Biochemical Pharmacology, Vol. 22, pp. 1133–1140. Pergamon Press, 1973. Printed in Great Britain.

BLOOD FLOW LIMITATIONS IN INTERPRETING MICHAELIS CONSTANTS FOR ETHANOL OXIDATION *IN VIVO*

ROBERT L. DEDRICK and DONALD D. FORRESTER

Chemical Engineering Section, Biomedical Engineering and Instrumentation Branch, Division of Research Services, National Institutes of Health, Public Health Service, United States Department of Health, Education and Welfare, Bethesda, Md. 20014, U.S.A.

(*Received* 22 *June* 1971; *accepted* 20 *October* 1972)

Abstract—A two-compartment model permitting localization of biochemical parameters and consideration of blood flow between compartments is used to simulate the concentration of ethanol in the blood as a function of time. The effect of the local Michaelis constant and hepatic blood flow on this concentration is illustrated by comparing model simulations with published data from rats. It is concluded that blood flow limitation can introduce a significant artifact in a commonly used method to measure the effective Michaelis constant *in vivo*.

ALTHOUGH ethanol oxidation has been studied extensively *in vitro* and *in vivo*, there remains considerable controversy over the details of the process *in vivo*. The prevailing view is that ethanol oxidation occurs primarily in the liver by alcohol dehydrogenase (ADH). Other enzyme systems such as a microsomal ethanol-oxidizing system (MEOS) and catalase have not been fully evaluated.

A major experimental method for elucidating the mechanism of ethanol oxidation compares the apparent Michaelis constant *in vivo* with those obtained *in vitro*. Lundquist and Wolthers[1] proposed this method, which considers ethanol oxidation to occur by a process with Michaelis–Menten kinetics in a homogeneous system of constant volume. They obtained an effective Michaelis constant *in vivo* for ten normal human subjects. The mean value of 2·03 mM compared favorably with the *in vitro* value of 1·75 mM for crystalline "liver" ADH. Recently, Makar and Mannering[2] used this method with a different computational technique to compare apparent Michaelis constants with those obtained *in vitro* from rats and monkeys. Agreement was reasonable: rat, 2·7 mM *in vivo*, 1·9 mM *in vitro*; monkey, 4·6 mM *in vivo*, 1·8 mM *in vitro*.

Lundquist and Wolthers[1] recognized the errors involved when considering the body to be a single homogeneous compartment for deriving kinetics *in vivo*. These errors become severe particularly when, at low levels, the concentration of ethanol in the blood no longer diminishes at a constant rate but rather decreases asymptotically toward endogenous levels. In fact, it has been observed in humans[3] and in cats[4] that almost all of the ethanol reaching the liver is oxidized at concentrations below 40–60 mg/l.

While the liver is extracting a large fraction of the ethanol reaching it, the mean concentration at the site of ethanol oxidation is significantly less than the arterial concentration. Approaching the limit of total extraction by the liver, the rate of

oxidation becomes dominated by hepatic blood flow and insensitive to the local enzyme kinetics. A valid *in vivo–in vitro* comparison of enzyme kinetics should consider the chemical environment at the site of the reaction to avoid artifacts caused by physiologic processes. Therefore, it is not rigorously correct to attempt enzyme analyses based on the concentration of ethanol in the arterial blood. The purpose of this paper is to show how the hepatic blood flow rate affects the interpretation of Michaelis constants determined *in vivo*. The analysis is based on a two-compartment mathematical model and is illustrated by comparison with published data from rats.

