First-Order Clearance of Plasma Galactose: The Effect of Liver Disease

J. MICHAEL HENDERSON, MICHAEL H. KUTNER, and RAYMOND P. BAIN

Departments of Surgery and Biometry, and the Clinical Research Facility, Emory University School of Medicine, Atlanta, Georgia

Galactose clearance kinetics at plasma concentrations of 0.01–0.1 mg/ml were studied during continuous infusion of 25–100 mg D-galactose per minute. In 10 subjects, plasma galactose vs. time curves during 140-min infusion, and 60 min thereafter, showed the data to fit a single-compartment model and attain 95% of plasma steady state by 80 min. Doubling the infusion rate in 14 subjects resulted in an 8% reduction in clearance at the higher rate. Hepatic extraction in normal subjects was 94%, while in cirrhotics it was 79%. Day-to-day reproducibility in 11 subjects gave a coefficient of variation of 4.5%. Extrahepatic clearance showed 2% of the total to occur in the urine, and 2.3% to occur by erythrocyte metabolism. The overall mean (±SD) clearance in the normal subjects of 1378 ± 218 ml/min was significantly ($p < 0.05$) greater than for the stable cirrhotics at 918 ± 279 ml/min, but not significantly different from patients with acute hepatocellular damage at 1186 ± 300 ml/min. This index gives flow-dependent hepatic clearance, and provides a noninvasive measure of effective liver blood flow.

Hepatic metabolism rapidly removes galactose from plasma and converts it to glucose by phosphorylation and epimerization (1). The two determinants in this clearance are hepatocyte function and liver blood flow, the relative importance of each depending on the sugar's plasma concentration (2–4). After a 30-g intravenous bolus of galactose, elimination over the initial 60 min is zero order, and the metabolic removal pathway is saturated. When plasma concentration vs. time is plotted in Cartesian coordinates, the linear decline in concentration, 0.4–1.5 mg/ml, is a measure of hepatocyte function (5). In contrast, when the plasma concentration is low (<0.3 mg/ml), elimination becomes a first-order process, the metabolic pathway is no longer saturated, and the exponential decline in plasma concentration with time depends on liver blood flow (4).

The kinetics of galactose elimination in the zero-order phase have been extensively studied in humans (5–8); the maximal rate of elimination so calculated, galactose elimination capacity (GEC), is widely used as a measure of hepatic function. In rats and pigs, the kinetics of elimination in both the zero-order and the first-order phases have been measured (9–12). First-order clearance in humans has received scant attention, and has been cursorily examined by one group (2), while others have presented a theoretical basis for elimination in the low plasma concentration range (3). The main barrier to full evaluation has been lack of an adequately sensitive assay for galactose in the appropriate concentration range, 0.001–0.1 mg/ml; we have recently developed such an assay (13). Galactose is unique in several respects among substances currently used to assess hepatic function and flow: (a) A galactose-specific metabolic pathway avidly removes it from plasma. (b) It is technically possible to study both zero- and first-order elimination. (c) It is water soluble, is not protein bound, and hence is freely available for exchange. In this study we have measured the kinetics of its distribution and elimination, in normal subjects and patients with cirrhosis, during infusion of 50 mg/min to steady state and in the postinfusion period. In further experiments we have confirmed first-order clearance, assessed day-to-day reproducibility, measured hepatic extraction, measured clearance in the face of severe hepatic dysfunction, and quantitated two pathways of extrahepatic removal.

Received May 11, 1981. Accepted June 3, 1982.

Address requests for reprints to: J. Michael Henderson, F.R.C.S., Department of Surgery, Rm. F-310, Emory University Hospital, 1364 Clifton Road, N.E., Atlanta, Georgia 30322.

This work was supported by Public Health Service Grant AM15736 and General Clinical Research Center Public Health Research Grant 5M01RR00039-22.

