

THE BLOOD GALACTOSE DISAPPEARANCE CURVE AS A TEST OF LIVER FUNCTION

LIONEL M. BERNSTEIN, M.D., PH.D., JOANNA X. WHEELER, B.S., EPPERSON E. BOND, B.S., MARVIN ROHMSDAHL, M.D., AND NORRIS DOUGHERTY, M.D.

Medical Research Division and Medical and Surgical Services, Veterans Administration Hospital, Hines, Illinois

The rate of disappearance from the blood stream of intravenously injected galactose has been used as a test of liver function. After a single rapid intravenous injection, the continuing decrease of blood galactose concentration is due to a combination of (1) diffusion from intravascular to some extravascular compartment, (2) utilization by liver (and possibly to a very small extent by other tissues), and (3) renal excretion. There is some variance in the interpretation of some of the details and mechanisms involved in these general agreements.

That galactose disappearance from the blood compartment immediately after injection is contributed to by diffusion into an extravascular space is indicated by the galactose volume of distribution reaching approximately 20 to 25 per cent of body weight within 10 to 15 minutes. The volume of distribution is calculated by dividing the injected dose of galactose by the zero time blood galactose concentration as obtained by extrapolation of the initial postmixing blood curve (whether this be considered linear¹⁻³ or exponential^{4, 5}). The contribution of such diffusion beyond a 10- to 15-minute initial mixing period is believed not to occur.²⁻⁵ Furthermore, after galactose concentration equilibrium between the intravascular and extravascular compartments is reached it is generally assumed¹⁻⁵ that a continuous concentration equilibrium between these compartments exists as the blood galactose level falls. Evidence to support this assumption has not, however, been provided.

Received January 4, 1960. Accepted April 22, 1960.

Address requests for reprints to: Dr. Lionel Bernstein, Veterans Administration Hospital, Hines, Ill.

Regarding metabolism of galactose, it appears that removal by the liver constitutes practically all of the galactose tissue removal. There is evidence of a very slow rate of galactose removal by other tissues,⁶ but this is quantitatively small enough to exert a negligible effect on the character of the blood galactose disappearance curve.

The renal clearance of galactose (in milliliters per minute) is essentially constant in an individual, being less than filtration rate.^{3, 5} The absolute amounts excreted are proportional to blood galactose levels. In the presence of near normal rates of liver galactose removal, renal excretion constitutes a very small portion of galactose removal.³ With marked reduction of liver galactose removal rates, the renal excretion may constitute a considerable part of total galactose removal from the blood vascular compartment. In calculations of galactose removal by the liver from blood galactose curves, correction is readily made for measured renal losses.³

After a single rapid intravenous injection, the blood galactose disappearance curve (after an initial mixing period) has been reported to be linear,^{1, 2} exponential,^{4, 5} and a combination of two phases (initial linear, subsequent exponential).³ In blood galactose curves regarded as exponential, the fractional disappearance constants have been used as indices of liver function, the constants being above 4.2 per cent in normal subjects and below 4 per cent in patients with liver disease.⁴ In galactose curves regarded as linear, determination of arteriovenous galactose differences across the liver by hepatic vein catheterization has been used for calculation of hepatic blood flow.²

