DEMONSTRATION OF HEPATIC MAXIMUM REMOVAL CAPACITY (L_m) FOR GALACTOSE IN HUMANS

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WHEN substances are transferred across living membranes by processes which use energy derived from cellular metabolism, the transfer is known as "active transport."[1] One important characteristic of such transport is that when increasing loads of the substance transported are presented, a fixed maximal transfer rate is eventually reached (saturation), beyond which transfer cannot be increased no matter how great the load. For the kidney, transport maxima (T_m's) for substances which are reabsorbed or secreted by the renal tubule have been demonstrated and carefully characterized.[2]

Several authors have shown that the liver has a maximum removal capacity for sulfobromophthalein (BSP) and have used the term "L_m" to represent it (and presumbably other hepatic transport maxima, as well).[3-5] Recently, the theory for a noncatheterization clearance method for estimating hepatic blood flow was presented, which is based upon the use of a test substance for which the liver has such an L_m.[6] However, direct evidence for the existence of hepatic L_m's for substances other than BSP which are removed from the blood, or secreted into the blood or bile by the liver, has been lacking. The purpose of the present report is to provide such direct evidence of an L_m in the normal human liver for galactose. The use of galactose to demonstrate an L_m (in this instance, a removal L_m) was suggested by the observed behavior of this substance after single intravenous injection.[6] Constant infusion to concentration equilibrium was used to minimize error caused by the rates of diffusion into, and mixing within the ultimate volume of distribution. Galactose titration was used to demonstrate the L_m in a manner similar to the classical use of glucose titration to demonstrate the glucose reabsorptive T_m of the renal tubule.[7]

MATERIALS AND METHODS

Fifteen men, between 22 and 62 years of age, convalescent from a variety of nonhepatic disorders, were the subjects of this study. Physical examination and a battery of liver tests were negative for hepatic abnormalities in every subject. All studies were done with the subject in a fasting condition and sedated with promazine hydrochloride 50 mg. intramuscularly.

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After voiding and discarding the urine, the subject drank 1 to 1½ L. of water to insure an adequate output, so that subsequent urine collection did not require urethral catheterization and its attendant hazards.[8] From time to time he drank additional water to maintain urine output. Urine was collected after a timed interval of approximately 30 minutes, the volume recorded, and an aliquot used to measure nongalactose-reducing substances, the rate of excretion of which varied from 0.5 to 3.5 mg. per minute (average 1.1 mg. per minute). It was assumed that the excretion of these substances was constant throughout the experimental period. The control rate of excretion was subtracted from the excretion rate of total reducing substances in the subsequent galactose-containing urine samples to obtain galactose excretion rates during the experimental periods.

Constant infusion of galactose at a predetermined rate was accomplished by means of a motor-driven syringe pump* which was known to deliver either 1.69 or 2.54 ml. per minute depending upon the gear ratio used. The 50 ml. pump syringe was refilled during the infusions from a reservoir connected to it by means of a threeway stopcock. The maximum time of interruption of an infusion in order to refill the syringe was 105 seconds, but interruptions averaged only 33 seconds. Priming doses were injected through the stopcock. The syringe was connected by polyethylene tubing of PE 240 gauge to an indwelling 19 gauge needle inserted into an antecubital vein of the left arm.

Peripheral blood samples, each 3 to 4 ml. in volume, were obtained by means of an indwelling 18 gauge Cournand needle inserted into an antecubital vein of the right arm. Samples from the hepatic vein were obtained by means of a F7 Nylon cardiac catheter which had been introduced into the left cephalic vein and advanced into a right hepatic vein under fluoroscopic guidance. Each blood sample was immediately transferred to a 50 ml. flask containing dried heparin as the anticoagulant and thoroughly mixed.

In 1 subject, a single infusion of galactose was carried out for 79 minutes to demonstrate that peripheral concentration equilibrium was attainable and maintainable. In each of the next 12 subjects, 2 rates of infusion (galactose titration) were carried out as follows: a 10 ml. control sample of galactose-free peripheral blood was obtained. The first (and slower) infusion was then begun and 30 minutes allowed for equilibrium to be reached, at which time the first urine sample was collected. Three to 7 peripheral blood samples were then collected at approximately 5 minute intervals, after which the second urine sample was collected. A second and faster infusion was then started. This was accomplished either by changing the gear ratio of the pump and using the original infusate, or by maintaining the original gear ratio and changing to an infusate of greater concentration. If the second rate of infusion was much greater than the first, a priming injection of 50 per cent galactose was given. After another 30 minutes to permit equilibration, urine and peripheral blood samples were obtained as before. All sample collections and interruptions of the pump for refilling or change of infusion were accurately timed by a stopwatch, with the start of the first infusion as reference time. It will be noted that the second and fourth urine samples were the ones collected during the equilibrium period. Finally, in each one of 2 additional subjects, hepatic vein catheterization was performed in conjunction with a single galactose infusion. In these, blood from peripheral and hepatic veins was sampled frequently throughout the entire infusion period.

