

# The Physiologic Basis for Clearance Measurements in Hepatology

K. WINKLER, L. BASS, S. KEIDING & N. TYGSTRUP

Dept. of Clinical Physiology, Hvidovre Hospital, and Division of Hepatology, Medical Dept. A, Rigshospitalet, Copenhagen, Denmark, and Dept. of Mathematics, University of Queensland, Brisbane, Australia

Winkler, K., Bass, L., Keiding, S. & Tygstrup, N. The physiologic basis for clearance measurements in hepatology, *Scand. J. Gastroenter.* 1979, **14**, 439-448.

Three hepatic clearance regimes (flow-limited, general, and enzyme-limited) can be defined from a model of hepatic perfusion-elimination relationships. Substances that can be used for clearance measurements can thus be classified into three categories according to the relation between their kinetic elimination constants ( $V_{max}$ ,  $K_m$ ) and hepatic blood flow. The pathophysiologic and clinical importance of the clearance regimes is discussed with special emphasis on the effect of changes in hepatic blood flow and liver function. The criteria for choosing test substances within each regime are stated. This choice depends on the object of study for a clearance measurement (blood flow, drug elimination, liver function). Only the enzyme-limited clearance regime is suited for direct assessment of quantitative liver function ('true clearance'), while the other regimes depend more or less on blood flow.

*Key-words:* Clearance; liver blood flow; liver function

*K. Winkler, M.D., Dept. of Clinical Physiology, Hvidovre Hospital, Kettegaard Alle 30, DK-2650 Hvidovre, Denmark*

Since Lewis (13) introduced the clearance of sulfo-bromophthalein (Bromsulphalein  $\text{R}^{\circ}$ ) as a measure of liver function, a multitude of publications on this subject have appeared. From these papers no unity of concept on methodology and interpretation has emerged. In contrast to the situation in nephrology, the difference in practically applicable methods becomes evident, because the use of renal clearances is founded on well-known kidney functions. The purpose of the present communication is to review and define the physiologic basis for the use of clearance measurements in hepatology, to serve as a guide for further use of the clearance principle or for the choice of alternative methods.

## DEFINITION OF CLEARANCE

As introduced in biology by Møller et al. (14), clearance (amount eliminated per time unit divided by blood concentration) was employed as a purely operational description of the removal rate of urea as a means to obtain a constant expression for removal of a substance with concentration-de-

pendent elimination.

However, to obtain an absolute independence of a clearance of a given substance from variations in the blood concentration, the elimination of the substance must kinetically obey the first-order reaction ( $dc/dt = -kc$ ), being that process in which the clearance (' $k$ ') is independent of the concentration ( $c$ ). The implication is that before a substance is chosen for clearance measurements, its removal kinetics has to be examined to ensure that it follows the first-order kinetics. The consequences of using clearance measurements by a substance whose kinetics deviates from the first-order process is that the calculated clearance is dependent on the dose given and, further, that the clearance varies with changes in liver function in a not easily comprehensible manner.

## CLEARANCE AND QUANTITATION OF LIVER FUNCTION

While qualitative diagnostic procedures, such as liver histology or common 'liver tests', rest on a rather well-established foundation, measurements

of quantitative liver function are difficult, and no agreement on methods exists today. As the 'vital' liver functions are at present unmeasurable in terms of known processes, the liver function must be measured by the rate of elimination from the body of indicator substances that are removed (or produced) by the liver, on the assumption that the processes responsible for the elimination vary with changes in the vital functions and, optimally, that they vary proportionally to variations in the functional liver cell mass.\*

It must be supposed that most processes in the liver are due to enzymatic reactions (or, for example, active transport mechanisms of a basically similar nature). They can be assumed to follow the simplest of such processes, the Michaelis-Menten reaction, which describes the relation of the conversion rate to the substrate concentration by two parameters, enzymatic activity ( $V_{max}$ ) and the affinity constant ( $K_m$ , the half-saturation concentration). This will be used as a basis for the following considerations.

The functional capacity can be evaluated by the  $V_{max}$  of a rate-limiting process of the liver by measuring the rate of elimination at a sufficiently high concentration, where the eliminatory mechanisms are saturated. (The usual procedure in biochemistry, where successively increasing loads below saturation are used and  $V_{max}$  is calculated from, for example, a Lineweaver-Burk plot, is less suitable for man, if only because of the time-consuming procedure.) The blood concentration to ensure saturation is determined by the  $K_m$  value, an estimate of which therefore has to be available (a concentration of  $19 K_m$  gives an elimination rate of 0.95  $V_{max}$ ). An example of a quantitative liver function test based on the above criteria is the galactose elimination capacity test (23).

