stimulation of the pancreas. J Physiol (Lond) 1967;190:519–30.
6. Henriksen FW, Worning H. The interaction of secretin and pancreozymin on the external pancreatic secretion in dogs. Acta Physiol Scand 1967;70:241–9.
7. Holst JJ, Schaffalitzky de Muckadell OB, Fahrenkrug J. Nervous control of pancreatic exocrine secretion in pigs. Acta Physiol Scand 1979;105:33–51.
8. Lenninger S, Ohlin P. The flow of juice from the pancreatic gland of the cat in response to vagal stimulation. J Physiol (Lond) 1971;216:303–18.
9. Meyer JH, Spingola LI, Grossman MI. Endogenous cholecystokinin potentiates exogenous secretin on pancreas of dog. Am J Physiol 1971;221:742–7.
10. Singer MV, Niebel W, Elashoff JD, Grossman MI. Does basal cholinergic activity potentiate exogenous secretin for stimulation of pancreatic bicarbonate output in dogs? Digestion 1982;24:209–14.
11. Beglinger C, Grossman MI, Solomon TE. Interaction between stimulants of exocrine pancreatic secretion in dogs. Am J Physiol 1983 (in press).
12. Wormsley KG. A comparison of the response to secretin, pancreozymin and a combination of these hormones in man. Scand J Gastroenterol 1969;4:413–7.
13. Foelsch UR, Wormsley KG. Pancreatic enzyme response to secretin and cholecystokinin-pancreozymin in the rat. J Physiol (Lond) 1973;234:79–94.

Reply: We thank Dr. Beglinger and colleagues for their interest in our work (1).

On the basis of their own studies and of reports in the literature dealing with secretin and cholecystokinin (CCK), they assert that there is little evidence supporting the occurrence of potentiation of pancreatic enzyme secretion in vivo in dogs. This may hold true for secretin and CCK, but we reported on neurotensin, which may act quite differently.

Dr. Beglinger et al. criticize that we did not present data on maximal pancreatic protein secretion for the combined stimulation. As stated, however, we have tried to approach "physiological" hormone levels and, therefore, avoided high-peptide doses. Maximal pancreatic stimulation may yield interesting results, but our interest was not in a pharmacologic study.

Similarly, the lack of potentiation of bicarbonate output after the combination of CCK and secretin in our study may represent a dose-related phenomenon. Other investigators gave 5 pmol · kg^{-1} · min^{-1} as the smallest dose of secretin (2–4), whereas we used 0.08 pmol · kg^{-1} · min^{-1} (basis of this calculation was that 3.6 μg of synthetic secretin represents 1 clinical unit).

We are sorry that our paragraph concerning methods was too short: the mean secretion ($\bar{y}$) stimulated by the three doses of neurotensin given alone or in combination with secretin/CCK was calculated from the analysis of regression as proposed by Elashoff (5).

The mean secretion of a combination was then compared with the sum of the mean secretion stimulated by the three doses of neurotensin plus the secretion stimulated either by secretin, CCK, or secretin and CCK from the same dog using the paired two-tailed Student's *t*-test. Basal secretion was always subtracted.

We admit that the problem of "wash-out" is not completely solved. Although we observed no further reduction of protein output after the 30-min infusion of secretin, a small residuum of intraductular protein cannot be excluded. This protein would then be washed out by the flow elicited by neurotensin. It would *add* to the protein secreted in response to neurotensin. But the protein output observed under these circumstances was greater than additive. Two more facts minimized any washout of protein during the secretin/neurotensin combination: the wash-out phenomenon is related to the ductular flow rate and the flow rate after the combination of secretin and neurotensin was identical (2.3 ml · 15 min^{-1}) with the added flow rates elicited by secretin and neurotensin each given alone.

Thus, while we agree that caution is needed, the evidence available points to a potentiation of pancreatic protein output in the dog when the combination of secretin and neurotensin is given in the doses described.

