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Key words: galactose clearance; hepatic blood flow; liver disease.

## Estimation of the hepatic blood "flow" by galactose plasma clearance in patients with liver disease

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**ABSTRACT** - Following intravenous administration of 500 mg/kg b.wt. galactose, Galactose Elimination Capacity (GEC, mg/min/kg) was determined in 24 subjects with chronic non-cirrhotic liver disease (CLD), 33 with liver cirrhosis and 11 controls. GEC was significantly ( $P < 0.01$ ) reduced in both CLD and cirrhosis. A statistically significant difference ( $P < 0.01$ ) was present between these two groups. Following the plasma disappearance curve at concentrations below 1.25 mmol/l, at which the extraction coefficient is assumed to be equal to one, the "Efficient Hepatic Blood Flow" (EHBF, ml/min) was determined in 11 consecutive cirrhosis patients, seven patients with CLD and 11 controls. EHBF was normal or slightly reduced in CLD as compared to controls ( $1046 \pm 216$  vs.  $1471 \pm 156$  ml/min, mean  $\pm$  SEM, n.s.) whereas it was markedly reduced in cirrhosis ( $846 \pm 96$  ml/min, mean  $\pm$  SEM,  $p < 0.001$ ). Interestingly, a significant linear correlation ( $r = 0.757$ ,  $p < 0.001$ ) was present between EHBF and the plasma clearance of sulfobromophthalein. No correlation was present, on the other hand, between the value of GEC and that of EHBF. These data indicate that after a single intravenous injection of galactose, the hepatic blood flow passing through the enzymatically active parts of the liver (i.e. excluding shunts) can be measured.

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The measurement of plasma clearances has been extensively applied in hepatology in order to set up a methodology for the quantification of the hepatic function. Winkler et al. (1) have given an excellent review of the physiological basis.

The plasma disappearance rate of galactose was introduced as a quantitative measurement of liver function by Tygstrup et al. (2). The principle of the test is based on the demonstration that the sugar is essentially metabolised by hepatocytes except for a small amount (around 10%) eliminated through the kidney (3). Once inside the cell, galactose is phosphorylated by the galactokinase and subsequently metabolized. It follows

therefore that, at plasma concentrations high enough to ensure near-saturation of the phosphorylating enzyme (2.0 mmol/l) (4, 5), the plasma disappearance is accounted for by the amount of the enzyme, i.e. by the number of metabolically active hepatocytes. On these grounds, the test allows the determination of the hepatic functional mass expressed as Galactose Elimination Capacity (GEC).

In healthy man, at plasma concentrations below 2.0 mmol/l, the rate-limiting step of the process will be the hepatic blood flow. At this concentration the hepatic extraction coefficient of the sugar may be assumed to be equal to one and

Table 1

Biochemical parameters of patients with chronic liver disease (CLD) and liver cirrhosis (C). Results are expressed as mean  $\pm$  SEM. (\*) =  $p < 0.01$  vs. CLD.

	No. of patients	Serum albumin (g/l)	Prothrombin time (s)	AST (U/l)	Total bilirubin (mmol/l)
CLD	24	40.6 $\pm$ 1.0	11.86 $\pm$ 0.29	187 $\pm$ 22	19.32 $\pm$ 2.05
C	33	36.0 $\pm$ 1.0	13.77 $\pm$ 0.37*	111 $\pm$ 12*	27.53 $\pm$ 6.33
Normal values		$\geq$ 40.0	$\leq$ 11	$\leq$ 40	$\leq$ 17.1

accordingly, the blood is completely cleared of the substance at every passage through the liver. The plasma disappearance therefore reflects the "Efficient Hepatic Blood Flow" (EHBF) (6).

In agreement with this assumption is the recent demonstration that the galactose hepatic extraction in healthy subjects is 94%, whereas it declines to 79% in patients with liver cirrhosis (7).

On these grounds we have determined the EHBF both in patients with chronic liver disease and cirrhosis and in healthy controls after a single galactose intravenous injection by following its plasma disappearance at concentrations at which the extraction coefficient is supposed to be equal or very near to unity.