DEVELOPMENT OF MATHEMATICAL DESCRIPTION

The simplest mathematical model which incorporates local enzyme kinetics and flow has two compartments. As shown schematically in Fig. 1, the hepatic circulation connects the liver to the remainder of the body. Assumptions in developing this model

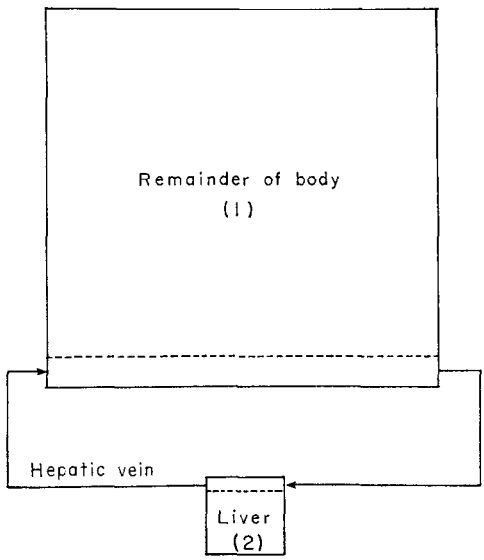

FIG 1. Compartmental model to show effect of localizing a metabolic reaction within the liver.

are that the compartments are homogeneous, that equilibration between blood and tissue water is rapid, that ethanol oxidation occurs in the liver and follows Michaelis–Menten kinetics locally, and that excretion through the lungs and kidneys is negligible. The latter assumption was shown to be sound even at high doses in the rat.[5] Based on these assumptions, mass balance equations were written for each compartment:

$$\text{Body: } V_1 \frac{dC_1}{dt} = QC_2 - QC_1 \qquad (1)$$

$$\begin{bmatrix}\text{Rate of change}\\ \text{of amount in}\\ \text{compartment 1}\end{bmatrix} = \begin{bmatrix}\text{Rate of flow to}\\ \text{compartment 1 from}\\ \text{compartment 2 with}\\ \text{blood}\end{bmatrix} - \begin{bmatrix}\text{Rate of flow from}\\ \text{compartment 1 to}\\ \text{compartment 2}\\ \text{with blood}\end{bmatrix}$$

$$\text{Liver: } V_2 \frac{dC_2}{dt} = QC_1 - QC_2 - \left[\frac{V_{max,2}C_2}{K_{m,2} + C_2}\right]V_2 \quad (2)$$

$$\begin{bmatrix}\text{Rate of change}\\ \text{of amount in}\\ \text{compartment 2}\end{bmatrix} = \begin{bmatrix}\text{Rate of}\\ \text{flow to}\\ \text{compart-}\\ \text{ment 2}\\ \text{from}\\ \text{compart-}\\ \text{ment 1}\\ \text{with}\\ \text{blood}\end{bmatrix} - \begin{bmatrix}\text{Rate of}\\ \text{flow from}\\ \text{compart-}\\ \text{ment 2 to}\\ \text{compart-}\\ \text{ment 1}\\ \text{with}\\ \text{blood}\end{bmatrix} - \begin{bmatrix}\text{Rate of}\\ \text{metabolism}\end{bmatrix}$$

where V = volume of compartment, milliliters; C = concentration in water, micrograms per milliliter; Q = hepatic (water) flow rate, milliliters per minute; V_{max} = maximum reaction rate, micrograms per (min) (milliliter); K_m = Michaelis constant, micrograms per milliliter; with the numerical subscripts designating the compartment. Equations (1) and (2) can be solved for C_1 and C_2 as functions of time on a digital computer after selection of the initial concentrations and the parameters (V_1, V_2, Q, V_{max} and K). These parameters were normalized for a 200-g rat.

SELECTION OF MODEL PARAMETERS

Volumes. To compare our results with those of Makar and Mannering,[2] we assumed that the rat was 70 per cent water and that ethanol distributes in body water. The value of 70 per cent is similar to the mean of 67 per cent reported for adult male rats by desiccation;[6] however, calculations from the data of Bustos *et al.*[7] suggest ethanol distribution in a volume almost 10 per cent less than body water. Van Harken and Mannering[5] reported that the liver represents about 4 per cent of the total body weight of Sprague–Dawley rats. The compartment volumes for a 200-g rat are thus: V_1 = 134·4 ml; V_2 = 5·6 ml.