0016-5085/82/111090-07$02.50

Materials and Methods

Subjects

Eleven subjects without and 28 with liver disease were studied under different experimental plans as outlined below. In the latter group, 22 had stable cirrhosis and 6 had acute hepatocellular damage as the predominant component of their liver disease. Cirrhosis was diagnosed on biopsy of the liver, and was judged to be stable at the time of study either by absence of inflammatory infiltrate and new collagen at biopsy, or by normal or marginal elevation of bilirubin and serum glutamic oxaloacetic transaminase (SGOT). Acute hepatocellular damage was diagnosed by elevation of bilirubin and SGOT, and confirmed at biopsy in 3 patients in that group. These patient populations were selected because of the prime role of the liver in galactose clearance. The normal subjects had both normal function and flow, while those with liver disease were impaired to variable degrees on one or both counts. The patients are defined in Table 1 and the etiologic characteristics, operative status, conventional clinical and biochemical data (14), and angiographic grade of portal perfusion are given for the patients with liver disease. Portal venous flow was graded semiquantitatively on the venous phase of the superior mesenteric artery angiogram (15) as I–IV: Grade I was normal perfusion, while grade IV showed no prograde flow or reversal of flow in the portal vein. Patients were studied under a protocol approved by Emory University Hospital Human Investigation Committee.

Method

All subjects ate a regular diet and were fasted for 8 h before and throughout the study. Studies were done with the subjects supine and their heads elevated 30°. Five percent D-galactose (Fischer Scientific Co., Pittsburgh, Pa., prepared with a 5% solution in pharmacy) was given by peripheral intravenous infusion at a constant rate by I-MED infusion pump (I-MED Corporation, San Diego, Calif.). Infusion rate varied from 25 to 100 mg/min as defined in the studies below. The galactose concentration of each solution prepared for infusion was analyzed concurrently with the plasma samples for that study. Blood samples (3 ml) were drawn from an indwelling catheter in the opposite arm, collected in fluoride (Vacutainer tube M3273 PS;

Table 1. The Clinical and Biochemical Data of the 28 Patients With Liver Disease

Patient	Type	Body weight (kg)	Galactose clearance (ml/min)	Albumin (g/dl)	SGOT (U/dl)	Bilirubin (mg/dl)	Alkaline phosphatase	Prothrombin time (s)	Ascites	Encephalopathy	Op. status	Portal perfusion
1–11	Normal	64–91	1378 ± 218	>3.5	<40	<1.0	30–95	<+2	0	0	0	I
Cirrhosis												
12	Alc	68	482	2.8	41	2.5	255	+4.4	0	0	0	III
13	PH	85	636	3.4	46	2.1	140	+3.1	0	+	Mesocaval	IV
14	PH	64	638	3.3	43	2.1	139	+2.7	0	0	DSRS	IV
15	PH	72	698	3.2	45	1.6	120	+3.0	0	+	Mesocaval	IV
16	Alc	65	715	2.1	127	3.4	201	+5.0	+++	+	0	a
17	Alc	103	776	4.1	45	2.4	108	+0.7	0	0	DSRS	III
18	Alc	70	780	3.3	25	1.3	77	+3.2	+	0	0	II
19	Alc	65	794	4.2	66	1.6	95	+2.5	+	+	DSRS	II
20	PH	86	803	3.1	81	2.4	98	+3.0	0	0	Mesocaval	IV
21	Alc	81	815	3.8	30	0.7	70	+0.1	0	0	0	a
22	PH	62	826	3.5	66	1.6	460	+1.7	0	0	DSRS	III
23	PH	64	863	3.1	54	1.2	143	+2.5	0	0	DSRS	IV
24	Alc	81	884	3.5	28	0.7	127	+3.1	++	0	DSRS	I
25	Alc	85	932	3.9	39	0.8	110	+2.1	0	0	Portacaval	IV
26	PH	76	932	3.3	49	3.0	113	+2.4	+	0	DSRS	II
27	Alc	86	936	3.3	129	4.7	239	+3.0	0	0	DSRS	II
28	Alc	70	984	2.2	135	1.7	117	+4.0	++	0	0	a
29	PH	67	987	4.1	35	1.3	53	+2.3	0	0	0	I
30	Alc	49	1282	3.2	36	1.1	172	0.0	0	+	Portacaval	IV
31	Alc	84	1342	3.7	142	2.6	191	+2.5	0	0	DSRS	I
32	Alc	96	1457	3.7	43	0.7	79	+1.3	+	0	0	I
33	Alc	110	1623	3.4	35	0.9	146	+1.7	0	0	DSRS	IV
Hepatitis												
34	CAH	45	784	2.8	140	23.2	210	+10.0	++	0	0	a
35	Alc	71	1031	2.7	438	30.1	341	+5.0	++	0	0	a
36	CAH	60	1071	2.5	200	8.2	170	+10.0	++	0	Mesocaval	IV
37	Acute	65	1165	3.1	2690	24.3	209	+1.0	0	0	0	a
38	CAH	70	1467	2.6	338	5.0	257	+4.0	++	0	0	a
39	Acute	53	1600	3.4	605	33.9	165	+4.0	+	0	0	a