It is not always possible to design tests of liver functions based on a measurement of  $V_{max}$  at high blood concentrations in man, because these

may be toxic or have unwanted haemodynamic or osmotic effects or because the large amount of test substance may be very expensive. In this case quantitative liver function may still be measured by the use of a clearance measurement employing a lower blood concentration.

The clearance principle can be deduced from the general Michaelis-Menten relation of a well-mixed system

$$v = \frac{V_{max} c}{K_m + c} \quad [1]$$

( $v$  = velocity of elimination,  $c$  = concentration,  $V_{max}$  and  $K_m$  kinetic constants of the enzyme). When the concentration is very low ( $c \ll K_m$ ), [1] is transformed to

$$v = \frac{V_{max} c}{K_m} \quad [2]$$

or, by rearranging, to

$$\frac{v}{c} = \frac{V_{max}}{K_m} \quad [2a]$$

The left-side expression is by definition the clearance (with the dimensions volume · time<sup>-1</sup>), which thus equals  $V_{max}/K_m$ . On the assumption that  $K_m$  is a non-variable parameter given by the physicochemical properties of the enzyme molecule, clearance is proportional to  $V_{max}$ .  $V_{max}/K_m$  is measured by the clearance, which then can be used as an expression of the functional hepatic mass. When a substance is given *in vivo* as a short-lasting intravenous injection resulting in a concentration so low that removal is proportional to concentration, the average blood concentration after mixing in its volume of distribution declines, following a simple exponential function (first-order process), from which clearance can be calculated. The constant infusion technique can equally well be used and has the advantage that calculation of the volume of distribution is not required.

Apart from the use in measurements of liver function, the clearance principle has been used for estimating the hepatic blood flow and for pharmacokinetic studies. These applications will be discussed later on.

\*The anatomical liver mass is made up of all liver cells, while the 'functional' mass is defined by their function—that is, by including only those cells that contribute to some degree to metabolic function, thus excluding, for example, those bypassed by the hepatic blood flow. It can be expressed in terms of the number of normal cells that would yield the same metabolic work as that of the actual case.

## A GENERAL THEORY OF CLEARANCE BY A MODEL OF HEPATIC PERFUSION-FUNCTION RELATIONSHIPS

For a further understanding of clearance measurements, and to be able to define the conditions during which substances can be employed for such measurements, a more detailed examination of the ways by which the liver handles these substances is necessary.

In eq. [2], where clearance was defined in terms of simple kinetics, the concentration ( $c$ ) refers to an *in vitro* system with complete mixing and thus, in the intact liver, to the mean intracellular concentration at the active sites of the enzymes. In the structured organ it is evident that a concentration gradient must exist between afferent and efferent blood flow when a substance is eliminated (or synthesized) by the liver. Therefore, when the concentration to be used in clearance expressions is estimated, this concentration gradient should be taken into consideration. At high concentrations the sinusoidal concentration approximates the input (and output) concentration, but in the low range of concentration, where clearance measurements have to be performed (for example,  $c < 0.05 K_m$ ), this average differs substantially from the input concentration. As the average sinusoidal concentration cannot be measured directly, and as the difference from the test tube conditions is caused by the architectural organization of the perfused organ, it is necessary to use a model describing the elimination in terms of both hepatic perfusion and enzymatic removal kinetics—the two main factors that determine the sinusoidal concentration profile. From this model, the sinusoidal concentration profile and the overall rate of elimination of the liver can be calculated.

The mathematics of the simplest model, which have been evaluated using galactose as a test substance, have been described elsewhere (2, 28). It describes the sinusoids (including the perisinusoidal space) as cylindrical tubes lined by one layer of liver cells, all sinusoidal units having identical functional properties. (A generalized model has been constructed for the case when these properties have statistical distributions (3).) It is assumed that instantaneous equilibrium of diffusion occurs at each transverse section. It is further assumed

that the rate-limiting step of the elimination of the substance follows Michaelis-Menten kinetics, whether due to transport into the cell or enzymatic conversion. The general solution, which contains all measurable parameters of the system and which describes the rate of elimination during steady-state conditions, is obtained by solving a differential equation for the concentration profile, with the resulting connection between the observables

$$v = FK_m \cdot \ln \frac{c_o}{c_i} + V_{max} \quad [3]$$

( $c_i, c_o$  = input, output concentration,  $v$  = rate of elimination,  $F$  = hepatic blood flow).