The galactose solutions that were infused were prepared to approximate concentrations by the addition of 0.9 N NaCl to 50 per cent galactose solution (w/v) for intravenous injection (Pfanstiehl). A sample of each infusion was later analyzed for exact concentration. This permitted calculation of exact infusion rate from the known volume delivery rate of the calibrated infusion pump. Infusions and urine samples were analyzed for galactose by the Nelson-Somogyi hexose method.[9] Galactose in whole blood also was analyzed by this method after glucose in blood had first been eliminated by incubation with highly purified glucose oxidase.[10] Repeated analyses done by this technique are accurate to within 5 per cent in our experience. Regardless of blood concentration, 5 per cent of the galactose was

*Aminco Motor-Driven Compensator, American Instrument Co., Silver Springs, Md.

lost because of the glucose oxidase,[10] and was corrected for in the final calculation of galactose concentration. The small amount of nonhexose-reducing substance present in the blood was measured after treatment of the control-blood sample with glucose oxidase. This amount was subtracted from all subsequent galactose-containing samples. A standard solution of galactose, added to the control blood, and analyzed with, and without, glucose oxidase treatment, served to demonstrate that neither enzyme inhibition, nor unusual enzyme activity against galactose, occurred in the samples tested.

Calculations from the results obtained during the equilibrium periods were: (1) $R = I - U$, (2) $Cl_H = \frac{R}{P_{eq}}$, and (3) $Cl_U = \frac{U}{P_{eq}}$, where R is the removal rate of galactose within the body (mg. per minute), I is the rate of infusion (8 mg. per minute), U is the averaged urine excretion of galactose (8 mg. per minute), Cl_H is the "hepatic" clearance of galactose (milliliter per minute), P_{eq} is the averaged peripheral blood concentration (milligram per 100 ml.), and Cl_U is the renal clearance of galactose (milliliter per minute). Only the urine samples collected during the equilibrium periods were used in the caculations.

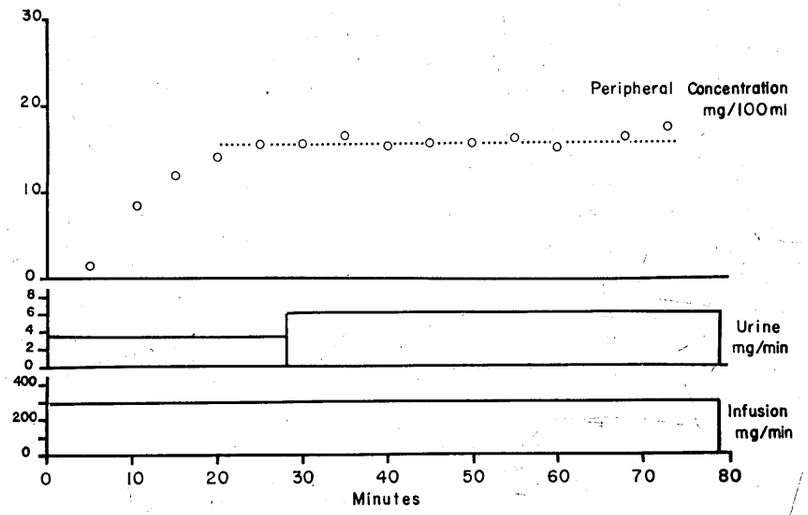

Fig. 1.—Results of infusion of galactose at the constant rate of 292 mg. per minute for 79 minutes. The dotted line represents the arithmetic mean of the 10 values for P obtained during the concentration equilibrium, and is referred to as P_{eq} in the text.

RESULTS

Fig. 1 illustrates that concentration equilibrium can be attained and maintained for a considerable period. In this subject, a constant infusion of galactose at the rate of 292 mg. per minute was given for 79 minutes. A priming injection of galactose was not given. The peripheral blood concentration of galactose (P) rose steadily, until a level of 15.65 mg. per 100 ml. was reached after 25 minutes of infusion. The galactose content of the urine collected at this time was 95.0 mg., averaging 3.4 mg. per minute for the first 28 minutes. However, this average has little meaning because it is an average of urine which was formed both when galactose levels in blood were very low and galactose excretion in urine was negligible, and when levels in blood were higher and excretion in urine was appreciable. The galactose concentration of the subsequent 9 peripheral

blood samples, drawn over the next 45 minutes, varied only slightly from the 25 minute sample. The arithmetic mean of these values was 15.95 mg. per 100 ml., with a standard deviation of 0.79 mg. per 100 ml. (The arithmetic mean will be referred to henceforth as P_{eq}, the mean equilibrium peripheral concentration, and is shown as the dotted line in Fig. 1.) Urine collected during the equilibrium period, from 28 to 79 minutes, contained 290 mg. galactose, or a mean galactose of 5.7 mg. per minute. This average means more, because peripheral blood concentration was constant during the period of formation of this urine. During the equilibrium, the rate of disposal of galactose through all routes must have equalled the rate of infusion, since the peripheral blood level neither rose nor fell. In this instance, therefore, extraurinary, intracorporeal removal of galactose (R) was equal to 292 (I) – 6 (U) or 286 mg.

