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Quantitative liver functions after 70% hepatectomy

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Abstract. Following 70% hepatectomy on rats the galactose elimination capacity, taken as a measure of the cytosolic liver function, was reduced from 2.55 ± 0.48 to 1.27 ± 0.19 (mean \pm SEM) $\mu\text{mol/min}$. Six hours later it was restored to control values. The prothrombin index, representing the function of the endoplasmic reticulum, was reduced from 1.13 ± 0.02 to 0.34 ± 0.02 (arbitrary units) after 12 h, and it was restored after 96 h. The rapid normalization of the initial fall in the capacity to metabolize galactose reflects a two- to three-fold increase of the galactose metabolizing capacity of the remaining liver.

This study demonstrates that liver functions are dissociated in time following 70% hepatectomy in the rat, and that the galactose elimination capacity is restored before regeneration can compensate for the loss in liver cell mass.

Key words. Hepatectomy, galactose elimination capacity, prothrombin index.

Introduction

The galactose elimination capacity (GEC) and the prothrombin index (PP) represent cytosolic and microsomal liver functions, respectively. In an earlier study [1] we have found that following paracetamol induced liver damage the PP is depressed after 12 h and GEC is unchanged. This dissociation of liver functions, especially the mechanism by which constant GEC is maintained in spite of necroses, is intriguing. The rapid increase in the activities of the galactose metabolizing enzymes *in vitro* following 70% hepatectomy [2] led to the hypothesis that GEC *in vivo* in the experimentally poisoned rat is restored to normal due to an increased metabolizing capacity of the surviving hepatocytes. In that case the time course of GEC after partial hepatectomy should be identical with that after paracetamol-

induced liver damage, and should be dissociated from the time course of PP. Sham operation itself is also able to stimulate the activity of some of the galactose metabolizing enzymes [2] *in vitro*.

In the present study we investigated the time course of the *in vivo* capacity to eliminate galactose and of the prothrombin index after 70% hepatectomy. Furthermore we investigated whether sham operation could induce an increase in the capacity for eliminating galactose.

Materials and Methods

Female Wistar rats weighing 171-212 g were fed Altromin® rat pellets and tap water *ad libitum*. Partial hepatectomy was performed according to Higgins & Andersson [3] under ether anaesthesia. On control animals an identical laparotomy was performed, the two anterior lobes of the liver were exteriorized, manipulated and replaced. The incision was closed with silk sutures.

The time course of GEC after partial hepatectomy was studied by determining GEC on groups of rats after 0, 6, 12, 18, 24, 48 and 72 h, the number of animals was 6, 5, 5, 5, 4, 6, and 6, respectively. In six control animals GEC was determined immediately after sham operation. The prothrombin index was measured in a similar control group and in groups 6, 12, 18, 24, 48, 72 and 96 h after hepatectomy, the number of animals in the groups was 6, 5, 5, 6, 6, 4 and 5, respectively. The groups were randomized to consist of six animals, the missing numbers reflecting technical failures.

A control experiment was performed in order to investigate whether the reported increase in the activity of galactose metabolizing enzymes [2] after sham operation would lead to an increase in GEC *in vivo*. This was done by comparing a group of animals in which GEC was estimated immediately after sham operation with another group in which GEC was estimated 6 h after sham operation, the number of animals was five and six in the two groups.

The GEC was determined during thiopental anaesthesia (100 mg/kg body weight) after tracheostomy and nephrectomy [4]. Galactose (Kabi, Sweden) was given as an intravenous priming dose (200 $\mu\text{mol}/100$ g

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body weight) followed by continuous infusion (1.0–2.5 $\mu\text{mol}/\text{min}$). After a distribution time of 20 min, seven duplicate arterial blood samples (50 μl) were drawn with an interval of 7 min for enzymatic determination of galactose [5]. The procedure was performed between 9 a.m. and 1 p.m.