Recently, a hypothesis has been presented

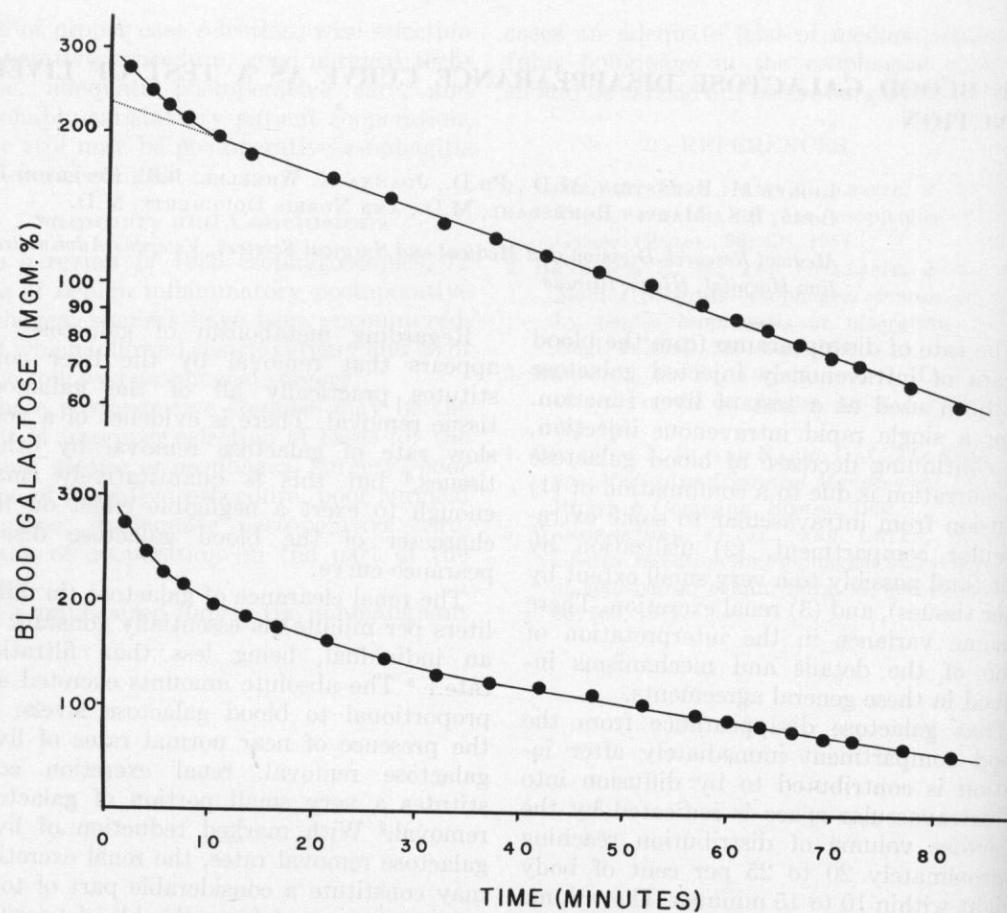


FIG. 1. Case 10: Single phase blood galactose disappearance curve, semilogarithmic and rectilinear plot. Extrapolation of semilogarithmic line to zero time was used for calculation of zero time galactose space.

for the interpretation of the initial linear-subsequent exponential two-phase curves which, without need for hepatic vein catheterization, allows calculation of both hepatic maximal removal capacity (Lm galactose) and effective hepatic blood flow.³ In this system it is assumed that (1) the injected galactose quickly (within 10 to 15 minutes) reaches its maximal volume of distribution, this volume then remaining constant throughout the test; and (2) after the maximal volume of distribution is reached the concentration of galactose is and remains identical in all parts of this volume of distribution. Accepting an unchanging volume of distribution and continuing galactose concentration equilibrium through-

out this volume of distribution, one can calculate absolute amounts of galactose disappearance from the decline of blood galactose concentration during the initial linear phase (while the liver galactose conversion mechanism is saturated). After correction for measured renal excretion, the disappearance rate represents liver removal of galactose. From the second (exponential) phase of the curve, during which the liver removes all galactose presented to it, the product of the fractional disappearance constant and the galactose volume of distribution represents effective hepatic blood flow plus renal clearance. Correction for the latter is accomplished from the measurements of urinary loss.³

The present study with single intravenous injections of galactose was undertaken in an attempt (1) to determine whether the postmixing blood galactose curve is linear, exponential, or a two-phase combination; and (2) to apply the above measurements of liver function to normal subjects and to patients with liver disease. Incidental to these main objectives were some interpretations of data shedding some darkness on the questions of the constancy of the galactose distribution volume and the maintenance of galactose concentration equilibrium throughout this distribution volume.

Methods

To 34 fasting subjects a solution of 50 per cent galactose (0.5 gm. per kg. of body weight) was administered intravenously. Accurate and rapid intravenous injection was facilitated by use of a sterile buret and air compression (sphygmomanometer bulb attached to top of buret). Total injection time was less than 90 seconds. Approximately 20 timed, 2-ml. samples of venous blood were drawn into dry heparin-containing syringes during the following 60 to 80 minutes from a Cournand needle placed in the opposite arm. Timed urines prior to and during the tests were also collected. Samples of the infused galactose, blood, and urine specimens were analyzed for galactose by the Nelson-Somogyi hexose method⁷ after incubation with glucose oxidase.⁸ Correction for galactose loss due to the glucose oxidase was assumed to be 5 per cent.⁶ Nonhexose-reducing substances present in control blood were assumed to be constant, and correction was made accordingly for all subsequent samples. Standard curves were made for each patient from galactose added to his own control blood sample.

