based on whether liver blood flow or liver function is the primary determinant of clearance [99]. If we require that $c \ll K_m$, then $v \ll V_{max}$ (i.e., elimination proceeding well below the maximal rate), and Eq. (16) reduces to

$$v/c_i = F(1 - e^{-V_{max}/FK_m}). \quad (17)$$

With $V_{max}/FK_m > 2.3$, Eq. (17) approximates

$$v/c_i = F, \quad (18)$$

which states that clearance = flow. Clearance of substances with values of $V_{max}/FK_m > 2.3$ can be classified as flow dependent. It is these substances that may be suitable for application to clearance determinations as estimates of flow. At the other extreme, when V_{max}/FK_m is less than 0.05, Eq. (17) reduces to

$$v/c_i = V_{max}/K_m, \quad (19)$$

which, if rearranged, is the Michaelis–Menten equation (Eq. (10)) when $c_i \ll K_m$.

When v_{max}/FK_m is small, clearance is not dependent on flow, rather clearance is dependent on function (V_{max} and K_m are flow independent values [40, 48]). Another way of stating this is when the biochemical efficiency for clearance of a substrate is high relative to

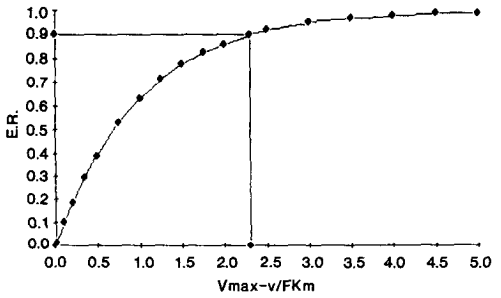

FIG. 4. Extraction ratio as a function of V_{max}/FK_m. Values of $V_{max}/FK_m > 2.3$ are associated with ER > 0.90.

flow, the ER will be high and perturbations in flow will be accompanied by proportionate changes in clearance. When the biochemical efficiency is low relative to flow, functional disturbances (i.e., V_{max}) will affect clearance rate. The schematic in Fig. 5 demonstrates these two extremes [99]. When substances have values $0.05 < V_{max}/FK_m < 2.3$ clearance depends to varying degrees on flow and function.

Table 1 shows values of V_{max}/FK_m for a variety of substances including galactose in the example sited earlier (from [43, 45, 47, 70]). Galactose can be seen to have values of $V_{max}/FK_m > 2.3$ for the *in vivo* preparations. The isolated-perfused liver preps tend to have lower values of V_{max}/FK_m as a result of a reduced

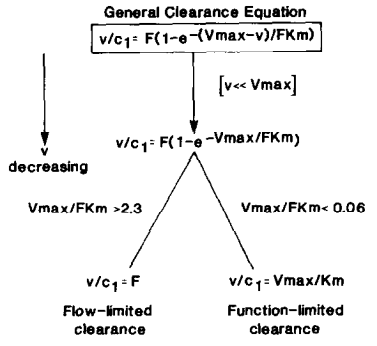

FIG. 5. General clearance equation. Clearance of substances with $V_{max}/FK_m > 2.3$ primary flow-dependent. Clearance of substances with $V_{max}/FK_m < 0.06$ function (enzyme) dependent. When $2.3 > V_{max}/FK_m > 0.06$, clearance is intermediate. (Adapted from Winkler *et al.* [99]).

TABLE 1

ESTIMATES OF V_{max}/FK_m FOR VARIOUS SUBSTANCES

Substance	Species, procedure	V_{max}/FK_m
Flow-dependent clearance, i.e., $V_{max}/FK_m > 2.3$		
Ethanol	Human liver, *in situ*	10 (5.5–13.3)
Propranolol	Rat liver, perfused	>10
Galactose	Human liver, *in situ*	3.5–12
Lidocaine	Rat liver, perfused	5
Galactose	Rat liver, *in situ*	4.0
Galactose	Rat liver, *in situ*	2–4
Intermediate clearance, i.e., $0.06 < V_{max}/FK_m < 2.3$		
Galactose	Pig liver, perfused	2.2
Ethanol	Pig liver, perfused	1.9
ICG	Human liver, *in situ*	1.0
BSP	Human liver, *in situ*	1.0
ICG	Cat liver, *in situ*	0.3
Antipyrine	Monkey liver, *in situ*	0.3
Function (enzyme)-dependent clearance, i.e., $V_{max}/FK_m < 0.06$		
Antipyrine	Pig liver, perfused	0.05

Note. Classification of sustances as flow-, intermediate-, or function-dependent based on relative value of kinetic constants to flow. (Adapted from Keiding *et al.* [43, 45, 47] and [70]).

V_{max} presumably caused by the isolation procedure.