Hepatic flow. A range of hepatic flow rates has been reported (see Table 1).

Maximum reaction rate. Van Harken and Mannering[5] reported a rate of ethanol disappearance from the intact rat of 325 mg/kg/hr, and Makar and Mannering[2] reported 330 mg/kg/hr based on the disappearance of ethanol from the blood. Thus, if the entire activity is assigned to a liver which is 4 per cent of a 200-g rat:

$$V_{max} = \frac{(325)\ (0{\cdot}2)}{(5{\cdot}6)\ (60)} = 0{\cdot}194 \text{ mg/(min) (ml liver water).}$$

Michaelis constant. A range of Michaelis constants *in vitro* has been reported for liver ADH in rats. Lundquist *et al.*[11] obtained a Michaelis constant of 0·3 mM at pH 7·1 and 21° for liver suspensions. Makar *et al.*[12] obtained a Michaelis constant of 0·1 mM at pH 10 and 23° with partially purified enzyme. A value of 2·13 mM has been measured by Markovic *et al.*[13] for purified enzyme at pH 10·0 and 23·5°. Arslanian *et al.*[14] reported an extraordinarily high 4×10^{-2} M for purified enzyme at pH 8·8 and 25°.

Makar and Mannering[2] obtained an apparent Michaelis constant *in vivo* of 2·7 mM. The apparent Michaelis constant *in vivo* is the unknown parameter in the two-compartment model.

TABLE 1. HEPATIC BLOOD FLOW IN RAT

Value reported	Method	References	Hepatic blood flow* (ml blood/min)	Hepatic blood flow* (ml water/min)
79 (75–92) ml/(min) (100 g liver)	Thermoelectric	8	6·3	5·3
1·2 ml/(min) (ml liver)	Chromic phosphate	9	9·6	7·68
66·2 ± 0·19 ml/(min) (kg rat)	^{131}I-albumin	8	13·2	11·1

* Based on a 200-g rat with blood 80% water, blood density 1·05 g/ml,[10] liver 70% water, liver density 1·05 g/ml.

RESULTS AND DISCUSSION

To investigate the effect of the Michaelis constant on ethanol elimination, equations (1) and (2) were solved for a dose of 1 g/kg to simulate the experimental results of Makar and Mannering.[2] Their data were recalculated to concentrations of mg/ml (of water) in the blood. The simulation was initialized to the first data point at 60 min.

Figure 2 shows the effect of the Michaelis constant on the kinetics of ethanol elimination at low ethanol concentrations. The Michaelis constant was varied within the reported range at a hepatic flow of 7·68 ml/min, which is the median of the flows reported. A Michaelis constant of 0·9 mM produces a satisfactory fit of the data at this hepatic flow. This is one-third the value obtained by Makar and Mannering[2] *in vivo* without allowing for the localization of the enzyme and for the flow of ethanol to the liver. This illustrates that a serious artifact can be introduced if the body is considered to be a single compartment while a rapid metabolic process is occurring, even in a well perfused region.

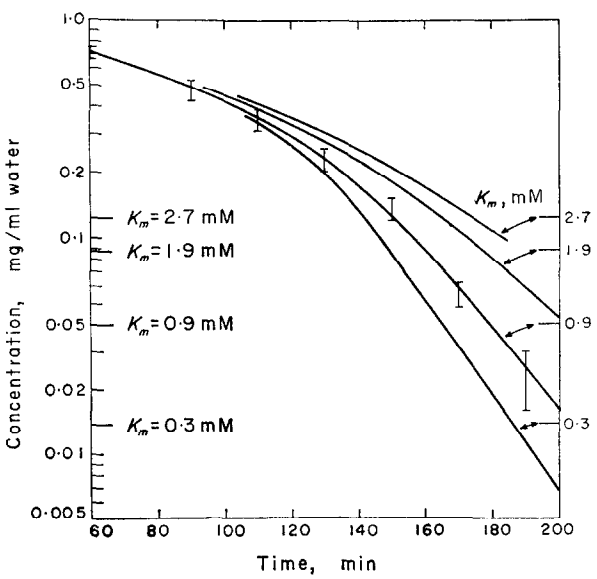

FIG. 2. Effect of Michaelis constant in the liver at a hepatic blood flow of 9·6 ml/min (7·68 ml water/min). The solid lines are computer simulations; the data are from Makar and Mannering[2] based on milliliters of water in the blood.