The blood galactose disappearance curves were subjected to statistical analysis. In group I (single phase) curves, regression analysis of the data of each curve was performed by using both arithmetic and logarithmically transformed values of blood galactose. Because inclusion of the first few points before mixing is complete (5 to 20 minutes) would tend to improve the fit of the logarithmic transformation and impair the fit of the arithmetic values, those early points were omitted. This determination was arbitrarily made by gross inspection, the semilogarithmic extrapolation being used as a guide (fig. 1). It is believed that this removes bias due to the clearly curvilinear falloff of blood galactose during the initial mixing period. Inclusion of all points beyond the initial mixing is believed proper for a fair and

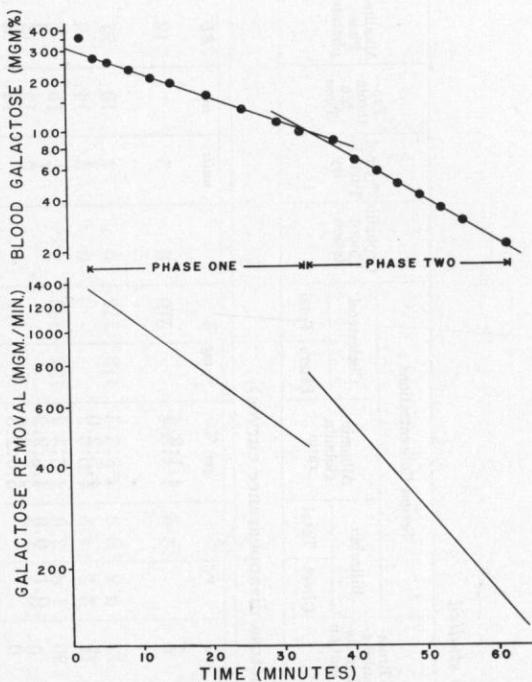


FIG. 2. Case 21: *Upper curve*, two-phase blood galactose disappearance curve, semilogarithmic plot. *Lower curve*, calculated absolute amounts per minute of galactose disappearance from zero time galactose space, assuming an unchanging galactose volume of distribution, and continuous concentration equilibrium throughout the galactose space during the test (see discussion for detailed explanation).

meaningful evaluation of the characteristics of such curves. If indeed the post-initial mixing should be exponential because of continued galactose diffusion from the blood compartment to some extravascular compartment throughout the test period, thus representing a previously unrecognized prolonged mixing, this is the information being sought.

The goodness of fit of the arithmetic and logarithmically transformed galactose data were evaluated by the analysis of variance method.^{9, 10} As an additional aid to evaluate goodness of fit, the ratios of the standard error of estimate to mean galactose were computed, smaller ratios indicating better fits of the data. Correlation coefficients were also calculated for the arithmetic and the logarithmically transformed data, and the Z-transformation test of significance of differences was applied to each blood galactose curve and to each group of curves.¹⁰

In group II (two-phase) curves, similar statistical analyses were made, the two phases being

TABLE I
Clinical data of patients studied

Subjects	Case No.	Height	Weight	Age	Clinical Diagnoses	Enlargement below Costal Margin		Brom-sulpha-lein Re-tention		Serum Concentrations		Cephalin-Flocculation	Thymol-Turbidity	Pro-throm-bin-Time	Alkaline Phos-phatase		
						Liver	Spleen	Bilirubin		Albumin-globulin ratio		Cholesterol Esters	Total				
								Direct	Total								
Group I (patients with one-phase blood galactose disappearance curves)																	
A. Controls (patients without liver abnormalities)	1	248	80.0	51	Hypertension; abdominal pain (cause unknown)	cm.	%	mg. %	gm. %	mg. %	mg. %	units	sec.	BU			
	2	303	92.7	35	Abdominal pain (cause unknown)		7		4.1:3.4								
	3	276	82.7	59	Old cholecystitis	6			4.5:2.0								
	4	268	91.8	38	Edema of hands (cause unknown)	2	0	0.1	0.6	4.6:2.2							
	5	283	95.7	38	Fistula-in-ano		0	0.2	0.7	5.0:2.3							
	6	272	79.5	35	Hemorrhoids	6	7	1.2	4.9:3.2	1.47	202	0	2				
	7	283	77.7	33	Fibrous dysplasia of femur	2	0	0.2	0.7	4.1:3.4	122	189	0	1			
	8	283	91.4	60	Ventral hernia		0	0.2	0.7	4.4:2.2				1	13	3	
B. Patients with liver abnormalities	9	283	69.1	62	Cirrhosis		28	2.7	3.0:3.1								
	10	256	80.0	48	Cirrhosis	15	3	21	1.5	2.3	3.6:3.9	220	4+	8	20		
	11	280	78.2	65	Postnecrotic cirrhosis	9	41	0.4	0.8	3.1:5.5	117	172	3+	12	13	7	
	12	283	87.3	56	Cirrhosis (ascites)	5	2	33	1.1	3.2	2.9:2.4	124	186	4+	16	18	10
	13	268	92.7	59	Cirrhosis (ascites); coronary artery disease	10	50	2.6	5.8	3.8:2.0	122	170	1+	5	14	10	
	14	272	72.3	44	Cirrhosis; chronic alcoholism	7	0	0.4	1.2	3.9:3.5				1	17	10	
	15	268	67.7	31	Cirrhosis (ascites)	9	4	12	0.7	0.9	3.0:4.1	117	4+	7	15	5	
	16	264	69.3	71	Postnecrotic cirrhosis	10	1.6	2.9	2.3:3.5	140	210	4+	6	3	18	15	8