As, from Fick's principle,

$$v = F(c_i - c_o) \quad [4a]$$

elimination of  $c_o$  from [3] and [4a] gives

$$v = Fc_i(1 - e^{-(V_{max} - v)/FK_m}) \quad [4b]$$

This solution is valid for  $c_i$  varying from zero to infinity and has the advantage of not containing the hepatic venous outflow concentration, which may be difficult to measure accurately when it is low. At high rates of removal (or concentrations) the solution approximates the condition where  $V_{max}$  can be measured directly.

From the equation [4b] the kinetic behaviour of any substance that satisfies the assumptions of the model can be predicted. By, for example, changing from a very high to a lower removal rate the kinetics changes from that of the saturated regime (where  $V_{max}$  can be measured directly) to that of the general regime (eq. [4b]), where the removal rate is related to the concentration in a complicated way.

## THE CLEARANCE REGIMES

From the general solution of the model a *general clearance regime* can be defined during certain restrictions of the parameters, which results in a simpler rate expression when the removal rate ( $v$ ) is low ( $v/FK_m \ll 1$ , or  $v/V_{max} \ll 1$ ):

$$v/c_i = F(1 - e^{-V_{max}/FK_m}) \quad [5]$$

It should be noted that this general clearance differs from the clearance expression of eq. [2].

While it is a correct clearance in the sense that removal rate is proportional to concentration, it is not proportional to  $V_{max}/K_m$ . It is furthermore seen that the clearance depends on hepatic blood flow (F). This dependence is caused by the organized perfusion of the intact liver ('manifolding' (28)). Substances of the general clearance regime therefore are not suitable for measurement of liver function (as defined above) or liver perfusion.

It is, however, possible, by further examination of the parameters of the model (2), to construct a scheme (Fig. 1) that defines different types of

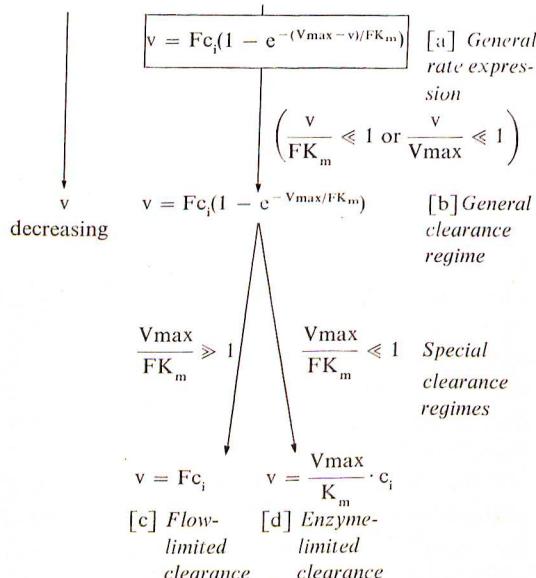


Fig. 1. The clearance regimes.

clearance and their special use. The limiting conditions of the scheme can also be stated both in terms of concentrations and in terms of rate of elimination. The reader should note that if a value of  $v$  is given, it is easy to calculate the corresponding value of  $c_i$  from eq. [4b] but not *vice versa* (because the equation is transcendental in  $v$ ).

#### *The enzyme-limited clearance regime*

When  $V_{max}$  of a given substance is small in relation to the product  $FK_m$ , the general clearance formula [eq. 5] by a series expansion can be approximated to

$$v/c_i = V_{max}/K_m \quad [6]$$

which is identical with eq. [2a].

This clearance does not vary with the perfusion and is proportional to  $V_{max}$ . This is the only type of clearance that can be used directly as a measure of quantitative liver function, in the sense used in this paper. Substances with  $V_{max}/FK_m$  much smaller than unity are eliminated in the enzyme-limited regime for all values of input concentrations used in practice.

#### *The flow-limited clearance regime*

When a substance is removed in the liver by a process with a large amount of enzyme and a high affinity (small  $K_m$ ) relative to the hepatic blood flow—that is, when  $V_{max}/FK_m \gg 1$ —the general clearance expression is reduced to  $v = c_i F$  (eq. [c] of Fig. 1). The removal is now wholly determined by the perfusion (all substance removed in a single passage). For practical purposes the flow-limited regime is one in which the elimination rate ( $v$ ) is determined to sufficient accuracy by the expression  $F \cdot c_i$ . (This does not necessarily mean that  $c_i$  is unmeasurably small.) This flow-limited regime can be used for measurement of the perfusion (but not of the function, as  $V_{max}$  and  $K_m$  have disappeared from the equation).