Figure 3 illustrates the simulated concentrations in the hepatic vein and the hepatic artery. It is evident that the fractional extraction of ethanol by the liver, which is represented by the vertical distance between the two lines, increases as the concentration of ethanol in the blood decreases. In this simulation, the extraction reaches 74 per cent at 40 μg/ml of ethanol in the blood. This is not consistent with the observation in humans[3] and cats[4] that extraction of ethanol by the liver is 96 per cent or more at blood ethanol concentrations below 40–60 μg/ml. If representative of the rat, this inconsistency has several possible explanations.

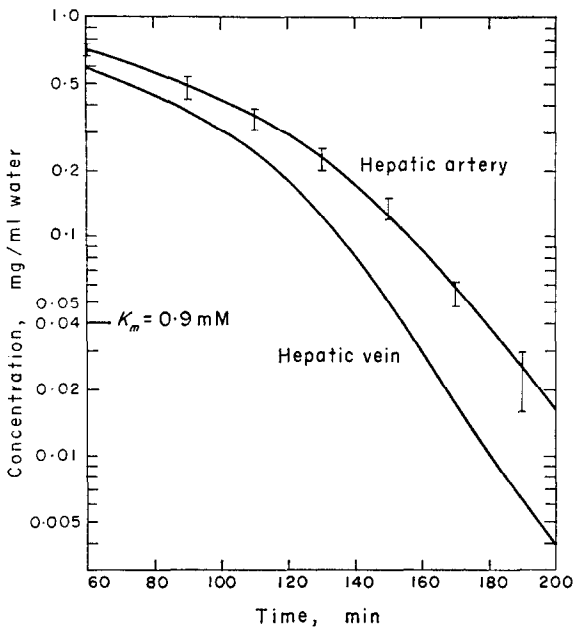

FIG. 3. Simulated concentrations in hepatic artery and hepatic vein for K_m = 0·9 mM and Q = 7·68 ml water/min. The solid lines are computer simulations; the data are from Makar and Mannering[2] based on milliliters of water in the blood.

First, the effective Michaelis constant may be lower. With a Michaelis constant of 0·3 mM, the predicted extraction is 88 per cent with 40 μg/ml of ethanol in the blood. Second, there may be a range of Michaelis constants *in vivo*, and a system may exist which has a low maximum rate and a low Michaelis constant. Third, the chemical reactions in the liver may be distributed. The assumption that the chemical reaction is occurring at a concentration equal to the average concentration in the liver may be conservative because, as the blood passes through the liver, chemical reactions are occurring at intracellular concentrations of ethanol that range from the level in the hepatic artery to less than that in the hepatic vein. A more sophisticated description of mass tansfer and chemical reaction in the liver will be required to settle this issue. It should be noted in this context that ADH is distributed nonuniformly in the liver. Morrison and Brock[15] reported that activity in the periportal quarter of the liver lobule was three-fifths that in the centrolobular quarter in the rat.