Group II (patients with two-phase blood galactose disappearance curves)

TABLE 2
Blood galactose disappearance curve data

Subjects	Case No.	Galactose B ₀ ^a	Galactose volume of distribution ^b	Galactose Disappearance Curves							
				Phase 1				Phase 2			
				n ^c	ANOV (P values) ^d	√ error M.S./Y ^e	k _{1f}	n ^c	ANOV (P values) ^d	√ error M.S./Y ^e	k _{2f}
	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)	(K)	(P)
Group I (patients with one-phase blood galactose disappearance curves)											
A. Controls (patients without liver abnormalities)	1	295	14.2	17.8	19	379	4405	10.86	0.72	2.48	
	2	255	17.4	18.8	16	167	742	22.81	3.13	4.47	
	3	233	21.6	26.1	15	327	480	10.06	1.95	2.89	
	4	272	15.6	17.0	15	109	2714	26.87	1.55	5.78	
	5	328	13.4	14.0	20	129	2643	31.56	2.07	4.33	
	6	401	10.9	13.7	15	238	696	13.06	1.69	3.46	
	7	270	16.0	20.6	9	137	392	12.16	1.89	2.57	
	8	260	15.6	17.1	17	268	3638	12.17	0.78	2.43	
Group IA mean											3.55
B. Patients with liver abnormalities	9	222	17.7	25.6	19	271	2956	10.89	0.73	2.04	
	10	223	17.2	21.5	16	390	1831	6.48	0.62	1.54	
	11	178	24.7	31.6	17	684	1243	3.25	0.67	0.63	
	12	220	19.6	22.3	14	338	430	4.53	0.80	1.39	
	13	178	28.4	30.6	18	691	2922	4.36	0.46	1.39	
	14	321	14.7	20.3	15	290	1601	7.50	0.61	2.07	
	15	220	20.8	30.7	19	662	624	5.73	1.30	1.49	
	16	242	17.4	25.1	18	826	2580	2.54	0.51	0.86	
	17	291	15.2	22.9	20	191	2090	19.23	1.46	2.95	
	18	323	14.1	19.4	19	501	2094	9.83	1.09	2.43	
	19	213	23.2	34.9	14	411	1276	4.56	0.58	1.65	

	Group IB mean			16.6		11.50	1.19	1.68			
	Group I (A + B) mean										
Group II (patients with two-phase blood galactose disappearance curves)											
A. Controls (patients without liver abnormalities)	20	180	24.7	41.2	8	1140	660	1.0	0.3	1.14	5
	21	309	13.6	13.6	10	68	394	14.2	1.2	3.46	9
	22	236	19.3	23.0	9	1119	5321	1.8	0.2	1.90	9
	23	330	13.5	18.0	9	159	632	6.4	0.6	3.01	11
B. Patients with liver abnormalities	24	226	17.8	36.3	7	306	210	1.1	0.3	0.63	7
	25	198	20.4	27.0	7	80	116	7.0	1.2	3.15	6
	26	231	17.4	21.4	10	1001	1462	2.1	0.4	1.92	9
	27	345	13.2	18.3	12	151	1136	12.7	1.0	3.85	8
	28	250	16.2	24.1	10	213	1110	6.5	0.6	2.77	10
	29	227	19.1	29.2	8	1185	6085	0.9	0.1	1.15	8
Group II (A + B) mean			25.1	9.0			5.4	0.6	2.30	8.2	
Group I + II mean			23.5							9.9	1.9

^a Zero time blood level obtained by extrapolation of phase 1 curves.^b Zero time distribution volume calculated as injected dose of galactose divided by Bo.^c Number of points per curve.^d Analysis of variance *F* values for fitting of data to regression lines.^e Ratio of the square root of the error mean square to the mean of *Y* (blood galactose values).^f Fractional disappearance constants of exponential curves (as percentage of fall of blood galactose concentration per minute)

analyzed separately. The division between the two phases was determined arbitrarily by gross inspection and free hand drawing of approximate regression lines on the data plotted semi-logarithmically (fig. 2). The initial points of the initial mixing period were excluded from the first phase as described for group I curves. All points were included in the analysis of the second phase that fell beyond the first phase and were above absolute galactose concentration levels of 20 mg. per 100 ml.

Results

In table 1 are summarized the clinical diagnoses and clinical biochemical laboratory findings of 12 normal controls (patients with no known liver disease or dysfunction) and 17 patients with definite liver disease or abnormality of liver function, or with both.