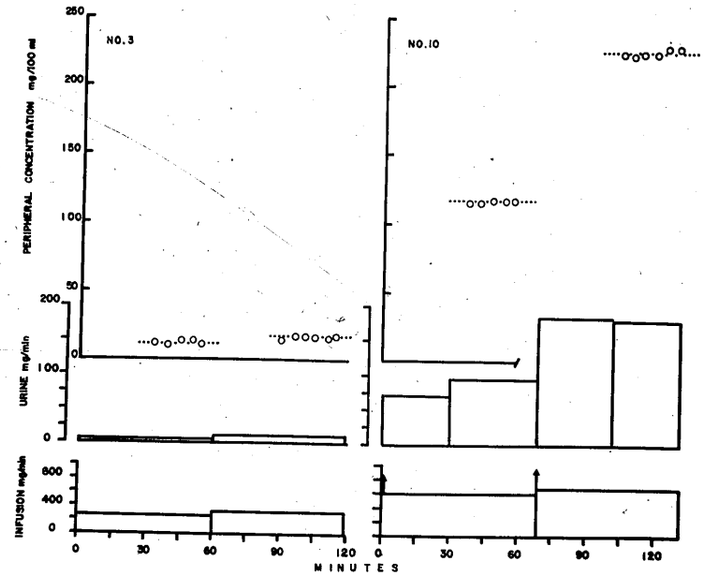

Fig. 2.—Typical results of galactose titration in 2 subjects. Left: in Subject 3, infusion of galactose at the rate of 244 and 302 mg. per minute respectively, resulted in little loss of galactose in the urine and low values for P_{eq}. Right: in Subject 10, infusion of galactose at the rate of 598 and 684 mg. per minute, respectively, resulted in large losses of galactose in the urine and high values for P_{eq}. The arrows indicate priming doses of 16 Gm. galactose each.

per minute. Renal clearance of galactose (Cl_U) $= \frac{5.7}{0.16} = 35.6$ ml. per minute. Finally, assuming for the moment that all intracorporeal removal was due to hepatic activity, "hepatic" clearance of galactose (Cl_H) $= \frac{286}{0.16} = 1{,}790$ ml. per minute.

Table I presents the data obtained in the next 12 subjects, in each of whom 2 levels of galactose infusion were done (galactose titration). Rates of infusion, mean equilibrium peripheral concentrations, standard deviations of P from P_{eq}, and urine excretions of galactose per minute, as well as the calculated

TABLE I. RESULTS OF GALACTOSE TITRATION IN 12 SUBJECTS WITHOUT LIVER DISEASE

SUBJECT	PERIOD	PRIMING DOSE (GM.)	INFUSION RATE (MG./MIN.)	LENGTH OF INFUSION (MIN.)	NO. OF SAMPLES	AT EQUILIBRIUM		CALCULATED		
						P_{eq} ± S.D. (MG./100 ML.)	U (MG./MIN.)	R (MG./MIN.)	Cl_U (ML./MIN.)	Cl_H (ML./MIN.)
	1		122	60	5	4.5 0.3		122		2710
	2		186	60	7	8.3 0.5		186		2250
1	Δ*		+ 64			+ 3.8		+ 64		- 560
	1		213	50	5	7.2 0.4	6	207	85	2880
	2		321	51	4	12.3 1.1	13	308	108	2550
2	Δ		+ 108			+ 5.1	+ 7	+ 101	+ 23	- 330
	1	3.3	244	58	5	10.5 0.6	4	240	36	2280
	2		302	55	5	16.5 0.5	7	295	43	1790
3	Δ		+ 58			+ 6.0	+ 3	+ 55	+ 7	- 490
	1		292	45	3	24.1 0.9	7	285	27	1180
	2		438	51	5	32.5 0.6	15	423	45	1300
4	Δ		+ 146			+ 8.4	+ 8	+ 138	+ 18	+ 120
	1	3.6	292	68	6	16.8 1.0	12	280	74	1665
	2		438	55	6	33.8 2.2	22	416	67	1230
5	Δ		+ 146			+ 17.0	+ 10	+ 136	- 7	- 435
	1	3.9	339	57	5	22.3 0.9	14	325	60	1460
	2	4.9	508	50	5	53.2 1.0	41	467	76	880
6	Δ		+ 169			+ 30.9	+ 27	+ 142	+ 16	- 580
	1	5.8	416	61	6	30.6 0.0	14	402	46	1315
	2		482	60	5	42.5 1.4	34	448	80	1055
7	Δ		+ 66			+ 11.9	+ 20	+ 46	+ 34	- 260
	1	2.9	547	56	6	40.2 0.9	63	484	156	1200
	2		582	50	3	76.1 0.6	73	509	96	695
8	Δ		+ 35			+ 35.9	+ 10	+ 25	- 60	- 505
	1	16.5	592	70	5	85.4 2.0	95	497	112	580
	2	8.3	646	61	5	122.9 0.0	140	506	114	410
9	Δ		+ 54			+ 37.5	+ 45	+ 9	+ 2	- 170
	1	16.0	598	68	5	116.6 1.3	94	504	81	430
	2	16.0	684	63	6	225.5 2.2	177	507	78	225
10	Δ		+ 86			+ 108.9	+ 83	+ 3	- 3	- 205
	1	15.5	598	62	6	66.2 1.9	88	510	132	765
	2	23.5	736	60	6	156.5 2.2	203	533	130	340
11	Δ		+ 138			+ 90.3	+ 115	+ 23	- 2	- 425
	1	26.2	671	62	5	131.3 1.8	159	512	121	390
	2	8.7	710	50	4	172.5 1.0	201	509	117	295
12	Δ		+ 39			+ 41.2	+ 42	- 3	- 4	- 95

*Δ = difference between periods (2 - 1).

values for R, Cl_U, and Cl_H for both periods in each subject, are listed. The first rates of infusion varied from 122 to 671 mg. per minute. The second rates of infusion varied from 186 to 736 mg. per minute. The differences between paired infusions varied from 35 to 169 mg. per minute. The times for infusions varied from 45 to 70 minutes, with an average of 58 minutes per infusion. The number of observations, from which P_{eq} was calculated, varied from 3 to 7, with an average of 5.1. The small standard deviations of P from P_{eq} indicate that satisfactory equilibria were attained.