Figure 4 illustrates the effect of changing the hepatic blood flow over the reported range. The results show that purely physiologic effects such as regional perfusion can

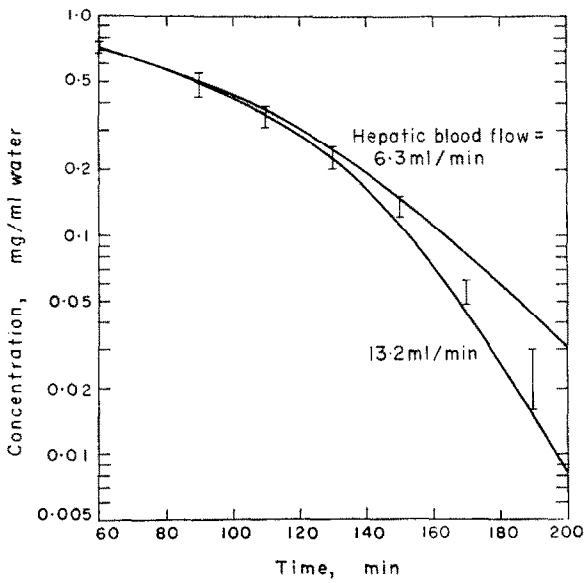

FIG. 4. Effect of hepatic blood flow at a Michaelis constant of 0·9 mM. The solid lines are computer simulations; the data are from Makar and Mannering[2] based on milliliters of water in the blood.

have a significant effect on observed rates of oxidation *in vivo*. This becomes particularly significant if the hepatic blood flow rate is altered by ethanol.

Two important aspects of the complete pharmacokinetics of ethanol in the rat are not incorporated in our two-compartment model: extrahepatic oxidation and its effects, and the concentration differences that may occur between well perfused and poorly perfused tissues.

Although extrahepatic oxidation can be mathematically simulated with ease, the data upon which estimates can be based are limited. Büttner[16] measured ADH activities in kidney at about 3 per cent of that in liver. Ferguson[17] and Mistilis and Garske[18] obtained substantial activities for ADH in the stomach and small intestine. Ferguson[17] also reported trace amounts in lung, heart, spleen and kidney. Extrahepatic ADH activities of a few per cent can be neglected at high concentrations of ethanol. At low concentrations, however, as the concentration becomes much less than the Michaelis constant, the rate becomes proportional to $V_{max}\,C/K_m$, i.e. first order in each tissue. Thus, the actual oxidation in tissues with high activities, such as liver, may occur at extremely low effective concentrations so that well perfused tissues with low activities, e.g. heart, lung and kidney, become relatively more significant.

The present model cannot simulate concentration differences between well perfused and poorly perfused tissues because they are combined into one compartment. However, large intertissue concentration differences in the rat are suggested by: (a) The comparison of the rates of ethanol oxidation from data published by Van Harken and Mannering[5] and Makar and Mannering.[2] The former, based on the loss of ethanol from the whole rat, indicate that the rate of ethanol oxidation is constant at 325 mg/(kg) (hr) until almost all ethanol is gone. The latter, based on blood levels of ethanol

in the rat, indicate a rate of ethanol oxidation of 480 mg/(kg) (hr) during the first hour. (b) A mean blood flow rate of 0·05 ml/(min) (g) to the gastrocnemius muscle of anesthetized rats as reported by Vogel.[19] If this flow rate represents muscle perfusion in rats under normal experimental conditions, then the muscle cannot closely follow rapid changes in the concentration of ethanol in the blood. (c) The failure of the model to predict the concentration in the blood (on a water basis) at 1 hr. For the work reported here, the 1-hr concentration is imposed as an initial condition.

One explanation for the occurrence of substantial intertissue concentration differences, is that the poorly perfused tissue might contain a significantly higher concentration of ethanol than would be expected on the basis of equilibrium. This can occur should ethanol enter the poorly perfused tissues while the concentration of ethanol in the blood is high, and if blood concentration decreases so rapidly that the concentrations in these tissues cannot follow closely. In particular, large poorly perfused tissues with negligible oxidation, such as skin and muscle, can serve as significant reservoirs. A thorough discussion of the linear two-compartment model for drug distribution is available.[20] Qualitatively, larger relative intercompartment concentration differences are expected during periods of rapid relative concentration change such as occur at low concentrations of ethanol. The concept of large intertissue differences during rapid changes in concentration should be considered in the interpretation of tissue to blood distribution ratios and whole body metabolism.