In table 2 are summarized the blood galactose disappearance curve data of the same patients. The blood galactose (post-mixing) disappearance curves were found to be of two main kinds. In 19 patients (8 controls and 11 patients with liver abnormalities) (group I, table 2), a single exponential line was present for the duration of the test (*e.g.*, case 10, fig. 1). In 10 patients (4 controls and 6 patients with liver abnormalities) (group II, table 2) a two-phase curve was found, both phases being exponential, the second phase having a greater slope than the first (*e.g.*, case 21, fig. 2).

In 5 other patients a variety of curves were obtained which contained multiple peaks and nadirs superimposed upon the general decline in concentration with time. In these cases the changes were systematic enough to preclude laboratory errors as the basis for the variation; this suggests that fluid shifts between the cells and extracellular fluid constituted the underlying cause. These data could not be satisfactorily interpreted and are not presented.

Statistical Analyses of Group I Blood Galactose Disappearance Curves

Analyses of the 19 regression lines of the group I cases by the analysis of variance method revealed *F* values which were very highly significant ($P \ll 0.001$) for both the arithmetic and logarithmically transformed galactose values. How-

ever, greatly higher *F* values for the regressions were generally obtained with logarithmically transformed data than with arithmetic data (table 2, columns E and F). In every instance but 1 (case 15) the *F* value was greater for the semi-logarithmic line than for the linear regression line. Further, the ratios of the standard error of estimate to mean galactose were much smaller for the logarithmically transformed data than for the arithmetic data in every one of the 19 cases, averaging 1.19 and 11.50 per cent, respectively (table 2, columns G and H). These statistical data, based on 19 regression lines composed of large numbers of individual points (average, 16.6 points per regression line), strongly indicate that in group I, after an initial 5- to 15-minute mixing period, the blood galactose disappearance curve follows an exponential function, not a linear one.

For direct comparison of the goodness of fit, correlation coefficients (*r*) were calculated for both the arithmetic and the logarithmically transformed regression data, the *r* values were converted to *Z* values, and the *t* test was applied to determine significance of differences of the *Z* values.¹⁰ The *r* was greater for the logarithmically transformed data (range, (-)0.986 to (-)0.998) than for the arithmetic data (range, (-)0.937 to (-)0.990) in all but 1 patient (case 15). Of these individual curves, the *Z* transformation test indicated these differences of *r* to be significant at the $P < 0.001$ level in 6 cases; $P < 0.05$ in 3; $P < 0.10$ in 3; and $P > 0.10$ in 6.

The derived *Z* values are normally distributed. Thus, a single *t* test of the significance of the differences of *Z* was applied to the entire group I. The mean difference (\bar{d}) of the *Z* values for the logarithmically transformed and the arithmetic data was 0.775. The standard deviation of the mean difference (S_d) of the *Z* values was 0.108. The $t_{(0.05)}$ value, $(\bar{d}/S_d) = 0.775/0.108 = 7.18$, indicated a very highly significant difference ($P \ll 0.001$) between the *Z* values (and the *r* values which they represent). This warrants the conclusion that the logarithmically transformed regression is statistically significantly better than the arithmetic regression.

These data strongly support the conclusion that the blood galactose disappearance curve is exponential in nature, not linear.

Statistical Analyses of Group II Blood Galactose Disappearance Curves

Phase 1. Analyses of the 10 regression lines (first phases) of the group II cases by the analysis of variance method revealed *F* values which were very highly significant ($P \ll 0.001$) for either the

gressions
arithmetically
tic data
tance but
the semi-
regression
error of
a smaller
ata than
of the 19
cent, re-
). These
nes com-
l points
strongly
al 5- to
galactose
ponential
unction.

ess of fit,
ated for
arithmetically
nes were
applied
of the Z
arithmetically
(-)0.998)
0.937 to
Of these
est indi-
cant at
5 in 3;

ributed.
of the
group I.
for the
arithmet-
of the
s 0.108.
= 7.18,
ference
d the r
nts the
formed
er than

on that
s expo-

od

n lines
nalysis
h were
ther the

arithmetic or logarithmically transformed galactose values. Generally, much higher F values for the regressions were obtained with logarithmically transformed data than with arithmetic data (table 2, columns E and F), although the F values of the arithmetic data were higher in 2 of the 10 cases. The ratios of the standard error of estimate to mean galactose were smaller for the logarithmically transformed data than for the arithmetic data in every one of the 10 cases, averaging 0.6 and 5.4 per cent, respectively (table 2, columns G and H). These statistical data, based on 10 regression lines composed of moderate numbers of individual points (average of 9.0 points per regression line), indicate that after the initial mixing period the first phase of the blood galactose disappearance curve follows an exponential function rather than a rectilinear function.