GERHARD E. FEURLE, M.D.
IVO BAĆA, M.D.
Medizinische Poliklinik
University of Heidelberg
D-6900 Heidelberg
Federal Republic of Germany

1. Baća I, Feurle GE, Haas N, Mernitz T. Interaction of neurotensin, cholecystokinin, and secretin in the stimulation of the exocrine pancreas in the dog. Gastroenterology 1983;84:556–61.
2. Meyer JH, Spingola LI, Grossman MI. Endogenous cholecystokinin potentiates exogenous secretion on pancreas of dog. Am J Physiol 1971;221:742–7.
3. Wormsley KG. A comparison of the response to secretin, pancreozymin and a combination of these hormones in man. Scand J Gastroenterol 1969;4:413–7.
4. Henriksen FW, Worning H. The interaction of secretin and pancreozymin on the external pancreatic secretion in dogs. Acta Physiol Scand 1967;70:241–9.
5. Elashoff JD. Down with multiple *t*-tests. Gastroenterology 1981;80:1615–20.

Galactose Clearance as a Measure of Hepatic Blood Flow

Dear Sir:

An improved assay for galactose has been used by Henderson et al. (1) to investigate the estimation of organ blood flow from organ clearance of a substance taken up by first-order kinetics (2), as applied to the estimation of the rate of blood flow through the liver from the hepatic clearance of galactose. Since the estimation is to be noninvasive (the hepatic vein is not to be sampled), it is highly model-dependent. Misunderstandings of the limitations of just this method have led, in the past, to notable errors in renal pathophysiology, such as the inference of severe renal ischemia in acute anuria (2). If such errors are to be avoided in hepatology, the kinetics underlying the limitations of the estimation must be made explicit. We propose to do this here, taking into account the fact that hepatic uptake of galactose follows Michaelis–Menten kinetics (3,4), whereby some of the questions raised by Henderson et al. (1) can be answered.

In this method, a steady infusion of galactose at the rate I is balanced by steady hepatic uptake; the resulting galactose concentrations, c_i upstream and c_o downstream of the liver, are also steady. Clearance is defined by $Cl = I/c_i$, and extraction fraction by $E = 1 - c_o/c_i$. If F is the rate of hepatic blood flow that is to be estimated, we can write Fick's principle, $I = F(c_i - c_o)$, in the form

$$\text{Clearance} = FE \leq F, \qquad (1)$$

where the last relation follows from $E \leq 1$. In the noninvasive method, one does not ascertain how much clearance falls short of F in each individual case because c_o (and hence E) is not measured. We therefore consider this shortfall theoretically by

using the sinusoidal perfusion model of hepatic uptake. We begin with the undistributed version of the model (5,6).

The theoretical relation between clearance, F, and I is (5):

$$\text{Clearance} = F - F \exp\left(-\frac{V_{max} - I}{FK_m}\right), \quad (2)$$

where V_{max} is the maximum rate of galactose uptake by the liver at saturation, and K_m is the half-saturation (Michaelis) constant. The rate-limiting step in the hepatic uptake of galactose is phosphorylation by galactokinase (3) and the value of K_m is known to be 0.03–0.04 mg/ml from in vitro (7) and in vivo (3,5) studies. Typical V_{max} of the normal human liver is 200–400 mg/min, but it can fall below 100 mg/min in liver disease (8) when extrahepatic elimination of galactose is taken into account. The infusion rates used by Henderson et al. (1), 25–100 mg/min, were smaller in each case than V_{max} (otherwise, a steady state could not be reached). The resulting values of c_i were all either of the order of K_m or larger, while most values of c_o were below K_m (1). Hence, the arterial–venous difference $c_i - c_o$ traversed most of the Michaelis–Menten range of elimination kinetics, and only the perivenous hepatocytes operated in the limit of first-order kinetics ($c_o \ll K_m$). Data presented by Henderson et al. (1) do not suffice for the calculation of V_{max}, K_m, and F from Eq. (2), but the insertion in Eq. (2) of the aforementioned typical values readily reproduces the main features of the data. For example, the question was raised by Henderson et al. (1) as to why the doubling of I from initial values in the range 22–55 mg/min resulted in reductions in clearance, which averaged 8% in 14 subjects. Such a reduction is accomplished according to Eq. (2), if we set, for example, $V_{max} = 200$ mg/min, $K_m = 0.035$ mg/ml, and $F = 1500$ ml/min. Then Eq. (2) predicts clearance $= 0.95F = 1425$ ml/min for $I = 45$ mg/min, and clearance $= 0.88F = 1320$ ml/min for $I = 90$ mg/min (a drop in clearance of ~8%).