#### *Classification of clearance substances*

The classification of substances for clearance measurements generally depends on the knowledge of their kinetics and specially of the  $V_{max}/K_m$  ratio. Adequate data in man are almost non-existent at present, and most have to be extrapolated to man from animal experiments. Examples of substances whose kinetics at low concentration approach the flow-limited clearance are galactose, ethanol, and taurocholate (all having  $V_{max}/FK_m$  above 2–3). Dyes like Bromsulphalein and indocyanine green ( $V_{max}/FK_m$  of approximately unity) belong to the general clearance regime, while antipyrine ( $V_{max}/FK_m$  about 0.05) is an example of the enzyme-limited regime. It should be noted that these three clearance regimes are in principle identical with zone A, B, and C as defined by Brauer (5).

The techniques by which kinetics are determined are principally those of biochemistry, applied to the perfused liver or to the intact organism. Paired data of rate of elimination and blood concentra-

tion may be used together with the hepatic blood flow to calculate the kinetic constants (10). A crude idea of the kinetics can be obtained by analysis of the blood elimination curve after a single intravenous injection (zero- or first-order reaction). Furthermore, the classification can be made roughly by an estimate of clearance, for example during constant-infusion technique. In normal man the clearance of the flow-limited regime should approximate the hepatic blood flow (approx.  $1000 \text{ ml} \cdot \text{min}^{-1}$  of plasma), while substances of the general clearance regime have a plasma clearance of about  $500 \text{ ml} \cdot \text{min}^{-1}$ . The enzyme-limited regime has a clearance below  $100 \text{ ml} \cdot \text{min}^{-1}$ . Other approaches for determinations of Vmax and  $K_m$  are described by Paumgartner et al. (15) and Goresky et al. (9).

#### *Other clearance concepts*

The clearance measurements are usually based on measurements of peripheral (arterial) blood concentrations, which during intravenous injections of the test substance equal hepatic input concentration ( $c_i$ ) during steady state. For substances belonging to the general or flow-limited regime this clearance ('apparent' (27) or 'systemic' (17) clearance) differs from that defined above (clearance =  $V_{\max}/K_m$ ), which is independent of hepatic blood flow and only depends on the enzymatic properties of the removal system ('true' or 'intrinsic' clearance\*).

Thus, in these two clearance regimes two different clearance expressions are available, and the choice between them depends on the actual problem to be studied. The 'systemic' clearance describes the removal rate of a substance from the organism and is important in pharmacokinetics, for example for prediction of the blood concentration of drugs. On the other hand, when an expression of liver function is wanted, this clearance is 'apparent', as liver function ( $V_{\max}/K_m$ ) cannot be directly estimated from this clearance. Here

'intrinsic' or 'true' ('true' with respect to liver function) clearance has to be employed, either calculated from determination of  $V_{\max}$  and  $K_m$  or by using a substance of the enzyme-limited regime, where the two clearance expressions coincide.

It should, however, be noted that calculation of 'true' or 'intrinsic' clearance depends strongly on the type of model used. This is due to the choice of the concentration at which the relevant enzymes operate. Thus several pharmacologists (17, 24) have chosen the hepatic venous outflow concentration as the ambient concentration throughout the liver, in contrast to the complete concentration profile used here. For details, including the mathematics (11) the readers should consult the papers quoted. At present this conceptual discrepancy has not been experimentally resolved (12, 18–20). Thus the model by which an intrinsic clearance is calculated should always be stated, to avoid ambiguity.

#### *Hepatic extraction and clearance*

When samples of hepatic venous blood are available, the arteriohepatic venous difference (the 'extraction') has been used to measure liver function. A large extraction generally indicates good function, a small one indicates bad elimination efficiency. It is customary to express the extraction as  $(c_i - c_o)/c_i$  ('extraction ratio', 'extraction percentage'). This expression in the clearance regime is constant during variations in peripheral blood concentration.

In the clearance regime the extraction fraction can be derived from equations [4a] and [5].

$$(c_i - c_o)/c_i = 1 - e^{-V_{\max}/K_m F} \quad [7]$$

It is seen that the extraction fraction depends on liver function in a curvilinear way as clearance does, and that it depends on hepatic blood flow. Thus the use of the extraction fraction to measure liver function offers no theoretical advantages over clearance measurements; it is more cumbersome to obtain; and its analytical accuracy, including 'catheter-induced errors,' is less than that of a clearance determination.