The correlation coefficients for the logarithmically transformed data ranged from (-)0.979 to (-)0.999, and for the arithmetic data from (-)0.946 to (-)0.997. For direct comparison of the goodness of fit, t tests were applied to the Z transformations of calculated r values. These indicated a significantly better fit of the logarithmically transformed data in only 1 of the 10 patients. A single t test of the differences of the Z values was applied to the entire group II, phase 1 curves. The mean difference (\bar{d}) of the Z values for the logarithmically transformed and the arithmetic data was 0.425. The standard deviation of the mean difference (S_d) of the Z values was 0.140. The $t_{(9)}$ value, $(\bar{d}/S_d) = 0.425/0.140 = 3.04$, indicated a highly significant difference ($P < 0.015$) between the Z values. Therefore, the logarithmically transformed regression is statistically significantly better than the arithmetic regression.

These statistical data, although less conclusive than the group I data presented above (because of smaller numbers of cases and of points per regression line) do support the conclusion reached with group I data. In view of the similarity of the data, it is reasonable to accept the first phase of group II curves as being exponential rather than rectilinear in character.

Phase 2. Analyses of the 10 regression lines (second phases) of the group II cases by the analysis of variance method again revealed F values which were very highly significant ($P \ll 0.001$) for either the arithmetic or logarithmically transformed galactose values. Generally, higher F values for the regressions were obtained with logarithmically transformed data than with arithmetic data (table 2, columns L and M). However, the arithmetic data F values were higher in 3 of the 10 cases. The ratios of the

standard error of estimate to mean galactose were smaller for the logarithmically transformed data than for the arithmetic data in every one of the 10 cases, averaging 1.9 and 9.9 per cent, respectively (table 2, columns N and O). These statistical data, based on 10 regression lines composed of moderate numbers of individual points (average of 8.2 points per regression line), indicate that the second phase of the blood galactose disappearance curve in the group II cases also describes an exponential function, not a rectilinear one. These data would seem to be of about the same level of reliability as were the statistical data of phase 1. However less strong this conclusion may be when group II data are compared with the data of group I, it must be recognized that these data cannot be interpreted as indicating a linear rather than an exponential function.

The correlation coefficients for the logarithmically transformed data ranged from (-)0.972 to (-)0.998, and for the arithmetic data from (-)0.930 to (-)0.998. The Z transformation and t tests of significance were applied. As in phase 1, a statistically significantly better fit of the logarithmically transformed data occurred only once in the 10 patients. The single t test of the differences of the Z values was applied to the entire group II, phase 2 curves. The mean difference (\bar{d}) of the Z values for the logarithmically transformed and the arithmetic data was 0.403. The standard deviation of the mean difference (S_d) of the Z values was 0.208. The $t_{(9)}$ value, $(\bar{d}/S_d) = 0.403/0.208 = 1.94$, indicated no statistically significant difference ($P < 0.09$) between the Z values (or the correlation coefficients they represent). Despite the absence of a clear statistically significant difference, these data do favor an exponential rather than a rectilinear blood galactose disappearance curve.

The marked similarity of the statistical data warrants the conclusion that the blood galactose disappearance curves in group I cases and in both phases of group II cases are exponential in character, not rectilinear.

Discussion

Character of Blood Galactose Disappearance Curves

The statistical analyses of the data (table 2) clearly indicate that the post-mixing blood disappearance curve following a single injection of galactose consists of either (1) a single exponential phase (group I, cases 1 to 19), or (2) two phases, both of which are exponential, the second phase

having a greater slope than the first (group II, cases 20 to 29). The exponential character of the curves in group I and in phase 1 of group II, agrees with the data of Colcher *et al.*⁴ and Dominguez and Pomerene.⁵ The conflict with the statistics of Tygstrup and Winkler,¹ whose data were interpreted as showing a rectilinear blood curve, may be due to their having used smaller numbers of points (average of 6.2 per regression line), and to an arbitrary selection of the most linear appearing portion of the curve. Detailed statistical analyses for their individual sets of data rather than for grouped data were not presented.¹ The conflict with the preliminary data of Waldstein and Arcilla³ also may be due to the smaller numbers of points used. Certainly, parts of the exponential curves in the range of values under consideration may greatly resemble rectilinear plots. It is by the use of larger numbers of individual points that the exponential nature of the blood galactose disappearance curve may be clearly demonstrated.

Evaluation of Liver Function with Blood Galactose Disappearance Curves

Use of fractional disappearance constants of exponential curves. In group I cases, consisting of single exponential phase curves, the controls tended to have larger fractional disappearance constants (k_1) than the liver abnormality group, averaging 3.55 and 1.68 per cent, respectively (table 2, column J). However, there was considerable overlap of the two groups. The separation of normal from abnormal at the 4.0 to 4.2 per cent level, as reported by Colcher *et al.*,⁴ was not found. Furthermore, a low k_1 value was often associated with normal Bromsulphalein retention. Over-all, the fractional disappearance constant was not found to be a clinically valuable index of liver function.