Alc = Alcoholic cirrhosis; PH = posthepatitis cirrhosis; CAH = chronic active hepatitis; DSRS = distal splenorenal shunt. 0 = None.
[a] = No angiogram.

Becton Dickinson Co., Rutherford, N.J.), placed on ice, and plasma separated within 1 h. Galactose analysis was by the method of Henderson and Fales (13). This automated, fluorometric assay, in a defined working range of 0.001–0.1 mg/ml, has a standard deviation of ±0.002 mg/ml over 20 samples assayed in duplicate. Careful blood collection is essential, since at the low plasma concentration under study, erythrocytes will metabolize a substantial fraction of plasma galactose in vitro unless fluoride is used as a preservative. Previous work has shown that plasma collected in fluoride loses <0.001 mg/ml galactose over 4 h (13) regardless of initial concentration. This assay uses galactose oxidase, which detects galactosamine and galactosides in addition to galactose. The measured fasting levels of galactose in the 39 subjects in this study ranged from 0.001 to 0.019 mg/ml.

Experiments

Six experiments were carried out. The first experiment was designed to define the kinetics of galactose clearance from the plasma concentration vs. time curve during a constant infusion of 50 mg/min, and subsequently in the postinfusion period. The second sought to confirm that clearance was a first-order process at plasma concentrations <0.1 mg/ml, by infusion of galactose at two rates. The third measured the hepatic extraction in both normal subjects and patients with cirrhosis. The fourth measured clearance in patients with clinically and biochemically severe hepatocellular disease. The fifth measured the day-to-day reproducibility of the clearance rate of galactose calculated by this method. The sixth quantitated the renal and erythrocyte clearances respectively.

Experiment 1: Plasma Galactose During and After Infusion at One Rate

This first phase of the study aimed to define the kinetics of galactose distribution and elimination during, and after continuous infusion at a rate that would give a steady-state plasma concentration <0.1 mg/ml.

Five normal subjects and 5 patients with cirrhosis were studied. Galactose was infused at 50 mg/min for 140 min. Blood samples were drawn at 0, 3, 6, 9, 12, 15, 20, 30, 40, 50, 60, 80, 100, 120, and 140 min. After the infusion was stopped, further samples were drawn at 3, 6, 9, 12, 15, 20, 30, 45, and 60 min.

The galactose plasma concentration vs. time curve for this group was analyzed by a curve fitting program of the statistical package BMD-PAR (16). This program allows data fitting to multicompartment models. From this analysis the plasma steady-state concentration (C_{ss}) and area under curve ($AUC_{0-\infty}$) were estimated.

Clearance was calculated in two ways: first, from the C_{ss} which was adjusted by subtracting the basal level at time (C_0):

$$\text{Clearance}_{ss} = \text{infusion rate}/(C_{ss} - C_0) \quad (1)$$

and second, from the area under the plasma concentration vs. time curve from 0 to ∞:

$$\text{Clearance}_{AUC} = \text{total dose infused}/(\text{AUC}_{0-\infty}). \quad (2)$$

The difference in clearance calculated by these two methods was compared by an analysis of variance, considering the two clearances as duplicates (17).

The clearance in all further experiments was calculated from Equation (1). The time taken to achieve steady state during continuous infusion can be shortened by giving a priming intravenous bolus injection. In all subsequent studies, a 500-mg intravenous bolus of galactose was given before the infusion. The specific criteria used to define C_{ss} in this study were that four of the five plasma samples drawn between 50 and 100 min were ±0.002 mg/ml of the mean.