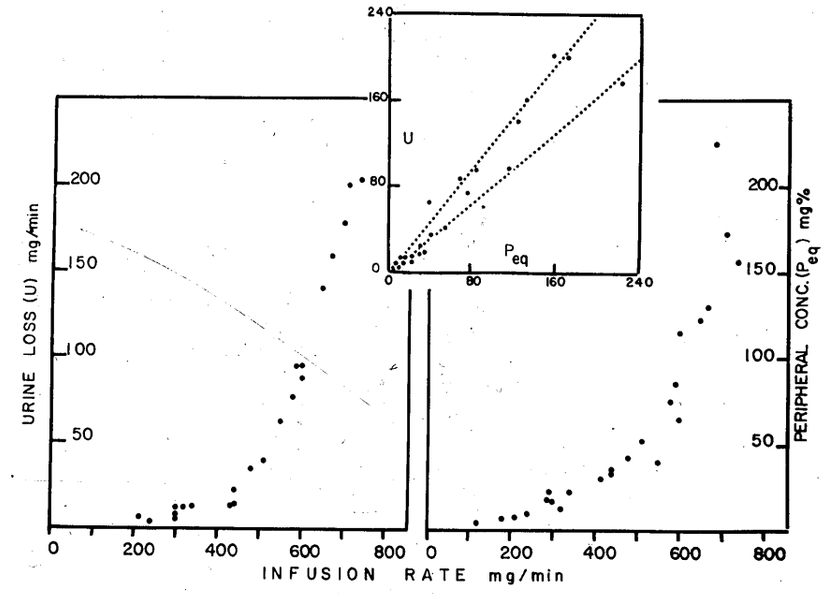

Fig. 3.—Relation of urine excretion rates and mean equilibrium peripheral concentrations to infusion rates of galactose. Inset: comparison of excretion rates to equilibrium concentrations. The upper dotted line represents a renal clearance of 120 ml. per minute, the lower dotted line, a clearance of 80 ml. per minute.

Representative of the results typical for the method are those plotted in Fig. 2. In Subject 3 (Fig. 2, left), the rate of infusion for the first period was 244 mg. per minute and for the second period 302 mg. per minute. U was 4 mg. per minute in the first period and 7 mg. per minute in the second. P_{eq} was 10.5 and 16.5 for the respective periods. With both infusion rates well below maximum removal capacity, P_{eq} and U were small. In Subject 10 (Fig. 2, right), by contrast, infusions at rates of 598 and 684 mg. per minute, respectively, effected much higher concentrations in the blood and excretions in the urine. The first infusion exceeded maximum hepatic removal capacity. The excess had to be eliminated in the urine in order to attain concentration equilibrium. At equilibrium, P_{eq} was 116.6 mg. per 100 ml., and U was 94 mg. per minute. With the second infusion, the increment could not be removed intracorporeally and had to be eliminated in the urine to attain concentration equilibrium. Now, P_{eq} was 225.5 mg. per 100 ml., and U was 177 mg. per

minute. Thus, in Subject 3, an increment of infusion of 58 mg. per minute effected a concentration increment of only 6.0 mg. per 100 ml., and a urine excretion increment of only 3 mg. per minute. In Subject 10, by contrast, an

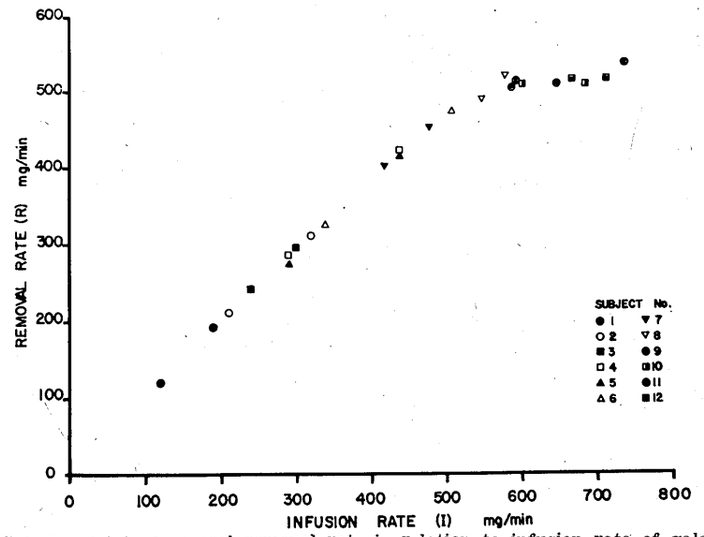

Fig. 4.—Calculated intracorporeal removal rate in relation to infusion rate of galactose in 12 subjects without liver disease.