A final comment concerns the relative effect of the hepatic MEOS reported by Leiber and DeCarli.[21] The Michaelis constant for this system has been reported to be 8 mM *in vitro*. The rate of reaction at very low substrate concentrations ($C \ll K_m$) is proportional to $V_{max}\ C/K_m$. If: (a) 8 mM is also characteristic of behavior of the MEOS *in vivo*; (b) the Michaelis constant for ethanol oxidation at low concentrations *in vivo* is 0·9 mM or less, as suggested by this work; and (c) the V_{max} for the MEOS is similar to the V_{max} for ADH or less; then, the MEOS would not be expected to play a significant role in ethanol oxidation *in vivo* at very low ethanol concentrations. This does not eliminate its possible importance at high ethanol concentrations.

In conclusion, the apparent Michaelis constants determined for rats by considering their ethanol concentration uniform in a single compartment are artifactual and highly dependent on hepatic blood flow. A satisfactory Michaelis constant *in vivo* based on localization of the activity in the liver is within the range reported for liver ADH *in vitro*. A more sophisticated model than that reported here will be required for elucidation of all major aspects of ethanol distribution and metabolism *in vivo*.

REFERENCES

1. F. Lundquist and H. Wolthers, *Acta Pharmac. tox.* **14**, 265 (1958).
2. A. B. Makar and G. J. Mannering, *Biochem. Pharmac.* **19**, 2017 (1970).
3. J. A. Larsen, *Nature, Lond.* **184**, 1236 (1959).
4. J. A. Larsen, *Acta physiol. scand.* **57**, 209 (1963).
5. D. R. Van Harken and G. J. Mannering, *Biochem. Pharmac.* **18**, 2759 (1969).
6. D. S. Dittmer (Ed.), *Blood and other Body Fluids*, p. 353. Federation of American Societies for Experimental Biology, Washington, D.C. (1964).
7. G. O. Bustos, H. Kalant, J. M. Khanna and J. Loth, *Science, N.Y.* **168**, 1598 (1970).
8. P. L. Altman and D. S. Dittmer (Eds.), *Respiration and Circulation*, p. 430. Federation of American Societies for Experimental Biology, Washington, D.C. (1970).
9. W. F. Hamilton and P. Dow, *Handbook of Physiology*, Vol. 2, section 2, p. 1405. American Physiological Society, Washington, D.C. (1963).

10. D. S. DITMER, *Blood and Other Body Fluids*, p. 15. Federation of American Societies for Experimental Biology, Washington, D.C. (1961).
11. F. LUNDQUIST, I. SVENDSEN and P. HYLTOFT PETERSON, *Biochem. J.* **86**, 119 (1963).
12. A. B. MAKAR, T. R. TEPHLY and G. J. MANNERING, *Molec. Pharmac.* **4**, 471 (1968).
13. O. MARKOVIC, H. THEORELL and S. RAO, *Acta chem scand.* **25**, 195 (1971).
14. M. J. ARSLANIAN, E. PASCOE and J. G. REINHOLD, *Biochem. J.* **125**, 1039 (1971).
15. G. R. MORRISON and F. E. BROCK, *J. Lab. clin. Med.* **70**, 116 (1967).
16. H. BÜTTNER, *Biochem. Z.* **341**, 300 (1965).
17. M. M. FERGUSON, *Q. Jl microsc. Sci.* **106**, 289 (1965).
18. S. P. MISTILIS and A. GARSKE, *Australas. Ann. Med.* **18**, 227 (1969).
19. T. T. VOGEL, *Cardiologia* **53**, 19 (1968).
20. K. B. BISCHOFF and R. L. DEDRICK, *J. theoret. Biol.* **29**, 63 (1970).
21. C. S. LIEBER and L. M. DECARLI, *J. biol. Chem.* **245**, 2505 (1970).