In the present paper it has been assumed that the hepatic input concentration ( $c_i$ ) is equal to that of an artery (mixed venous blood). This is

\*In the general clearance regime 'true' or 'intrinsic' clearance can be calculated as  $v/\bar{c}$ , where  $\bar{c}$  is the logarithmic average of  $c_i$  and  $c_o$ :  $(c_i - c_o)/\ln c_i/c_o$ , with  $c_o$  determined by hepatic venous catheterization (2, 10). This holds also in the flow-limited regime but is useful only when  $c_o$  can be measured with sufficient accuracy.

evidently not the case when a test substance or a drug is given perorally and absorbed from the gastrointestinal tract. In this case portal venous concentration is higher than that of an artery, and this means that in the clearance regime hepatic elimination is generally higher for identical doses compared with that of an intravenous injection. This is the 'first-pass effect' (17), which is specially important in clinical pharmacokinetics. The resulting difference in peripheral blood concentration becomes more important with increasing extraction fraction, and for drugs of the flow-limited regime nothing reaches the peripheral circulation during oral administration (when no porto-caval shunting is present). The 'first-pass' effect should be distinguished from the effect caused by quickly saturated binding of the drugs in the liver (22), which indicates more complicated kinetics than those assumed in the present paper.

In the following sections the above considerations will be discussed from the point of view of their pathophysiologic and clinical importance.

#### A. THE GENERAL CLEARANCE REGIME

The removal of a substance at a rate which corresponds to this type of kinetics (eq. [5]) depends both

on the enzyme function ( $V_{max}/K_m$ ) and on flow (F). It is well known that many liver test substances such as Bromsulphalein and indocyanine green are removed by processes that are dependent on flow, contributing to the decreased clearance observed during haemorrhagic shock, an effect similar to that of drugs which decrease hepatic blood flow, such as vasopressin. This is in accordance with the cited estimates of  $V_{max}$  and  $K_m$  of these dyes. It should be stressed, however, that these constants describe the removal of the dyes from the blood into the liver cells, and that the kinetics of these dyes are far more complicated (16, 26), which means that the simple kinetics of the present model are not valid.

The variation in clearance and extraction fraction of the general regime with changes in hepatic blood flow can be predicted from eq. [5], as shown in Fig. 2. In the enzyme-limited regime the extraction fraction is inversely proportional to the flow, and the clearance remains constant [eq. 6]. By contrast, in the flow-limited regime the extraction remains constant (and near unity), and clearance varies proportionally to the flow (eq. [c], Fig. 1).

In the general clearance regime the response of

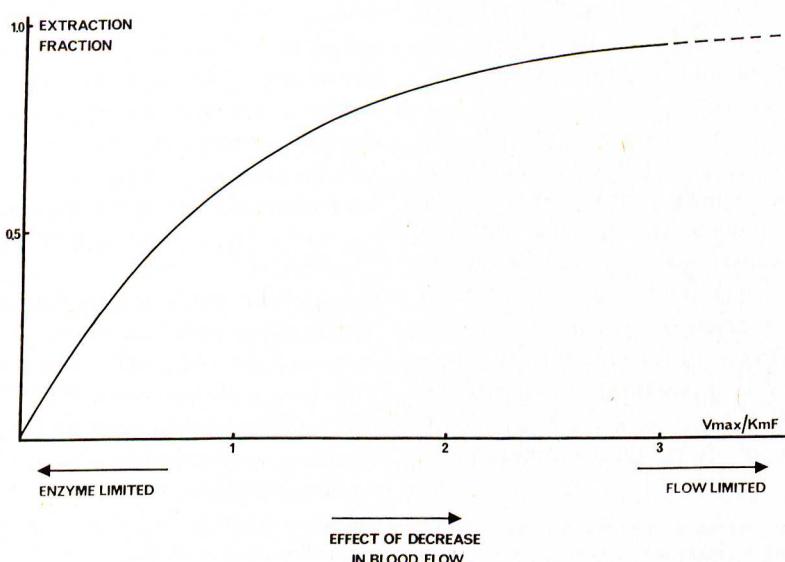


Fig. 2. The variation of hepatic extraction fraction with  $V_{max}/K_m F$ . A decrease in hepatic blood flow results in the enzyme-limited regime in a proportional increase in the extraction fraction, while it is almost unchanged in the flow-limited regime.