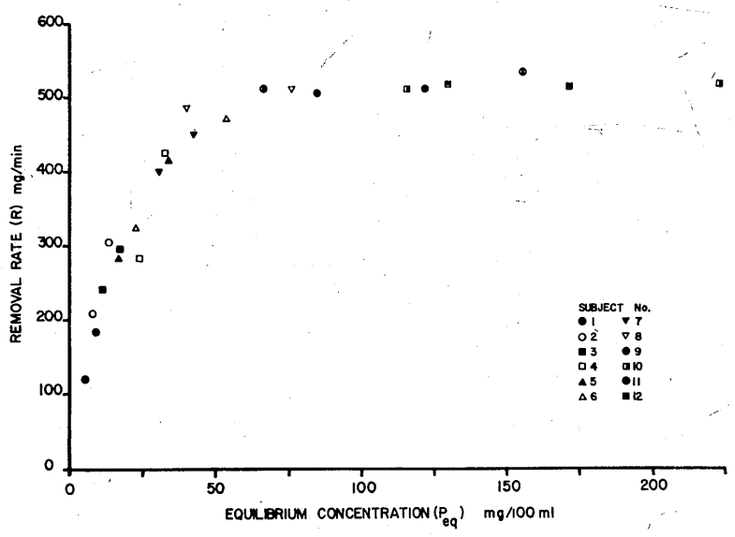

Fig. 5.—Galactose titration curve, derived from data obtained, in 12 subjects without liver disease. The coordinates differ from those classically used to plot titration curves (e.g., for the kidney) in that the abscissa is P_{eq} instead of load per removal maximum ratio, and the ordinate is removal instead of removal per removal maximum ratio. Because removal maxima and loads could not be determined for each subject, these ratios could not be calculated for each individual. In the absence of extrahepatic removal, for an individual having a removal maximum of 500 mg. per minute and a hepatic blood flow of 1,000 ml. per minute, a removal per L_m ratio of 1.0 on the ordinate would correspond to the point labeled 500, and a load per L_m ratio of 1.0 on the abscissa would correspond to the point labeled 50.

infusion increment of 86 mg. per minute effected a concentration increment of 108.9 mg. per 100 ml., and a urine excretion increment of 83 mg. per minute. The latter thus accounted for more than 96 per cent of the increment infused, which indicates that further intracorporeal removal of galactose was not accomplished, despite the faster rate of infusion.

The graphs (Fig. 3) were obtained when U and P_{eq} for each set of experiments were plotted against I. At rates of infusion up to 500 mg. per minute only small amounts of galactose were lost in the urine, and P_{eq} increased only moderately with the increasing rates of infusion. At rates of infusion above 500 mg. per minute, excretion in the urine markedly increased, and equilibria were obtained at greatly increased peripheral concentrations. As illustrated in the inset of Fig. 3, U was, in general, proportionate to P_{eq}.

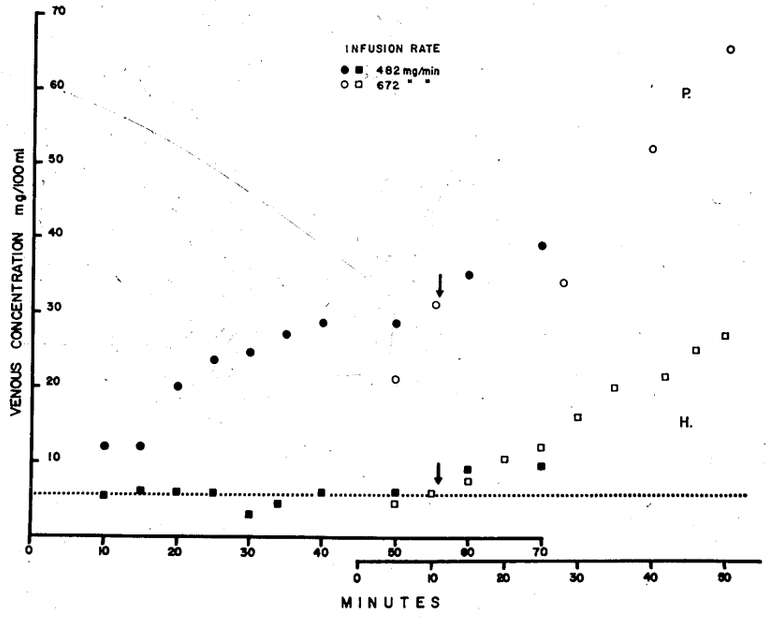

Fig. 6.—Peripheral (circles) and hepatic vein (squares) concentrations of galactose during constant infusion of galactose in 2 subjects of equal height and weight. No priming injections were given, and sampling was discontinued before concentration equilibrium was attained. The dotted line is an average of the initial hepatic vein concentrations. That these were not the zero expected by theory may have been due to sampling error caused by reflux from the vena cava or by arteriovenous shunts. The arrows indicate the time at which P first exceeded 30 mg. per 100 ml.