Calculations of liver function from two-phase curves. The systems of Hansen *et al.*² and of Waldstein and Arcilla³ for calculation of liver mass (L_m galactose) and effective hepatic blood flow are both based on the demonstration of an initial (postmixing) rectilinear galactose disappearance curve. Since the data presented in this report

clearly have been shown to be exponential and not rectilinear in character, these systems could not be applied.

Implication of Blood Galactose Disappearance Curves Having Two Exponential Phases

The finding of two phases, both exponential, in the galactose disappearance curves of 4 controls and 6 patients with liver abnormalities (table 2, group II) was unexpected. The frequent and large numbers of blood samples obtained allowed conclusive demonstration by statistical analyses of the exponential nature of both phases. In each the second phase had a greater slope than the first (fig. 2).

If one tentatively accepts the two propositions underlying the calculations of Tygstrup and Winkler² and Waldstein and Arcilla,³ that (1) galactose has a fixed volume of distribution throughout the duration of the test, and (2) galactose is in continuous concentration equilibrium throughout this volume of distribution, certain information can be inferred. From the absolute blood levels and the slopes, the rates of change of blood galactose concentration may be determined for any time, t :

$$\frac{dB_t}{dt} = B_t k_1$$

where B_t represents the galactose concentration in blood (and throughout the calculated volume of distribution) at any time, t ; and k_1 (or k_2) is the slope of the first (or second) phase. The rate of change may then be transformed to absolute amounts of galactose removed per minute by assuming this rate of concentration change for the entire galactose volume of distribution (fig. 2).

Such calculations applied successively to all points of the curve indicate that, at the point between the two phases, the greater slope of phase 2 must represent a sudden increased rate of utilization (disappearance from blood stream) (fig. 2). To test this, two repeat experiments were performed (on cases 23 and 27) in which two full doses of galactose were injected 50 minutes apart. In both experiments the slope of the exponential disappearance curve following

ential
these

urance
ses

onent-
urves
er ab-
unex-
bers of
usive
of the
each
than

position-
strup
cilla,³
one of
on of
uous
this
ation
blood
ge of
be

acen-
calcu-
ime,
(or
then
s of
ning
the
ation

y to
the
ater
lden
ance
two
(on
s of
part.
ex-
ving

the second galactose injection was *less than*, not greater than, the slope following the first galactose injection. Therefore, there was not an accelerated rate of utilization following a latent period to explain the increased slope of the second exponential phase.

If no absolute acceleration of galactose utilization occurs, the increased rate of change of the blood galactose during the initial part of phase 2 as compared with the terminal part of phase 1 must mean that one or both of the propositions tentatively accepted must be incorrect; that is, the galactose volume of distribution does not remain constant, or the concentration of galactose is not in continuous equilibrium throughout the galactose space, or both.

A hypothesis has been formulated to explain the data. Assume that injected galactose rapidly diffuses throughout red cell water, extracellular fluid, and an additional compartment, *g* (fig. 3). The calculated zero time galactose space (*G*) is known to be greater than extracellular space (*ECF*).^{2, 3} Assume that there exists another space, compartment *U*, into which galactose diffuses slowly. During phase 1 the saturated liver galactose conversion mechanism removes a fixed amount of galactose per minute. Waldstein *et al.*⁶ have demonstrated the existence of such an *Lm* galactose by constant infusion studies. Superimposed upon this linear component is an exponential component due to continuing diffusion from *G* into compartment *U*, this diffusion being more rapid initially because of the large concentration gradient immediately after the galactose injection. As the blood level falls (and the concentration in *U* rises) the gradient between *G* and *U* diminishes to zero (equilibrium). Subsequent to this equilibrium, the blood level continues to fall because of rapid removal by liver and kidney, creating a concentration gradient in the reverse direction (*U* greater than *G*). Because of the lower absolute blood levels at this time, the concentration gradient must be small. Therefore, the movement of galactose from *U* to *G* during phase 2 is considerably slower than it was from *G* to *U* during phase 1. Because of the continued rapid fall of blood level (due to liver

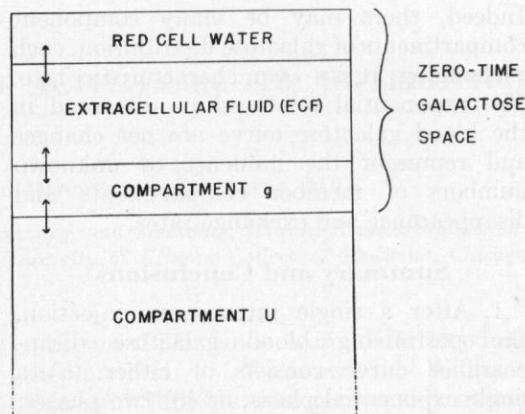


FIG. 3. Compartments into which galactose diffuses. Arrows represent movement of galactose in both directions across compartmental boundaries.

conversion and renal excretion), and the low concentration gradient, there occurs a lag in the return of galactose from *U* to *G*. Therefore, the fall in blood galactose level during phase 2 reflects the fall in concentration of galactose in compartment *G* and in some incomplete portion of compartment *U*. This would explain the apparent (but not real) increased rate of galactose utilization during the initial portion of phase 2.