peripheral concentration and hence clearance to changes in blood flow depends on the change of blood flow relative to the magnitude of  $V_{max}/K_m$ . The actual experimental experience with dyes like Bromsulphalein and indocyanine green is probably explained by this statement. Sometimes the extraction fraction changes, while peripheral concentration remains essentially constant (and clearance thus remains constant). In other cases the extraction fraction is unchanged while peripheral concentration changes (and clearance changes considerably). These two situations can be predicted from Fig. 3. The first happens if the flow change occurs for a substance of the general regime, but near that of the enzyme-limited (right side of Fig. 3), while the second occurs near the flow-limited regime (left side of Fig. 3). In any actual case the variation of clearance with flow can only be predicted if the kinetic constants and the blood flow are known.

Expected change during disease	Flow-limited	General	Enzyme-limited
$\frac{V_{max}}{FK_m} \gg 1$ (ex: colloids)	$\frac{V_{max}}{FK_m} \sim 1$ (ex: BSP)	$\frac{V_{max}}{FK_m} \ll 1$ (ex: antipyrine)	
$V_{max} \downarrow$	→	→	→
$K_m \uparrow \downarrow ?$	↔	↔	↔
Flow ↓ (total or effective)	←	←	←

Fig. 3. The changes in clearance regimes during disease.

### B. THE REGIME OF ENZYME-LIMITED CLEARANCE

Substances with a small clearance will be well suited for measurements of liver function even if the slow removal rate implies that the time for performing the test must be rather long for an accurate estimate of the removal rate. This clearance is not influenced by changes in hepatic blood flow.

This class of substances has until now not been much explored as compared with the more rapidly cleared bile-excreted dyes. It has been demon-

strated (1) that the clearance of antipyrine is well correlated with quantitative liver function (galactose elimination capacity). Bilirubin may belong to this class of substances, but the complex kinetics of this substance will probably prevent the design of a simple clinical test.

### C. THE REGIME OF FLOW-LIMITED CLEARANCE

The flow-limited elimination by the liver is made possible by the small distance from sinusoid to hepatocyte, which makes diffusion from the central blood stream to the liver cell membrane fast. For some substances  $V_{max}/FK_m$  is large enough to allow practically complete removal at sufficiently low inputs [eq. 5], where the exponential term is rapidly reduced as  $V_{max}/FK_m$  is increased. In this regime large differences exist between apparent and true clearance, and the theoretical maximally obtainable clearance by the enzyme present in the liver ( $V_{max}/K_m$ ) is larger than what is possible at a normal hepatic perfusion rate.

A practical criterion for flow-limited uptake is that the concentration in the hepatic veins is close to zero. For several substances this happens in the normal organism. When the blood removal curves of such substances (for example, galactose and ethanol) are followed after a single intravenous injection, as concentration declines there is a continuous but rather steep transition from zero- to first-order kinetics at the time when the hepatic venous concentration becomes small. This class of substances represent removal by various partial functions of the liver, including cytoplasmatic phosphorylation, conversion in the microsomal system, transport for later biliary excretion, and phagocytosis by the reticuloendothelial system of the liver.

Measurements of hepatic blood flow by clearance techniques are based on the principles of flow-limited elimination, as originally suggested by Dobson & Jones (6). It has been shown that in normal individuals correct values are obtained by these methods. Difficulties occur, however, when these results are extrapolated to liver disease, where decreased clearance of these substances (colloids) usually is found. The conditions for a flow-limited regime may be invalidated if the

removal capacity of the system ( $V_{max}$ ) is decreased and, thus, hepatic venous concentration is no longer close to zero. Furthermore, extrahepatic removal, if present, increases with diminished liver function (cf. the increased uptake of colloids in bone marrow used for liver scintigraphy in liver disease). These effects are not easily quantitated, but they make it difficult *a priori* to accept changes in, for example, colloidal clearance in liver disease as quantitative changes in liver blood flow.