We now turn to the central question: under what conditions in clearance a good or bad estimate of F? Clearance approaches F from below as the exponential factor in Eq. (2) is diminished; that is, as the fraction $(V_{max} - I)/FK_m$ is increased. Clearance will be >95% of F when that fraction is >3, and <80% of F when the fraction is <1.5. The former case has been exemplified earlier with $I = 45$ mg/min. The latter case is illustrated by the same example if we reduce V_{max} to 120 mg/min. This shows how rapidly clearance can fall below F with falling V_{max}. In general terms, clearance is a good estimate of F at low F and high V_{max}, and a poor one at high F and low V_{max} (in liver disease). Reducing I in order to approach first-order (linear) kinetics, ($I \ll V_{max}$) brings clearance closer to F, but not decisively so: $(V_{max} - I)/FK_m$ may be small even for negligible I, and large even for I comparable with V_{max}. In the latter case, clearance $\approx F$ will be independent of I (equivalently, of c_i) even when many (periportal) hepatocytes operate in the nonlinear range of Micahelis–Menten kinetics. Then, despite that nonlinearity, the ratio of dose to area under the $c_i(t)$ curve obtained in a transient measurement will give the same value of clearance as I/c_i in the steady-state measurement on the same subject (1).

The foregoing discussion in terms of Eq. (2) is oversimplified. Consider an intrahepatic shunt, through which some part pF ($0 < p < 1$) of the flow rate F carries the concentration c_i undiminished into the vein. Suppose also that the substrate is eliminated completely, in one pass through the liver, from the remaining part $(1 - p)F$ of the flow. Then $E = 1 - p$, and clearance $= (1 - p)F$, which is independent of I [so that such cannot be the case (1) discussed earlier]. We see that clearance only estimates the flow rate $(1 - p)F$ through the enzymatically active part of the liver, and that is satisfactory. But what if the shunt is replaced by a vascular pathway of flow rate pF associated with some qV_{max} ($0 < q < 1$), leaving the remainder of the liver with $(1 - q)V_{max}$? We must then use Eq. (2) separately for each part of the liver. Since c_i is common to both parts, and since the appropriate partial infusion rates are pI and $(1 - p)I$, the total clearance will be the sum of the two clearances so calculated. If $p = q$, we recover readily Eq. (2) for the complete liver, but one can show that whenever $p \neq q$, the resulting clearance is always less than at $p = q$. For example, if we take the first of the foregoing examples (with $I = 45$ mg/min) and choose $p = 1/3$ and $q = 2/3$, we find clearance $= 0.85F$ (in place of clearance $= 0.95F$).

Proceeding in this way to consider a set of vascular pathways, we arrive at the distributed model of hepatic uptake [(9) and references therein], for which there is the general result: *any* heterogeneity of extraction in a set of parallel vascular pathways (without change in the total organ V_{max}, F, K_m) must diminish the organ extraction fraction E, and hence also clearance by Eq. (1). For a real liver, there is therefore an additional determinant of the accuracy of the estimation of F by clearance: the greater the heterogeneity of extraction [which can be quantified precisely (9)], the farther does the clearance Cl fall below the rate F of hepatic blood flow.

SUSANNE KEIDING
Medical Department B
Frederiksborg County Hospital
DK-3400 Hillerod, Denmark

LUDVIK BASS
Department of Mathematics
University of Queensland
Brisbane, Australia

1. Henderson JM, Kutner MH, Bain RP. First-order clearance of plasma galactose: the effect of liver disease. Gastroenterology 1982;83:1090–6.
2. Lassen NA, Perl W. Tracer kinetic methods in medical physiology. New York: Raven Press, 1979:18–9.
3. Keiding S, Johansen S, Winkler K, et al. Michaelis-Menten kinetics of galactose elimination by the isolated perfused pig liver. Am J Physiol 1976;230:1302–13.
4. Keiding S, Chiarantini E. Effect of sinusoidal perfusion on galactose elimination kinetics in perfused rat liver. J Pharmacol Exp Ther 1978;205:465–70.
5. Bass L, Keiding, S. Winkler K, et al. Enzymatic elimination of substrates flowing through the intact liver. J Theor Biol 1976; 61:393–410.
6. Winkler K, Bass L, Keiding S, et al. The physiologic basis for clearance measurements in hepatology. Scand J Gastroenterol 1979;14:439–48.
7. Ballard FJ. Purification and properties of galactokinase from pig liver. Biochem J 1966;98:347–52.
8. Andreasen PB, Ranek L, Statland BE, et al. Clearance of antipyrine—dependence on quantitative liver function. Eur J Clin Invest 1974;4:129–34.
9. Bass L, Robinson PJ. Effects of capillary heterogeneity on rates of steady uptake of substances by the intact liver. Microvasc Res 1981;22:43–57.