Fig. 4 shows a comparison between the calculated intracorporeal removal rate, R, and the rate of infusion. It can be seen that the removal rate was proportionate to the rate of infusion for all infusions up to approximately 580 mg. per minute. At faster rates of infusion, intracorporeal removal did not increase significantly above 500 mg. per minute. The attainment of a maximum removal rate was shown more strikingly in the plot of R against P_{eq} (Fig. 5). Removal rate increased proportionately to the equilibrium concentration, until

the latter was approximately 70 mg. per 100 ml., and R was about 500 mg. per minute. At all concentrations above this level (the highest being 225.5 mg. per 100 ml.), intracorporeal removal did not significantly exceed 500 mg. per minute. It should be noted that the ascending portion of the curve, shown in Fig. 5, joins the horizontal portion by a gradual smooth curve, rather than by an acute angle.

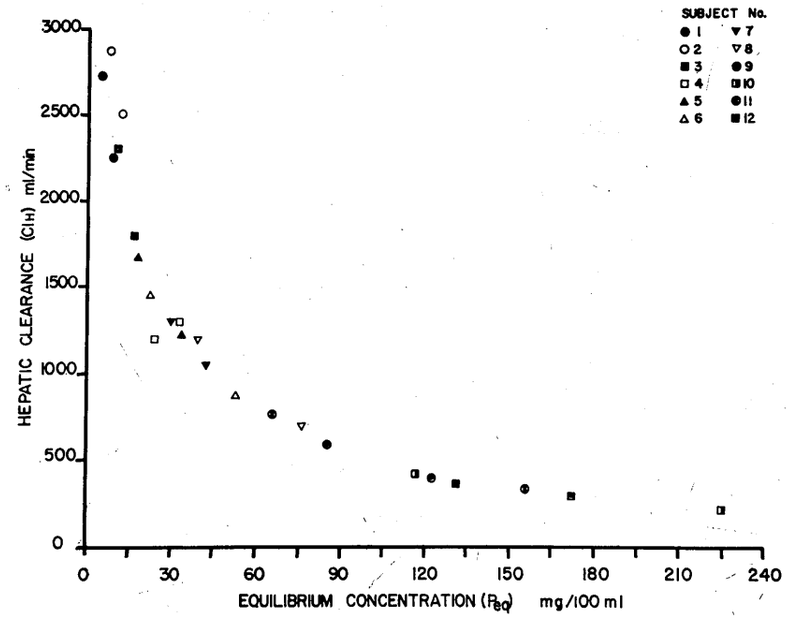

Fig. 7.—Relation of calculated "hepatic" clearance of galactose to P_{eq} in 12 subjects without liver disease. In all but 1 subject (No. 4) an inverse relation between concentration and clearance was found. The fact that this relationship holds as well for the range of P_{eq} below 30 mg. per 100 ml. suggests the presence of a small amount of extrahepatic removal of galactose.

Hepatic vein catheterization was performed in conjunction with galactose infusion in 2 subjects, who were of similar height and weight. In each, priming doses were not given, and blood from both peripheral (P) and hepatic (H) veins was sampled frequently, in order that the appearance of galactose in the hepatic vein in relation to its concentration in the periphery (Fig. 6) could be studied. In each subject, the first hepatic vein concentrations obtained were not truly zero, but were 5.4 mg. per 100 ml. This base line value is shown as the dotted line in Fig. 6. In the first subject (solid symbols), infused at a rate of 484 mg. per minute, H did not exceed the initial value in the 8 samples drawn during the first 55 minutes of infusion. During this period, P was less than 30 mg. per 100 ml. When P exceeded this concentration (arrow), H first rose above the base line. In the second subject (open symbols), a faster galactose infusion, at a rate of 684 mg. per minute, was given. P rose much faster and exceeded 30 mg. per 100 ml. in 10 minutes. It was at this time that galactose concentrations, greater than the baseline, first appeared in blood from the

hepatic vein. H increased steadily thereafter in keeping with the rising P. However, the slope of the ascending curve of H was shallower than that of P, and even at the termination of the experiment the 2 curves were not parallel.

The curve (Fig. 7) was obtained when intracorporeal clearance ("hepatic" clearance) of galactose was calculated for each pair of infusions in each subject, and the results plotted against P_{eq}. In all, with the exception of Subject 4, an inverse relation between clearance and equilibrium concentration was found at all levels of P_{eq}, including those below 30 mg. per 100 ml.

DISCUSSION

It is generally agreed that substances move across biologic membranes by simple diffusion, facilitated diffusion, or active transport.[1] Active transport implies that a substance is transferred across these membranes against a concentration gradient by using energy derived from cellular metabolism. However the gradient may be difficult to demonstrate, and the other mechanisms of transfer may appear to have an effect on this. Other important features of active transport are that the transfer is unidirected and that there is a nonlinear relation between rate of transfer and concentration, so that eventually saturation of the transport system occurs, and a fixed maximal rate of transfer is attained. The renal tubular epithelium has been shown to utilize active transport, for transfer of a number of substances. Determination of the maximal rate of transfer (T_m) for tubular reabsorption of some (e.g., glucose), and tubular secretion of others (e.g., paraaminohippurate), has become a standard procedure in the study of renal physiology and pathology.[2]

The role of active transport, and particularly of transfer maxima, by the liver epithelial cells has not been so clearly defined or demonstrated. Mason and co-workers,[3] Lewis,[4] and Verschure[5] have each shown that the liver has a limited capacity for the uptake of sulfobromophthalein (BSP) from the blood, and have calculated the maximum rate from known rates of infusion and changing peripheral blood concentrations. By analogy to the term T_m for tubular mass, the term L_m was proposed for "functional hepatic mass."[3] Although T_m may also be taken to stand for "transport maximum" and thereby be perfectly suitable to the liver, as well as to the kidney or to any other tissue, its usual identification with renal tubular maxima makes the use of distinct designations for other organs desirable. Since L_m has been previously used for the liver, its use has been continued in the present study.