## Patients and methods

GEC was determined in 11 controls (six males and five females, mean age 28 years, age range 18–36), 24 patients with chronic liver disease (CLD) (18 males and six females, mean age 47, age range 18–72) and 33 with cirrhosis (23 males and 10 females, mean age 53, age range 35–72). Some of the biochemical tests performed in the two groups of patients are reported in Table 1. In all patients diagnosis was assessed by liver biopsy. Sixteen CLD were of alcoholic origin, the remaining eight related to HBV infection. In the case of cirrhosis, 26 were alcoholic and seven post-necrotic. None of the patients had diabetes, ascites or heart failure at the time of the test.

The morning after an overnight fast, 500 mg/kg b.wt. galactose (Kabi, Stockholm, Sweden) were injected intravenously within 5 min; after 20 min, 3 ml of blood were collected every 5 min up to 60 min in heparinized tubes in the presence of fluoride from a contralateral arm vein via an indwelling needle. In order to minimize the suspected erythrocytic metabolism of the sugar, blood samples were stored at +4°C and plasma was separated and collected within 15 min after sampling. Under these conditions, plasma was shown to lose less than 0.1  $\mu$ mol/l/h (8). Galactose determination was enzymatically performed within 1 h after the end of the test according to Wallensfels & Gerhart (9). Before and 240

min after the galactose injection, the subject was invited to urinate and all urine was collected. Urinary galactose elimination was determined in each subject with the above-mentioned method (9).

Plasma concentrations were plotted against time, and the line fitted from a least-square regression line using a linear scale. GEC was calculated according to Tygstrup (10) on the basis of the following formula:

$$\text{GEC (mg/min/kg)} = I - U/t_{c=0} + 7 \quad [1]$$

where  $I$  is the injected dose,  $U$  urinary elimination,  $t_{c=0}$  the intercept with the abscissa and 7 is a correction for uneven distribution.

Initial Distribution Volume (DV) was calculated according to the relationship:

$$\text{DV (ml/kg)} = \frac{\text{injected dose (mg/kg)}}{\text{plasma concentration at 0 time (mg/ml)}} \quad [2]$$

EHBF was determined in 11 consecutive patients with cirrhosis of the liver (seven males and five females, mean age 47, age range 36–68), seven with CLD (five males and two females, mean age 38, age range 18–70) and 11 controls (six males and five females, mean age 28, age range 18–36). 500 mg/kg b.wt. galactose were injected and blood samples collected as previously described. In this case, in order to follow the plasma disappearance at concentrations lower than 1.25 mmol/l, blood samples were collected every 5 min up to 120 min.

Plasma concentration values below 1.25 mmol/l were determined in duplicate and plotted against time on a semilogarithmic scale. The plasma fractional disappearance rate ( $k_1$ ,  $\text{min}^{-1}$ ) was determined from a least-square regression line. In each case, the correlation coefficient of the regression line was found to be higher than 0.97 ( $p < 0.001$ ). EHBF, as indicated from the volume of plasma from which galactose is irreversibly removed by the liver per time unit, was calculated according to the formula:

$$\text{EHBF (ml/min)} = k_1 (\text{min}^{-1}) \times \text{DV (ml)} \quad [3]$$

Sulfobromophthalein (BSP) plasma clearance was determined by injecting 5 mg/kg b.wt. and following the plasma disappearance by blood sampling every 3 min up to 12 min. From the regression line of the plasma values plotted against time on a semilogarithmic scale, the plasma fractional disappearance rate ( $k_1$ ,  $\text{min}^{-1}$ ) and

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CLD	Cirrhosis	Controls
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liver cirrhosis (C). Results are expressed

AST (U/l)	Total bilirubin (mmol/l)
187 ± 22	19.32 ± 2.05
111 ± 12*	27.53 ± 6.33
≤ 40	≤ 17.1

galactose injection, the subject was invited to urinate. Urine was collected. Urinary galactose was determined in each subject with the method (9).