The implications of the above speculative hypothesis (which fits the data) are that: (1) during at least phase 1 there is a continuing increase in galactose distribution volume (into compartment *U*); and (2) during both phases 1 and 2 there is *not* a continuing concentration equilibrium between the various compartments (red cell water, *ECF*, *g*, and *U*) (fig. 3) comprising the galactose volume of distribution.

Group I single phase exponential galactose disappearance curves could represent the first phase of group II curves in patients with very large *U* compartments. This is likewise speculative. The nature of compartment *U* is unknown. A similar compartment has been inferred from disappearance curves following injection of radiosulfate.¹¹

The data have been interpreted to mean that there is neither a single volume of galactose distribution nor a continuing concentration equilibrium throughout the components of any single galactose space.

Indeed, there may be many component compartments of galactose distribution, each exchanging at its own characteristic rate. The exponential relationships discerned in the blood galactose curve are net changes and represent the influence of unknown numbers of member compartments and disappearance and exchange rates.

Summary and Conclusions

1. After a single intravenous injection, the postmixing blood galactose disappearance curve consists of either (a) a single exponential phase, or (b) two phases, both exponential, the second phase having a greater slope than the first.

2. The fractional disappearance constant of the blood galactose exponential curve does not appear to be a clinically valuable index of liver function.

3. The data were interpreted as indicating that after a single galactose injection there is neither (a) a constant galactose volume of distribution throughout the duration of the test, nor (b) a continuous galactose concentration equilibrium throughout the galactose volume of distribution. These interpretations preclude calculation of hepatic maximal removal capacity for galactose (L_m galactose) or effective hepatic blood flow from the blood galactose disappearance curves following *single intravenous* injections.

Acknowledgments. The authors wish to express appreciation to Dr. Sheldon S. Waldstein for the many helpful discussions of this work, and to Dr. Herbert C. Batson for his advice on the statistical treatment of the data.

REFERENCES

- TYGSTRUP, N., AND WINKLER, K. Kinetics of

- galactose elimination. *Acta Physiol. Scand.* **32:** 354, 1954.
- HANSEN, A. T., TYGSTRUP, N., AND WINKLER, K. Determination of the hepatic blood flow by galactose. *Danish Med. Bull.*, **1:** 146, 1954.
- WALDSTEIN, S. S., AND ARCELLA, R. A. Measurement of hepatic blood flow by clearance methods; a review and a theoretical basis for a new noncatheterization method. *Amer. J. Dig. Dis.*, **3:** 137, 1958.
- COLCHER, H., PATEK, A. J., JR., AND KENDALL, F. E. Galactose disappearance from the blood stream; calculation of a galactose removed constant and its application as a test for liver function. *J. Clin. Invest.*, **25:** 768, 1946.
- DOMINGUEZ, R., AND POMERENE, E. Kinetics of the disappearance of galactose from the plasma after a rapid intravenous injection. *Amer. J. Physiol.*, **141:** 368, 1944.
- WALDSTEIN, S. S., GREENBERG, L. A., BIGGS, A. D., JR., AND CORN, L. Demonstration of hepatic maximum removal capacity (L_m) for galactose in humans. *J. Lab. Clin. M.*, **55:** 462, 1960.
- NELSON, N. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.*, **153:** 375, 1944.
- WALDSTEIN, S. S., ARCELLA, R. A., AND DUBIN, A. The effect of purified glucose oxidase on galactose. *Quart. Bull. Northw. Univ. Med. Sch.*, **31:** 323, 1957.
- BATSON, H. C. *An Introduction to Statistics in the Medical Sciences*. Burgess Publishing Company, Minneapolis, 1956.
- SNEDECOR, G. W. *Statistical Methods Applied to Experiments in Agriculture and Biology*, Ed. 4. Iowa State College Press, Ames, Iowa, 1950.
- RYAN, R. J., PASCALE, L. R., INOUYE, T., AND BERNSTEIN, L. M. Experiences with radio-sulfate in the estimation of physiologic extracellular water in healthy and abnormal man. *J. Clin. Invest.*, **35:** 1119, 1956.