Experiment 2: Plasma Galactose During Infusion at Two Rates

If the clearance of galactose in this plasma concentration range is a first-order process, i.e., is independent of concentration, and is independent of hepatic extraction, doubling the infusion rate will result in a twofold increase in the plasma steady-state concentration.

To verify this ideal, 5 normal subjects and 9 patients with cirrhosis were studied. After an intravenous priming bolus of 500 mg, the fusion rates were 50 mg and 100 mg galactose/min in the normal subjects, and 25 and 50 mg galactose/min in the patients with cirrhosis. The lower rate was chosen for the latter group to keep their plasma galactose concentrations in our defined working range. Blood samples were drawn at 50, 60, 70, 80, and 90 min during each infusion. Clearance at each infusion was calculated using Equation (1). The ratio $\text{Clearance}_2/\text{Clearance}_1$, was calculated in each subject; the ideal should be 1.0. For the whole group, the ratio was then tested equal to 1 by Student's *t*-test (17).

Experiment 3: Hepatic Extraction Studies

The purpose of this phase of the study was to measure the extraction of galactose across the liver in our defined range. If extraction by functioning hepatic tissue is 100%, this should be confirmed in normal subjects. However, it is impossible to verify 100% extraction by functioning hepatocytes in patients with liver disease, as hepatic venous outflow comes from functioning and nonfunctioning tissue: extraction measures the percent of total liver blood flow which perfuses functioning tissue.

Eleven subjects, 2 without liver disease and 9 with cirrhosis, were studied. The normal subjects were patients undergoing cardiac catheterization studies who had no history of liver disease, no congestive cardiac failure, and normal serum bilirubin, albumin, SGOT, lactate dehydrogenase (LDH), and alkaline phosphatase. The cirrhotic patients were studied at the time of angiographic evaluation of their portal hypertension.

Galactose was given as a priming intravenous bolus of 500 mg, followed by continuous infusion at 50 mg/min in the normal subjects and 40 mg/min in the patients with cirrhosis. At the end of the primary procedure, when the patient was hemodynamically stable, four paired blood samples were drawn from a peripheral and hepatic vein.

Extraction was calculated:

$$\text{Extraction} = \frac{(C_{ss} - C_{hv})}{(C_{ss} - C_0)} \times 100, \quad (3)$$

where C_{hv} was the measured hepatic vein concentration.

Experiment 4: Acute Hepatocellular Dysfunction and Galactose Clearance

Extraction of a test substance by the liver will become progressively impaired with increasing hepatic damage. These studies measured galactose cleared in patients with severe hepatocyte damage, in support of the hypothesis that, in this population, galactose is being given at a sufficiently low concentration for extraction by functioning hepatocytes to be complete on each pass.

Six subjects, 34–39 in Table 1, were studied. Galactose was given as an initial 500-mg i.v. bolus followed by a 40-mg/min continuous infusion for 100 min. Plasma steady state was measured from 60 to 100 min, and clearance was calculated from Equation (1).

Experiment 5: Day-to-Day Reproducibility of Galactose Clearance

Two normal subjects and 9 patients with cirrhosis were studied under identical conditions twice within 1 wk. In each study, galactose was given as defined in experiment 4 above, and clearance was calculated from Equation (1). The day-to-day reproducibility was assessed by an analysis of variance (17).

Experiment 6: Extrahepatic Clearance

Two routes of extrahepatic clearance of galactose are by renal excretion and erythrocyte metabolism. These were quantitated as now outlined:

Renal clearance studies. Two normal subjects and 4 patients with cirrhosis were studied during infusion of 50 mg galactose/min as in experiment 2 above. Urine was collected from 30 to 90 min; its volume and galactose concentration were measured.

Renal clearance was calculated from the equation:

$$\text{Renal clearance} = uv/(C_{ss} - C_0),$$

where u is the urine concentration of galactose in milligrams per milliliter, v is the urine flow in milliliters per minute, and $C_{ss} - C_0$ is the plasma concentration of galactose in milligrams per milliliter.