In the example presented the $V_{max}/FK_m = 4.00$ for galactose elimination in the rat [70]. The limit of galactose elimination that can be tolerated before $(V_{max} - v)/FK_m$ is less than 2.3 is when $v/V_{max} = 0.40$. For values of v up to 40% of V_{max} the ER will be greater than 0.90 and clearance will approximate flow. Galactose elimination less than 40% of the GEC is achieved with inflow concentrations less than 60 mcg/ml. As v increases toward V_{max}, the value $(V_{max} - v)/FK_m$ becomes progressively smaller and flow becomes a progressively less important factor in clearance. This model is consistent with earlier studies that demonstrated the flow independence of the GEC (i.e., when $v = 95\%\ V_{max}$) [40]. GEC and antipyrine clearance correlate well as tests of liver function as would be expected from the low values of V_{max}/FK_m for antipyrine [1].

As an example of how this model can be used to validate and set limits for GCLC as an estimate of EHBF in disease, we studied the galactose elimination kinetics in a septic rat model [70]. The V_{max} fell 25% relative to controls while K_m was unaltered. The decreased galactose clearance produced a value of $V_{max}/FK_m = 3.82$ which meant that the extraction ratio remained quite high at low concentrations (i.e., low values of v). This provides a good example of how galactose elimination is still flow dependent at low concentrations despite a modest functional impairment. A reduction in V_{max} of 60% relative to control without a change in flow or K_m would be required to reduced the ER, at low concentrations, to less than 0.90. GCLC in this example of sepsis is a valid estimate of EHBF.

The V_{max} for galactose elimination is much higher in humans than in rats [47, 82, 96], with the K_m being similar [47]. Values for V_{max}/FK_m in humans are therefore higher than in rats. The lower range for *in situ* human liver presented in Table 1 represents the extreme of liver dysfunction in cirrhosis as estimated from reported values of GEC [47]. What this means is that higher values of v relative to V_{max} can be tolerated in humans, still retaining an ER > 0.90. This expands the concentration range within which GCLC estimates EHBF in humans compared to the relatively narrow range tolerable with rats [70].

Table 1 gives values for the V_{max}/FK_m of ICG. Even in the most ideal situation where v is infinitesimally small, ICG clearance is only approximately 70% dependent on flow. Any reduction in V_{max} or increase in v or K_m will lessen the degree to which ICG clearance reflects EHBF. That is not to say that ICG cannot be used for EHBF determination. As long as hepatic vein catheterization is performed to determine c_o, flow can be calculated by the Fick principle and Eq. (2). However doubt must be shed on the interpretation of results obtained from determination of the ICG clearance half-life, a commonly employed method for estimating EHBF [54, 65]. Paumgartner *et al.* [65] showed that ICG half-life at low doses of ICG was flow dependent and at high doses was function dependent. Although this may more or less be the case, it is not nearly as sensitive as is required to make statements about the magnitude of flow changes. In their own study they reported the effects of disease on both V_{max} and K_m that would make ICG decay or half-life almost completely function dependent even at the smallest doses.

Little mention has been made of how flow changes alone would affect clearance. An increase in F would reduce the value of V_{max}/FK_m and reduce the ER. This is a general principle of clearance which recognizes that the ER varies inversely with flow. As transit time increases (i.e., decreased flow rate) a higher percentage of substrate is eliminated in a single pass. Decreased transit time results in less efficient elimination. Clearance, however, will always change in the same direction as flow (assuming constant V_{max} and K_m) with the limiting elimination rate equaling V_{max}/K_m (Fig. 6). Because increased flow reduces ER, one must work at smaller values of v relative to V_{max} to satisfy the requirements of an ER > 0.90, otherwise flow will be underestimated.

OTHER CONSIDERATIONS IN GALACTOSE CLEARANCE

On theoretical grounds GCLC is an extremely good estimate of hepatic blood flow without hepatic vein catheterization. We require steady-state galactose concentrations between 20 and 60 mcg/ml in the rat due to loss of sensitivity of the assay below 20 mcg/

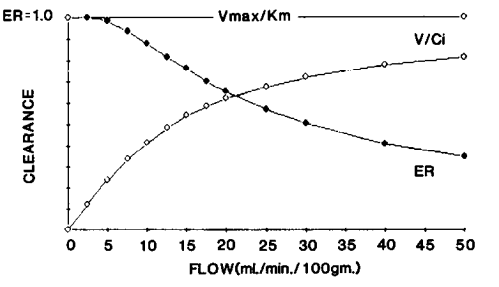

FIG. 6. Clearance and extraction ratio vs flow. Extraction ratio decreases as flow increases. Clearance increases with flow to a maximum of V_{max}/K_m.

ml [34]. The theoretical framework of the sinusoidal perfusion model which is supported by experimental observations allows prediction of the usefulness and limitations of GCLC as an estimate of EHBF in disease based on knowledge of how the disease affects the elimination kinetic constants.