When a substance is infused intravenously at a constant rate, concentration equilibrium is attained only when the rate of disposal by all means is equal to the infusion rate, for if disposal is slower than infusion, the concentration will rise, and if it is faster, the concentration will fall. Infusion at a constant rate to concentration equilibrium not only affords a means of easily measuring disposal rate, but avoids, as nonequilibrium states do not, estimation of such factors as volume of distribution, rate of diffusion, and rapidity of mixing, which may be very difficult, if not impossible, to measure. Berger and co-workers[11] have indicated that even constant infusion studies may be misleading, and the

apparent equilibrium may be an illusion. Within the bounds of practical clinical studies, however, this technique seems to avoid many pitfalls, and is the best available. It was used, therefore, in the present study. Galactose was chosen to be the substance used to study L_m in the liver for several reasons. Repeated clinical experience has shown that galactose removal from the blood depends primarily upon hepatocellular function.[12, 13] Recently, the liver cell has been shown to contain a specific enzyme system which converts galactose to glucose.[14] Enzyme systems are often a feature of active transport, and renal transport, at least, often conforms to the behavior predicted on the basis of enzyme-substrate interactions.[1] Although the red blood cell and the brain also contain some parts of the same enzyme system,[15] the liver has been shown to be the major site, quantitatively, of galactose uptake.[16] Finally, the observed behavior of galactose after single intravenous injection suggested the presence of a removal process with a maximum limiting rate.[6]

To demonstrate the L_m, galactose titration was employed. By titration is meant the measurement of removal at successively increased loads to determine the load at which maximum removal is reached. Smith and co-workers[7] used the titration technique to demonstrate reabsorptive maxima for glucose by the renal tubule. They were able to titrate each subject with many different loads. In the present study each galactose load was infused for an extended period (average 58 minutes) to assure adequate equilibrium. It was not practical, therefore, to do more than 2 infusions for each subject, as this would have extended the procedure to intolerable lengths. Nevertheless, the data from the 24 infusions into 12 subjects (Figs. 4 and 5) were in close agreement, and the individual values deviated very little from a single curve.

When the removal rate (R) was compared to rate of infusion (Fig. 4) and to equilibrium concentration (P_{eq}) (Fig. 5), it was found that intracorporeal removal did not significantly exceed 500 mg. per minute. Note that in Fig. 5 the abscissa is P_{eq}, not load. Because blood flow was not simultaneously measured, load (which is the product of flow times concentration) could not be known exactly. It is load rather than concentration with which we are concerned, for it is necessary to know that load was excessive and that every opportunity for maximal removal was afforded, in order to conclude that a maximum removal capacity was shown. A few simple calculations assure that load was indeed well in excess of removal capacity in these experiments. For blood galactose concentrations between 70 and 225 mg. per 100 ml., the maximum intracorporeal transfer rate was 500 mg. per minute. Hepatic removal by itself could, therefore, have been no greater. If hepatic blood flow was 1,500 ml. per minute (the average normal), then load varied from 1,050 to 3,375 mg. per minute, and load: removal maximum ratios varied from 2.10 to 6.75 in this range of concentration. If flow was as low as 500 ml. per minute (quite extreme for normals), load: removal maximum ratios varied from 0.70 to 2.25. If flow was as high as 3,000 ml. per minute, ratios varied from 4.20 to 13.50. It seems reasonable to assume, therefore, that the load presented to the liver was well in excess of

hepatic removal capacity at all concentrations corresponding to the horizontal portion of the curve (Fig. 5). Similar reasoning can be applied to all other possible, though quantitatively less important, sites of galactose removal.

The fact that intracorporeal removal did not increase, despite increasing loads, demonstrates the presence of a fixed maximum transfer (removal) rate, presumably caused by saturation of the transport system involved. The presence of a total body maximum removal rate implies that each of the sites effecting removal individually has a maximum rate, for if the removal rate of even one site was unlimited, total body removal would be unlimited. A body of evidence attests to the fact that the liver is the major site of removal of galactose from the blood. The liver thus appears to have a maximum capacity for removal of galactose.