Red blood cell metabolism of galactose. Seven studies on blood from patients in experiments 1 and 2 above were performed. At 80 min, during infusion of 50 mg galactose/min, 20 ml of blood was collected in heparin and incubated at 37°C in a water bath; no preservative was added and the samples were not oxygenated. Six-milliliter aliquots were centrifuged immediately, at 1 h, and at 2 h. Each was analyzed for plasma galactose concentration. From this the total metabolized each hour was calculated, and this corrected to a hematocrit of 45.

The potential in vivo loss of galactose by erythrocyte metabolism was calculated as follows from the in vitro data:

Corrected in vitro metabolism = x mg/ml · h.
Blood volume = 5000 ml.
∴ Total erythrocyte metabolism = (5000×) mg/h.

This was then expressed as the percent of the infused dose.

Results

The mean galactose clearance ± SD for all normal subjects (1378 ± 218 ml/min) was significantly ($p < 0.05$) greater than for the stable patients with cirrhosis (918 ± 279 ml/min), but not significantly different from the patients with hepatitis (1186 ± 300 ml/min). In the 9 normal subjects this correlated significantly with their body weight ($r = 0.64$, $p < 0.05$).

Pairwise correlations of the variables in Table 1 showed that galactose clearance did not significantly correlate with any of the conventionally measured indices of liver damage.

The following are results for each experimental phase of this study.

Experiment 1

Curve fitting showed both the infusion and postinfusion data to best fit a single-compartment model. All subjects achieved 95% of steady state between 60 and 80 min. Clearances, calculated by the two methods, for the 10 subjects are given in Table 2. For both normal subjects and cirrhotic patients the clearances are not significantly different when calculated by the two methods. Analysis of variance for the clearances gave a coefficient of variation of 17.6%: this was heavily weighted by 3

Table 2. Galactose Clearance in Experiment 1 Calculated From Steady-State and Area-Under-Curve Data

Subject No.	Infusion rate (mg/min)	Clearance_{ss} (ml/min)	Clearance_{AUC} (ml/min)
Normal			
1	50	1502	920
2	50	1572	2062
3	46	1144	1002
4	50	1333	1696
5	61	1279	1300
Mean ± SD		1366 ± 172	1396 ± 481
Cirrhotic			
16	47	715	668
18	47	780	793
20	50	859	733
24	49	884	908
27	52	936	1020
Mean ± SD		835 ± 87	824 ± 140

Table 3. Galactose Clearance Measured at Two Infusion Rates (Infusion 2 = Infusion 1 × 2) in Each Subject on the Same Day

	Subject													
	5	6	7	8	9	12	15	20	21	22	25	26	28	29
Infusion rate 1 (mg/min)	52	49	54	52	26	22	30	27	33	23	50	55	28	36
$Clearance_1$ (ml/min)	1156	1531	1862	1268	1130	500	714	844	825	885	962	932	1120	1000
$Clearance_2$ (ml/min)	954	1531	1543	1368	945	463	682	761	805	767	901	932	848	973
$\frac{Clearance_2}{Clearance_1}$	0.825	1.000	0.829	1.079	0.836	0.926	0.955	0.902	0.976	0.867	0.937	1.000	0.757	0.973

patients (1, 2, and 4) whose clearances were markedly different by the two methods.

Experiment 2

The results from 5 normal subjects and 9 cirrhotic patients are summarized in Table 3. All subjects achieved steady-state plasma galactose concentrations at both infusion rates. The ideal, a doubling of the plasma galactose concentration with a doubling of the infusion rate, was approached in all subjects. The mean (±SD) ratio $Clearance_2/Clearance_1$ was 0.919 ± 0.088. This ratio was significantly different from the ideal of 1.00 ($p < 0.005$).

Experiment 3

The results of the hepatic vein extraction studies are summarized in Table 4. The mean extraction for patients with normal liver function was 94%, while the mean extraction for those with cirrhosis was 79%. This latter figure implies an intrahepatic shunt of 21%, whether or not due to direct inflow/outflow shunting, or to loss of extraction by nonfunctional liver tissue.

Experiment 4

The clearances for the 6 patients with acute hepatocellular damage are given in Table 1. All reached a satisfactory plasma steady-state concentration between 60 and 100 min. The mean (±SD) clearance in this group was 1186 ± 300 ml/min. While this is not significantly different from the control group, it differs in that there is no correlation between clearance and body weight.