Correlations were plotted against time, and a least-square regression line using a was calculated according to Tygstrup of the following formula:

$$U = I - U/t_{c=0} + 7/kg \quad [1]$$

ected dose, U urinary elimination,  $t_{c=0}$  the abscissa and 7 is a correction for

concentration. Urinary Elimination Volume (DV) was calculated according to:

$$DV = I - U/t_{c=0} \times 7/kg \quad [2]$$

Concentration at 0 time (mg/ml)

determined in 11 consecutive patients with liver (seven males and five females, mean age 36–68), seven with CLD (five males and two females, mean age 38, age range 18–70) and 11 males and five females, mean age 28, age 20 mg/kg b.wt. galactose were injected intravenously. Samples were collected as previously described. In order to follow the plasma disappearance rate lower than 1.25 mmol/l, blood samples were taken every 5 min up to 120 min.

Disappearance values below 1.25 mmol/l were duplicate and plotted against time on a scale. The plasma fractional disappearance ( $k_1$ ) was determined from a least-square fit. In each case, the correlation coefficient of the line was found to be higher than 0.97. As indicated from the volume of plasma, galactose is irreversibly removed by the liver, it was calculated according to the formula:

$$k_1 = (min^{-1}) \times DV (ml) \quad [3]$$

Sulfobromophthalein (BSP) plasma clearance was determined 5 mg/kg b.wt. and following the disappearance by blood sampling every 3 min from the regression line of the plasma disappearance rate ( $k_1$ , min<sup>-1</sup>) and

Table 2

Galactose elimination capacity (GEC, mg/min/kg), distribution volumes (DV, ml/kg) and urinary elimination (UE, % of the injected dose) in the three groups. Results are expressed as mean ± SEM. (\*\*) = p < 0.01 vs. controls; (\*\*\*) = p < 0.01 vs. CLD.

	No. of patients	GEC (mg/min/kg)	DV (ml/kg)	UE (%)
CLD	24	5.21 ± 0.37**	330 ± 15	11.25 ± 1.01
Cirrhosis	33	4.02 ± 0.21***	364 ± 17	12.02 ± 0.98
Controls	11	7.28 ± 0.28	365 ± 18	8.75 ± 0.83

the plasma clearance (ml/min) were calculated according to formula [3].

## Data analysis

Data are expressed as mean ± SEM. Statistical analysis was performed using Student's *t*-test for unpaired data.

## Results

Table 2 reports the values of GEC, distribution volume (DV) and urinary elimination (UE) in controls, CLD and cirrhosis. GEC was significantly reduced in both CLD and cirrhosis, the reduction in the former being, on the other hand, significantly lower as compared to that observed in the latter (p < 0.01, n = 57). On the contrary, comparable values of the DV and in the UE were observed in the three groups. A linear statistically significant correlation was found to be present between GEC and serum albumin concentration (r = 0.894, p < 0.001). On the other hand, GEC was not correlated with any of the other biochemical parameters explored, with the exception of prothrombin time which was significantly negatively correlated with GEC (r = -0.872, p < 0.001).

Table 3 gives the EHBF (ml/min) and BSP

Table 3

Efficient hepatic blood flow (EHBF) and sulfobromophthalein clearance (Cl<sub>BSP</sub>) in patients with liver cirrhosis and chronic liver disease (CLD) and controls. Results are expressed as mean ± SEM. (\*) = p < 0.001 vs. CLD; ND = not determined.

	No. of patients	EHBF (ml/min)	Cl <sub>BSP</sub> (ml/min)
CLD	7	1406 ± 216	371 ± 40
Cirrhosis	11	846 ± 96*	143 ± 18*
Controls	11	1471 ± 156	ND

clearance (ml/min) values in the three groups. In the case of controls, BSP clearance was not performed for ethical reasons. The value of EHBF in CLD was superimposable on controls whereas it was significantly reduced in cirrhosis (846 ± 96 vs. 1406 ± 216 ml/min, mean ± SEM, n = 18, p < 0.001). Again no correlation was found to be present between EHBF and any of the explored biochemical tests, with the exception of BSP clearance. In fact, a linear and significant correlation linked these two parameters (r = 0.757; p < 0.001).