Extrahepatic, intracorporeal removal is also likely. In animals, which have undergone hepatectomy, nephrectomy, and evisceration, galactose disappears from the blood, although at a slow rate.[17] Studies in which radioactive C^{14} galactose has been used have demonstrated metabolism of this compound by tissues other than the liver.[18] The results of the present study are also in keeping with a small amount of extrahepatic removal of galactose. By hepatic vein catheterization, it was shown that significant galactose did not appear in the hepatic vein until P reached 30 mg. per 100 ml. (Fig. 6). When P was greater than 70 mg. per 100 ml. there was constant removal of galactose (Fig. 5). There were, therefore, progressively smaller extraction ratios for rising values for P. Given a constant hepatic blood flow, clearance can be expected to have decreased with increasing values for P above 30 mg. per 100 ml. Below this concentration a constant hepatic clearance equal to hepatic blood flow could be expected, if the liver were the sole site for galactose removal. Examination of the plot of clearance against P_{eq} (Fig. 7) shows an inverse relation of clearance to P_{eq} at all levels, including those under 30 mg. per 100 ml. One explanation for this finding is the presence of a small, but constant, amount of extrahepatic removal.*

*Assume that intracorporeal removal, R, is caused by both liver removal, L_r, and extrahepatic removal, E_r. Further assume that E_r has a maximum capacity (E_m) that is 10 per cent of total intracorporeal removal capacity, and that liver maximum capacity (L_m) is 90 per cent of intracorporeal capacity. If R maximum is 500 mg. per minute, then E_m is 50 mg. per minute, and L_m is 450 mg. per minute. If hepatic blood flow (HBF) is 1,500 ml. per minute, load (flow $\times$ concentration) will exceed L_m at $P > 30$ mg. per 100 ml. As P is decreased below this concentration, load to the liver will become progressively smaller and less than L_m, and complete removal will be effected. Because of the magnitudes, however, of extrahepatic flow and E_m, load to these sites will exceed E_m at almost any measurable P. Hence, as P is decreased, L_r will be proportionately decreased, whereas E_r will be constant and at its maximum, E_m. Since $HBF = \frac{L_r}{P-H}$ (Fick), and $Cl_H = \frac{L_r}{P}$, when H is zero, $Cl_H = HBF$. Thus, when P is between 1 and 30 mg. per 100 ml. and H is zero, Cl_H should be constant and equal to HBF. But if R is really L_r plus E_r, then, substituting R for L_r in the clearance equation will result in a progressively larger overestimate of L_r as P is lowered, because of the progressively larger contribution to R made by E_m. In the example cited, when $P = 30$ mg. per 100 ml. using L_r, $Cl_H = \frac{450}{0.30} = 1{,}500$ ml. per minute, but apparent clearance, using R instead of L_r, is: $Cl_H = \frac{500}{0.30} = 1{,}666$ ml. per minute, an estimate that is 111 per cent of the true value. When $P = 5$ mg. per 100 ml., $L_r = 75$, and $E_m = 50$ mg. per minute. Using L_r, $Cl_H = \frac{75}{0.05} = 1{,}500$ ml. per minute, but using R, $Cl_H = \frac{125}{0.05} = 2{,}500$ ml. per minute, an estimate that is 167 per cent of the true value.

The theoretical plot of concentration (or load) against removal of a substance transferred by active transport shows increasing removal with increasing load, until the maximum transfer rate is reached. At this point of saturation of the transport system, the ascending straight line has an abrupt point of inflection, and meets the horizontal line of constant removal. Departure from this ideal curve, with a gradual transition from the ascending to the horizontal portion, instead of an abrupt, acute angle, has been termed splay.[7] The significance of splay in titration curves of renal tubular function has been discussed extensively.[7, 19, 20] The significance of splay in the galactose titration curve (Fig. 5) is, for the present, unknown. In part, it may be due to the fact that the curve was derived from galactose titration of 12 subjects. Another possibility is that there exists a range in the liver, as in the kidney, wherein saturation of the transport mechanism is completed in some areas before it is completed in others. This would give rise to a minimum threshold, below which no area spills galactose, as well as a maximum threshold, above which all areas spill galactose. From the data of the present study, the minimum threshold can be expected to be near 30 mg. per 100 ml. and the maximum near 70 mg. per 100 ml. As shown by catheterization, galactose does not appear in the hepatic vein in significant amounts below a peripheral concentration of 30 mg. per 100 ml. (Fig. 6).[21, 22] This can be taken to mean that no area of the liver is saturated and, therefore, no spill occurs. Above 70 mg. per 100 ml., maximum removal is reached (Fig. 5). Hence, all areas must be saturated and be spilling. It can be supposed that between 30 and 70 mg. per 100 ml. some areas are saturated and some are not. This is partly supported by the fact that up to peripheral levels of 68 mg. per 100 ml., the slope of hepatic vein galactose appearance is less steep than the slope of rising peripheral concentrations (Fig. 6). However, further studies will be necessary before the interpretation of these findings can be made with any confidence.

SUMMARY

Evidence for the existence of a maximum removal capacity (L_m) for galactose in the human liver has been obtained from the results of galactose titration in each of 12 subjects, who had no liver disease. Intracorporeal removal of galactose was calculated from the known rate of infusion, and from the measured rate of excretion in urine during the period of blood concentration equilibrium, for each of 2 infusions in each subject. At equilibrium, excretion of galactose in urine was proportionate to its peripheral blood concentration.

From the data, a titration curve can be constructed which primarily represents hepatic transport of galactose. It is splayed from the theoretical concentration–transfer rate curve. The maximum intracorporeal removal capacity for galactose is normally approximately 500 mg. per minute. Although the greatest part of this removal capacity is hepatic, a small amount of extrahepatic removal probably occurs.

Saturation methods appear to be applicable to the study of the liver and to afford another means of assessing hepatocellular function.

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