Reply: In response to the letter from Keiding and Bass concerning our paper "First-order clearance of plasma galactose: the effect of liver disease" in GASTROENTEROLOGY 1982;83:1090–6, we would make the following points.

We agree that it is important to understand the limitations of clearance methodology in applying such to blood flow estimation. While we accept their model for galactose elimination, and acknowledge the group's enormous contribution in this field, we do not agree with their typical values applied to this theoretical model. First, the normal V_{max} from their own source is 406 ± 91 mg/min; in more than cirrhotic patients we have found only 2 patients with a value <150 mg/min. Second, the reported K_m

values for galactokinase vary from 0.017 to 0.036 mg/ml in animal studies, and in human studies galactokinase has been purified with a K_m = 0.018–0.027 mg/ml (1). The limits of the rates of liver blood flow (F), in normal subjects and patients with liver disease, lie between 500 and 2000 ml/min. When these parameter values (which are within acceptable pathophysiologic limits), are used in Eq. (2) at I = 45 mg/min, clearance is, at worst, $\sim 0.94F$ for F = 2000, V_{max} = 250, and K_m = 0.036. The theoretical example they cite to explain the 8% drop in clearance with a twofold increase in I is, we believe, based on an unrealistic combination of V_{max} and F. The clearance-to-V_{max} ratio of galactose usually lies in the 3:1 or 4:1 range (2,3), and although this will vary with different liver diseases, we have never measured the 7.5:1 ratio they suggest. While increasing the infusion rate will contribute to the clearance reduction by the mechanism they suggest, we submit that the full explanation is more complex.

In response to their question as to what conditions are required to best estimate flow by a clearance method, we would add the following. Substitution of the cited values of V_{max} and K_m for normal subjects satisfies the criteria of $(V_{max} - I)/FK_m > 3$; in patients with advanced liver disease (e.g., a V_{max} = 150 mg/min, flow maintained at 1000 ml/min), $(V_{max} - I)/FK_m > 2.92$, i.e., clearance still approximates flow. On a theoretical basis, Keiding and Bass undersell the capability of galactose clearance to approximate liver blood flow. The practical application of the method in advanced liver disease, as presented in our paper, negates their theoretical argument.

Finally, the limitations of the oversimplification must be recognized, particularly in respect to intrahepatic shunting. Total liver blood flow cannot be measured by a clearance method without knowing hepatic extraction, which may well vary in different parts of the liver. Any clearance method applied without hepatic vein catheterization and within the constraints outlined previously has the limitation of measuring flow to functioning liver tissue: this is the important flow metabolically. The data presented in our paper, and reinforced by the preceding examples, show that galactose clearance at these concentrations approaches the ideal of measuring functional liver blood flow.

J. MICHAEL HENDERSON, F.R.C.S.
MICHAEL H. KUTNER, PH.D.
Emory University School of Medicine
Emory University Hospital
Atlanta, Georgia 30322

1. Blume KG, Beutler E. Purification and properties of galactokinase from human red blood cells. J Biol Chem 1971;246:6507–10.
2. Henderson JM, Millikan WJ, Wright-Bacon L, Kutner MH, Warren WD. Quantitative estimation of metabolic and hemodynamic hepatic function: the effects of shunt surgery. Surg Gastroenterol 1982;1:77–85.
3. Henderson JM, Millikan WJ, Wright-Bacon L, Kutner MH, Warren WD. Hemodynamic differences between alcoholic and nonalcoholic cirrhotics following distal splenorenal shunt—effect on survival. Ann Surg (in press).