Experiment 5

The day-to-day reproducibility of the calculated galactose clearance is summarized in Table 5. The coefficient of variation was 4.5%.

Experiment 6

The mean (±SD) renal clearance of galactose in this concentration range for the 6 subjects was 24 ± 17 ml/min; this is equated to 2% of the total clearance rate. The in vitro erythrocyte metabolism showed 0.013 ± 0.005 mg/ml to be metabolized in the first hour and 0.015 ± 0.008 mg/ml in the second hour of incubation. The loss of galactose from plasma was independent of the initial concentration, and was not significantly different in the two periods. Extrapolated to the in vivo situation, erythrocyte metabolism equaled 2.3% of the infused dose of 50 mg/min.

Discussion

Continuous intravenous infusion of galactose, at rates varying from 25 to 100 mg/min, proved to be the most practical and accurate method of measuring distribution and elimination kinetics at plasma concentrations <0.1 mg/ml. The short half-life of galactose, 10–30 min, made analysis of bolus data impractical in this range, because of the rapid fall off to very low concentrations. The disadvantage of steady-state kinetic analysis was that it required 60–80 min to attain stable plasma levels, and thus did not allow measurement of short-term changes in clearance. However, this time interval was shortened by a loading intravenous bolus injection (18,19) in the

Table 4. Hepatic Vein Extraction Data in Two Patients Without Liver Disease and Nine Patients With Cirrhosis

	Subject										
	10	11	13	14	17	23	25	30	31	32	33
C_0	0.01	0.009	0.01	0.001	0.011	0.014	0.009	0.004	0.009	0.008	0.004
$C_{ss} - C_0$ (mg/ml)	0.027	0.021	0.066	0.118	0.081	0.051	0.040	0.039	0.036	0.035	0.039
$C_{hv} - C_0$ (mg/ml)	0.002	0.001	0.013	0.047	0.008	0.017	0.004	0.008	0.006	0.010	0.002
Extraction (%)	93	95	80	60	90	67	90	79	83	71	95
$Clearance_{ss}$ (ml/min)	1851	1905	636	638	776	863	1285	1282	1342	1457	1623

Table 5. Day-to-Day Reproducibility of Galactose Clearance in Two Normal Subjects and Nine Patients With Cirrhosis

	Subject										
	5	8	12	13	15	19	20	24	25	29	32
Infusion rate, day 1	61	52	55	42	60	33	50	56	45	36	46
Infusion rate, day 2	62	50	47	42	50	27	54	40	66	54	51
$Clearance_1$ (*ml/min*)	1156	1268	463	661	682	825	761	848	917	1000	1484
$Clearance_2$ (*ml/min*)	1279	1250	470	636	725	794	859	851	893	915	1457

Mean of duplicates = 917.9. SD of duplicates = 41.05. Coefficient of variation (CV) of duplicates = 4.5%.

later studies, but even then sufficient time must elapse to define steady state.

Central to the accuracy of this method is the biochemical assay: is it adequately sensitive? Reproducibility studies in our previous paper (13) showed a within-run coefficient of variation of 2.1% at 0.04 mg/ml and 5% at 0.1 mg/ml, and day-to-day coefficient of variation of 2.2% and 8%, respectively. These figures equate to an "accuracy" of ±0.002 mg/ml with this assay in the range where the majority of the steady states were defined, which in turn amounts to a ±4% variability in clearance. The within-run coefficient of variation at 0.01 mg/ml, the level at which most of the hepatic vein and fasting samples were assayed, was 8.6%. The very low concentrations in Table 4 are calculated from the measured hepatic vein concentrations minus the basal concentration. We believe the sensitivity of the assay lies within the required range for these studies.