## Discussion

The data presented confirm the already reported utility of the determination of the galactose elimination capacity as a quantitative liver function test (10, 11). In fact, this test provides a sensitive tool in assessing the total capacity of the liver in the phosphorylation of galactose, a step which is critically dependent on the presence of ATP within the hepatocyte. As far as diagnostic efficacy is concerned, GEC has been shown to have a rather low sensitivity (11). Our results are in line with these findings mostly because a rather large scattering of results in the two groups of patients is present. The finding that in both patients and controls the urinary elimination was superimposable suggests that the extra-hepatic elimination of galactose was in the same range. The distribution volume was also comparable in the three categories.

An interesting point emerging from our investigation is the possibility of determining the EHBF by following the plasma disappearance at concentrations below the assumed Vmax of the phosphorylating enzyme. This method was previously applied by Tygstrup et al. (6) in 1958. In that study, galactose was infused intravenously at a

constant rate in order to maintain its blood concentration below the Vmax of the enzyme and, thus, to allow a complete removal of the substance at every passage through the liver. Under these conditions a value of hepatic blood flow superimposable on that we have found in controls was observed. In addition, a close correlation between galactose plasma clearance and hepatic blood flow, as determined by BSP infusion, was present in subjects with normal galactose elimination. Our data support and extend these previous findings with the demonstration that again a close correlation exists between BSP plasma clearance and EHBF determined by a single injection of galactose also in patients with various degrees of hepatic function.

In healthy subjects, the galactose plasma concentration at which the hepatic extraction coefficient is equal to 1 has been demonstrated to be below 2.0 mM (6). By using plasma concentrations below 0.5 mM, Henderson et al. (7), showed that the hepatic extraction of galactose was 94% in controls and 79% in cirrhotics (7). That study was done, however, with a constant intravenous infusion of galactose and therefore with a quite complicated experimental design. The decreased hepatic extraction at the low galactose plasma concentration present in liver cirrhosis may be regarded as the result of an alteration in perfusion together with a reduced hepatocellular function and an alteration in the vascular permeability. In our study we have followed the plasma clearance of the sugar at concentrations below 1.25 mM and assumed that, under these conditions, the hepatic extraction of the substance was equal to 1. Admittedly this assumption has yet to be experimentally proved. However, if a reduction around 15% in the hepatic extraction was demonstrated to be present in cirrhosis (7), it seems conceivable that at the concentrations used in the present study (below 1.25 mM), the hepatic extraction coefficient would be around 100% also in patients with chronic liver disease.

It is interesting that EHBF determined in healthy controls correlates well with the values of hepatic flow obtained with other techniques (12). EHBF seems to discriminate well between patients with cirrhosis and those with CLD, no overlapping existing between the two classes. It must

be stressed, however, that this could be due to the rather low number of patients, and probably some false positive and negative cases will be found, thus extending the number of cases.

The close correlation we found between EHBF determined by plasma galactose clearance and by BSP clearance suggests that both tests may be considered as markers of hepatic delivery of substances the liver has to handle. Even if there are many differences in both the uptake and the subsequent intracellular handling between the two substances, it may be assumed that the plasma disappearance of galactose and BSP is critically dependent on the blood flow to the "functional hepatocyte". The lack of correlation between GEC and EHBF underlines how different the two entities are. The first reflects principally the number of functioning liver cells, the second is mostly accounted for by the blood flow delivering the substance to the cell and represents the plasma volume from which galactose is irreversibly cleared per time unity by the liver.

In conclusion, from the data presented it appears that with a single injection of galactose it is possible to determine two different parameters of basic importance from a theoretical and clinical point of view. The already demonstrated prognostic value of GEC (11, 13) could be reinforced by the determination, at the same time and in the same patient of EHBF. Prospective studies could provide us with more information about this clinically relevant topic.

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this could be due to the patients, and probably some of these cases will be found, or of cases.

found between EHB<sup>F</sup> lactose clearance and by that both tests may be hepatic delivery of substrate. Even if there are the uptake and the subcellular binding between the two it is assumed that the plasma bile and BSP is critically low to the "functional" correlation between how different the two tests principally the numbers, the second is mostly blood flow delivering the represents the plasma lactose is irreversibly the liver.

The data presented it appears that in the detection of galactose it is different parameters of theoretical and clinical studies demonstrated prognostic could be reinforced by same time and in the prospective studies could make a statement about this clin-

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