The physiologic importance of flow-limited removal in the normal organism may from a functional point of view be that it allows for complete hepatic disposal of substances that are absorbed from the gut and which are potentially toxic for the organism, without any spillover into the general circulation. For galactose this protection may be in early milk-drinking childhood (cf. congenital galactosaemia), and for ethanol prevention of alcoholhaemia in animals, where intestinal fermentation occurs. The efficient removal of the bile acids participating in the enterohepatic circulation is an energy-saving mechanism in addition to probably being a protective one. Of special clinical importance is the almost complete  $NH_3$  clearance by the normal but not by the diseased liver. For certain hormones (glucagon) and drugs (propranolol, lidocaine) a rapid clearance by the liver is a prerequisite for their short-lasting action. The flow-limited removal of particulate matter by the Kupffer cells is the physiological basis of the concept of the 'filter function' of the liver.

It should be noted that flow-limited elimination offers a built-in type of 'autoregulation', as seen in the equation of the flow-limited regime  $v = Fc_i$ . When hepatic blood flow decreases, the clearance of a flow-limited substance decreases correspondingly, but at the same time the upper limit for the condition of flow limitation increases (cfr.  $V_{max}/FK_m \gg 1$ ); thus complete elimination (of the same amount) may be maintained even if the input (that is, portal venous) concentration increases. Further exploration of the physiologic range of flow-limited removal by the liver is necessary for confirmation of these aspects of the model.

## CLEARANCE REGIMES IN LIVER DISEASE

Variation in three factors ( $V_{max}$ ,  $K_m$ , and  $F$ ) induced by liver disease or by the effect of drugs and physicochemical effects may modify the clearance regimes. These effects can be seen in Fig. 3.

Throughout this paper hepatic blood flow ( $F$ ) has been taken to represent 'effective' flow as defined by Goldring et al. (8)—that is, only that fraction of total splanchnic inflow which comes into contact with functioning liver cells. Portosystemic or intrahepatic shunts, which are not easily measurable, thus tend to decrease clearance relative to the normal situation. Even if flow-limited conditions may be maintained in functioning sinusoids, hepatic venous concentration rises if intrahepatic shunts are created. It should be noted that acute decrements in hepatic blood flow will have different effects on removal rates in the flow-dependent regimes depending on whether they are caused by diminishing overall sinusoidal volume or by closing some of the sinusoids (5).

It is not known whether and to what extent changes in  $K_m$  occur during disease. It must be assumed that liver disease will result in a decrease of  $V_{max}$ , due either to decreased cellular function or to impeded transport of substrates to the liver cells (for example, due to 'capillarization' of the sinusoids). The effect of the described changes may be that, apart from decrease in clearance caused by the change in  $V_{max}$ , the clearance regime may be changed if the parameters defining the regimes are sufficiently affected. This is illustrated in Fig. 3. The general implication is that when a substance is chosen for measurement within the assumptions of a regime, it must be known whether these are maintained during disease. The pathologic consequences of the above-mentioned changes of regimes await experimental and clinical studies.

## GENERAL CONCLUSIONS

To be able to perform physiologically satisfactory clearance measurements, the following points should be taken into consideration.

Since the range of concentration (or removal rate) within which clearance is constant depends on

the kinetic removal constants ( $V_{max}$ ,  $K_m$ ) and the hepatic blood flow, estimates of these must be available. Since it is often impossible in clinical work to estimate these constants, removal of the test substance by a first-order process should be established. To ensure this, the blood concentration curve after a single intravenous injection must be studied for a sufficiently long time, as it is frequently possible with these types of elimination curves to delimit segments that will display an apparent linear course in a semilogarithmic system. Another way to ensure that a substance follows first-order kinetics is to verify the constancy of clearance at substantial increases of the amount given. It should, furthermore, be possible to extrapolate such evidence from the normal state to the different pathologic states in which the clearance must be measured.

As the general regime cannot be easily employed to obtain quantitative information on the functional state of the liver, test substances should be chosen with a suitable  $V_{max}/FK_m$  ratio according to the demands, which are different if function or perfusion is to be evaluated. The problem of how great a part of a reduced blood removal is caused by decreased blood flow or decreased liver function can be sorted out by employing two test substances, one in the enzyme-limited, the other in the flow-limited clearance regime. However, experimental data on this clinically important problem are not available. For further discussion see Wilkinson & Schenker (25).

Not many clearance substances employed today fulfil these requirements. More experimental work is clearly wanted to discover suitable substances. It should be stressed that the above considerations apply to an elementary model of hepatic perfusion, and further that this model assumes simple compartments and kinetics of removal. (Descriptions of more advanced models have been published regarding bilirubin (4), Bromsulphalein (7), fructose (21), and galactose (3).) The interpretation of clearance measurements with substances having more complicated removal kinetics adds great difficulties to practical solutions.