Clearance is defined as the volume of blood from which a test substance is completely removed in unit time. In this study, galactose clearance measures the sum of all its removal processes from plasma. The major route is hepatic metabolism, but renal excretion and erythrocyte metabolism contribute 4%, and other extrahepatic metabolic sites may add further to the total clearance. Hepatic clearance for any substance is affected by the intrinsic elimination capacity of the liver and hepatic extraction of that substance, and by liver blood flow (20–22). Intrinsic elimination capacity measures the maximal ability of the liver to remove the substance, and has been well documented for galactose in the zero-order kinetic phase (5,6). In normal subjects this capacity is 450–550 mg/min, with progressive fall in the face of liver damage. However, even with severe hepatic dysfunction, it rarely falls below 200 mg/min (7,23). All studies in this paper have measured clearance at plasma steady state during continuous infusion rates well below the intrinsic elimination capacity in a deliberate attempt to study flow-dependent clearance. Hepatic extraction, measured by hepatic vein catheterization, includes test substance which has not traversed sinusoids because it has passed through intrahepatic shunts. It is impractical to measure sinusoidal extraction, but this is the index required to define functional liver blood flow. The high plasma hepatic extraction ratio measured in this study is compatible with an even higher sinusoidal extraction, indicating flow to be the limiting factor in the calculated clearance. The role of liver blood flow in hepatic clearance is more complex. For substances which are incompletely extracted, decreased flow will increase extraction: for those with a low intrinsic elimination capacity, changes in liver blood flow will have a small effect on clearance, but for those with a high intrinsic elimination capacity and high extraction, flow becomes the primary factor in alteration in clearance. The data suggest that galactose falls into the latter category.

Plasma clearance cannot be equated to plasma flow for a test substance that equilibrates in erythrocytes, as whole blood acts as a single compartment. The rapid reequilibration from erythrocytes into plasma down a concentration gradient (24) makes the erythroycte galactose available for clearance in addition to the plasma concentration. The necessary step to translate the plasma clearance measured in this study to blood clearance requires either measurement of whole blood galactose concentrations or correction for erythrocyte distribution. We have previously shown that our assay is equally applicable to whole blood as to plasma (13), and suggest that clearance from whole blood may ultimately be of more importance physiologically.

The variability of the ratio $Clearance_2$ to $Clearance_1$ in experiment 2 from the ideal of 1.0 requires further comment. Three circumstances could reduce this to the observed ratio of 0.919. First, fasting galactose (0.002 – 0.019 mg/ml), as measured by galactose oxidase methods, probably comprises amino sugars and galactosides, and is not free galactose. We have assumed that these remain constant in the face of available free galactose in plasma and have subtracted them from steady-state values. If the alternative assumption was made, that these fall to zero in the presence of freely available galactose, the mean (±SD) ratio $Clearance_2/Clearance_1$ becomes 0.996 ± 0.092. Second, the ratio will be reduced if hepatic extraction falls when twice as much galactose per

minute is presented to the sinusoids. If this was the sole factor, the 8.1% reduction in clearance at the higher infusion rate could be accounted for by a parallel reduction in the extraction ratio. A third potential explanation is that the sum of all extrahepatic removal processes follows saturation kinetics even at these low plasma concentrations. The erythrocyte incubation studies indicate that a constant 0.014 mg/ml · h is metabolized by this route regardless of the initial plasma concentration. Other minor metabolic routes of galactose removal may utilize it in a similar way. Under such circumstances the contribution of such removal to the total clearance will be disproportionately higher at lower plasma concentrations. It is unlikely that any single one of these factors alone accounts for the discrepancy, but rather a combination of all three. The data, with the above provisos, show elimination is first order and that, within the defined ranges in this study, the high extraction of experiment 3 is maintained when the infusion rate is doubled.

What is the clinical usefulness of such a measure? As a flow-dependent index, the main use will be in measuring changes in hepatic perfusion. In patients with normal livers, drugs known to alter splanchnic blood flow (25–27) could be evaluated. In patients with liver disease, is there a critical galactose clearance level below which perfusion is inadequate and which is of prognostic value, akin to creatinine clearance in renal failure? An important criterion for the usefulness of any test is its reproducibility. Galactose clearance in this plasma concentration range is a reproducible index. The day-to-day reproducibility studies show a low coefficient of variation and support the use of this method in the longitudinal evaluation of patients. However, attention to detail in delivery rates, sample collection, and analysis is essential to maintain this accuracy. The present study has demonstrated the feasibility of measuring galactose clearance in this flow-dependent elimination phase, opening the way to answer these questions.