GCLC has been shown not to be significantly different than flow probe or roller pump determinations [31, 76]. Henderson *et al.* demonstrated that doubling the infusion rate at low concentrations doubled the clearance rate with a slight drop in ER as would be expected from the model [35].

In addition to the theoretical advantages, galactose has characteristics that lend it well to routine use. Galactose is a normal constituent of all mammalian diets and is safe administered orally or parenterally [19, 72, 77] except in those rare individuals with hereditary enzyme deficiencies who suffer from galactosemia [71]. There have been no reported complications with its use in humans, unlike with ICG and BSP [78, 79]. Also hepatic vein catheterization and its potential complications are avoided [69].

Many processes in the liver have been shown to be inducible by endogenous substances or drug therapy. Phenobarbital has been shown to increase BSP transport [25], decrease ICG half-life [25, 59], and increase antipyrine clearance [90]. Galactose metabolism, on the other hand, has clearly been demonstrated not to be affected by phenobarbital induction [46]. Adult rats fed a 40% galactose diet for 5 days did not show an increase in GEC although 20 days on the diet resulted in a 20% increase in V_{max} suggesting that adaptive mechanisms are slow [18]. This lack of inducibility and relatively constant V_{max} is desirable in clearance methodology as a fluctuating V_{max}/FK_m would certainly complicate clearance interpretations.

Galactose is involved in carbohydrate metabolism and the effects of perturbations in this system on galactose metabolism have been studied. Insulin has been shown not to affect galactose elimination rate [5, 67]. A large galactose bolus produces a transient rise in glucose levels, but a large glucose load does not affect galactose elimination rate [32].

Galactose elimination is an energy-dependent process utilizing ATP in the rate-limiting step [27, 28, 50]. The reaction of galactokinase with ATP at fixed galactose concentrations obeys Michaelis–Menten kinetics with a K_m = 0.12–0.20 mM [2, 3, 81]. The approximate ATP concentration in rat liver studied in our lab is 5.3 mM in sham-operated animals and 3.3 mM in the livers of septic rats [80] (estimated cytosol concentration from whole liver freeze clamped values 10 hr after cecal ligation and puncture). Therefore, the ATP concentration in this severe stress state is more than an order of magnitude greater than the *in vitro* K_m of galactokinase-ATP. It would take a reduction in ATP concentration below compatibility with cell life to desaturate ATP from galactokinase making it unlikely that a reduced hepatic ATP alone will affect galactose clearance in clinical situations.

Elevated ADP [2] and elevated NADH/NAD ratios [39] *in vitro* have an inhibitory effect on conversion of galactose to galactose 1-P, however, these effects have not been quantitated *in vivo*. The septic rat model discussed earlier has a decreased hepatic ATP, elevated ADP, and chemical evidence of a more reduced state [80]. The V_{max} in that case was down 25% but the K_m was unaffected [70]. The etiology of the reduction in V_{max} is not clear; however, the V_{max}/FK_m remained high validating GCLC as an estimate of flow despite this severe perturbation in the bioenergy status of the liver.

Ethanol is the only substance shown clinically in man to alter galactose elimination kinetics [39, 49, 86]. It acts as an uncompetitive inhibitor producing a reduction in both V_{max} and K_m [49]. Concomitant ethanol ingestion may affect the validity of galactose clearance as a flow estimate.

Table 1 lists propranolol, lidocaine, and ethanol as other substances whose clearance is flow dependent and these substances are cleared primarily by the liver [7, 15, 51, 73]. However, unlike galactose, these substances are known to affect systemic and/or regional

blood flow or produce untoward physiologic effects rendering them inappropriate for use as test substances to determine hepatic blood flow [54].

CONCLUSION

Methods for determining hepatic blood flow have varying degrees of invasiveness and accuracy. Galactose is nearly an ideal test substance for clearance at low concentrations to approximate EHBF without the need for hepatic vein catheterization. The sinusoidal perfusion model provides a means of predicting the limitations of the test based on determination of the kinetic constants—this is particularly useful when the size of the animal precludes hepatic vein catheterization. A moderate functional impairment can be tolerated with the test retaining its validity due to the liver's efficency at removing galactose. The safety and simplicity of the procedure make it suitable for repeated application to either humans or animals. Development of the fluorometric assay for galactose has made determination of low galactose concentrations possible. Modification of commonly available laboratory analyzers has made galactose assay in whole blood possible on a routine basis expanding the potential for clinical and investigational application of GCLC as an EHBF estimate.

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