It is the aim of this survey that, by developing a physiologically based general theory of clearance including effects of hepatic perfusion, further pro-

gress can be made in this field, so that more appropriate methods for studying liver function can be developed.

#### ACKNOWLEDGEMENTS

This work has been supported by grants from the Danish Medical Research Foundation (512-2097). A brief account of the paper was given at the 2nd International Gstaad Symposium, 1975, and at the International Association for the Study of the Liver, Pecs, Hungary, 1976.

#### REFERENCES

1. Andreasen, P. B., Ranek, L., Statland, B. E. & Tygstrup, N. *Europ. J. Clin. Invest.* 1974, 4, 129-134.
2. Bass, L., Keiding, S., Winkler, K. & Tygstrup, N. *J. Theor. Biol.* 1976, 61, 393-417
3. Bass, L., Robinson, P. & Bracken, A. *J. J. Theor. Biol.* 1978, 72, 161-184
4. Berk, P. D., Howe, R. B., Bloomer, J. R. & Berlin, N. I. *J. Clin. Invest.* 1969, 48, 2176-2190
5. Brauer, R. W. pp. 113-127 in *Liver Function*. American Institute of Biological Sciences, Washington, D.C., 1958
6. Dobson, E. L. & Jones, H. B. *Acta Med. Scand.* 1952, 144, 1-71
7. Forker, E. T. pp. 229-240 in Goresky, C. A. & Fisher, M. M. (eds.) *Jaundice*. Plenum Press, New York, 1975
8. Goldring, W., Clarke, R. S. & Smith, H. W. *J. Clin. Invest.* 1936, 15, 221-228
9. Goresky, C. A., Bach, G. G. & Nadeau, B. E. *J. Clin. Invest.* 1973, 52, 991-998
10. Keiding, S., Johansen, S., Winkler, K., Tønnesen, K. & Tygstrup, N. *Amer. J. Physiol.* 1976, 230, 1302-1313
11. Keiding, S. *Scand. J. Clin. Lab. Invest.* 1976, 36, 113-118
12. Keiding, S. & Chiarantini, H. *J. Pharmacol. Exp. Ther.* 1978, 205, 465-470
13. Lewis, A. E. *Amer. J. Clin. Path.* 1948, 18, 789-795
14. Møller, E., McIntosh, J. F. & Van Slyke, D. D. *J. Clin. Invest.* 1929, 6, 427-466
15. Paumgartner, G., Probst, P., Kraines, R. & Leevy, C. M. *Ann. N.Y. Acad. Sci.* 1970, 170, 134-146
16. Richards, T. G., Tindall, V. R. & Young, A. A. *Clin. Sci.* 1959, 18, 499-511
17. Rowland, M., Benet, L. Z. & Graham, G. G. *J. Pharmacokinet. Biopharm.* 1973, 1, 123-136
18. Pang, K. S. & Rowland, M. *J. Pharmacokinet. Biopharm.* 1977, 5, 625-654.
19. Pang, K. S. & Rowland, M. *J. Pharmacokinet. Biopharm.* 1977, 6, 655-680
20. Pang, K. S. & Rowland, M. *J. Pharmacokinet. Biopharm.* 1977, 6, 681

21. Sestoft, L. & Fleron, P. *Biochim. Biophys. Acta (Amst.)* 1974, **345**, 27-38
22. Shand, D. G., Branch, R. A., Evans, G. H., Nies, A. S. & Wilkinson, G. R. *Drug Metab. Dispos.* 1973, **1**, 679-686
23. Tygstrup, N. *Scand. J. Clin. Lab. Invest.* 1966, **18**, Suppl. 92, 118-125
24. Wilkinson, G. R. & Shand, D. G. *Clin. Pharmacol. Ther.* 1975, **18**, 377-390
25. Wilkinson, G. R. & Schenker, S. *J. Biochem. Pharmacol.* 1976, **25**, 2675-2681
26. Winkler, K. & Gram, C. *Acta Med. Scand.* 1965, **178**, 439-452
27. Winkler, K., Keiding, S., Tygstrup, N. pp. 144-155 in *The Liver. Quantitative Aspects of Structure and Function*. Karger, Basel, 1973
28. Winkler, K., Bass, L., Keiding, S. & Tygstrup, N. pp. 797-805 in Lundquist, F. & Tygstrup, N. (eds.) *Regulation of Hepatic Metabolism*. Munksgaard, Copenhagen, 1974

Received 22 January 1979

Accepted 10 February 1979