References

1. Goresky CA, Bach GG, Nadeau BE. On the uptake of materials by the intact liver. The transport and net removal of galactose. J Clin Invest 1973;52:991–1099.
2. Tygstrup N, Winkler K. Galactose blood clearance as a measure of hepatic blood flow. Clin Sci 1958;17:1–9.
3. Waldstein SS, Arcilla RA. Measurement of hepatic blood flow by clearance methods. Am J Dig Dis 1958;3:137–58.
4. Tygstrup N, Winkler K. Kinetics of galactose elimination. Acta Physiol Scand 1954;32:354–62.
5. Tygstrup N. Determination of the hepatic elimination capacity (L_m) of galactose by single injection. Scand J Clin Lab Invest 1966;92(Suppl):118–25.
6. Waldstein SS, Greenburg LA, Biggs AD, Corn L. Demonstration of hepatic maximum removal capacity (L_m) for galactose in humans. J Lab Clin Med 1960;55:462–75.
7. Tygstrup N. The galactose elimination capacity in control subjects and in patients with cirrhosis of the liver. Acta Med Scand 1964;175:281–9.
8. Colcher H, Patek AJ, Kendall FE. Galactose disappearance from the blood stream. Calculation of a galactose removal constant and its application as a test for liver function. J Clin Invest 1964;25:768–55.
9. Keiding S. Galactose elimination capacity in the rat. Scand J Clin Lab Invest 1973;31:319–26.
10. Keiding S, Chiarantini E. Effect of sinusoidal perfusion on galactose elimination kinetics in the perfused rat liver. J Pharmacol Exp Ther 1978;205:465–70.
11. Keiding S, Johansen S, Winkler K, et al. Michaelis–Menten kinetics of galactose elimination by the isolated perfused pig liver. Am J Physiol 1976;320:1302–10.
12. Keiding S, Johansen S, Tonnesen K. Kinetics of ethanol inhibition of galactose elimination in perfused pig liver. Scand J Clin Lab Invest 1977;37:487–94.
13. Henderson JM, Fales FW. Continuous-flow fluorometry of low galactose concentrations in blood and plasma. Clin Chem 1980;26:282–4.
14. Campbell DR, Parker DE, Anagnostopoulos CE. Survival prediction in portocaval shunts: a computerized statistical analysis. Am J Surg 1973; 126:748–51.
15. Nordlinger BM, Nordlinger DF, Milliken WJ, et al. Angiography in portal hypertension: clinical significance in surgery. Am J Surg 1980;139:132–41.
16. Dixon WJ, Brown ME, eds. Biomedical Computer Programs P-Series. Berkeley: University of California Press, 1979.
17. Snedecor GW, Cochran WG. Statistical methods. 6th ed. Ames, Iowa: The Iowa State University Press, 1967.
18. Greenblatt DJ, Koch-Weser J. Clinical pharmacokinetics. N Engl J Med 1975;293:964–70.
19. Wagner JG. A safe method for rapidly achieving plasma concentration plateaus. Clin Pharmacol Ther 1974;16:691–700.
20. Keiding S, Andreasen PB. Hepatic clearance measurements and pharmacokinetics. Pharmacology (Basel) 1979;19:105–10.
21. Wilkinson GR. Pharmacokinetics of drug disposition: hemodynamic considerations. Ann Rev Pharmacol 1975;15:11–27.
22. Winkler K, Bass L, Keiding S, Tygstrup N. The physiologic basis for clearance measurements in hepatology. Scan J Gastroenterol 1979;14:439–48.
23. Nordlinger BM, Fulenwider JT, Ivey GL, et al. Tyrosine metabolism in cirrhosis. J Lab Clin Med 1979;94:832–40.
24. Miller DM. The kinetics of selective biological transport. Further data on the erythrocyte-monosaccharide transport system. Biophys J 1971;11:915–23.
25. Gleman S, Ernst EA. Nitroprusside prevents adverse hemodynamic effects of vasopressin. Arch Surg 1978;113:1405–71.
26. Feely J, Wilkinson GR, Wood AJJ. Reduction of liver blood flow and propranolol metabolism by cimetidine. N Engl J Med 1981;304:692–5.
27. Sogaard PE. Propranolol in portal hypertension. Lancet 1981;i:1204.