The GEC was calculated as $\text{GEC} = I - (dc/dt \times 0.4 \times \text{body weight})$, where I is the infusion rate, dc/dt is the linear slope of the galactose blood concentration–time curve, and $0.4 \times \text{body weight}$ is the estimated volume of distribution of galactose [4]. After the final sample was taken the liver was removed, blotted on filter paper, and weighed. The total DNA [6] and protein [7] content was determined by the methods indicated.

Prothrombin index (PP) was determined by Owren's method [8] on aortic blood diluted 1 to 1 with isotonic saline. Blood was sampled between 9 a.m. and 1 p.m.

Comparisons between several groups were performed by one-way analysis of variance. Regression analysis was done by the least square method. P -values less than 0.05 were considered statistically significant.

Results

Immediately after hepatectomy the GEC was decreased to 50% of control values ($P < 0.05$) and rose to control values within 6 h (Fig. 1). From 6 to 72 h there was a tendency to a fall in GEC, which did not, however, reach statistical significance ($P > 0.05$). The PP was decreased to 27% of control ($P < 0.05$) after 12 h and rose to about control value after 96 h (Fig. 1).

Fig. 2 shows GEC calculated per gram of remaining liver tissue. The value from control animals and the value from animals immediately after hepatectomy

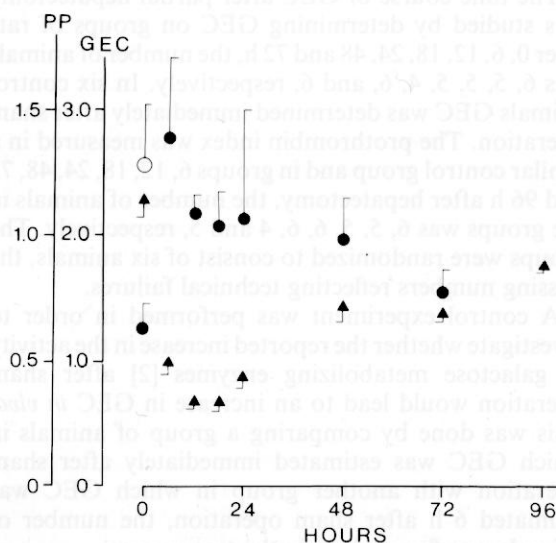


Figure 1. Galactose elimination capacity (●) in $\mu\text{mol}/\text{min}$ and prothrombin index (▲) in arbitrary units at various times after partial hepatectomy. Symbols indicate mean of four to six animals, bars indicate one SEM. The open circle indicate control value of galactose elimination capacity.

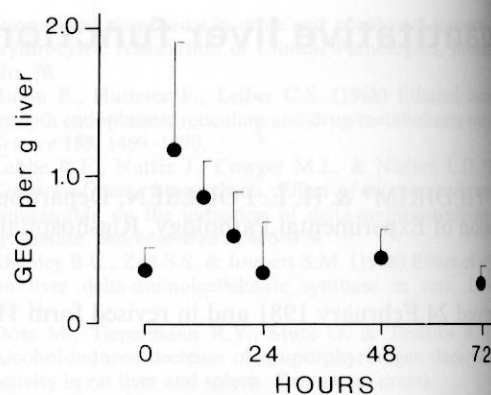


Figure 2. Galactose elimination capacity in $\mu\text{mol}/\text{min}$ and g liver at various times after partial hepatectomy. Dots indicate mean of four to six animals, bars indicate one SEM. Control value and zero hour value are identical (not shown).

were identical (not shown). Six hours after 70% hepatectomy the capacity of 1 g liver to metabolize galactose was increased 2–3 times ($P < 0.05$); it returned to control values after 24 h, and maintained this value.

The control experiment—to evaluate whether sham operation as such stimulated galactose metabolism—showed no difference between a control group, in which sham operation was performed immediately before the GEC determination, and the group where the sham operation was performed 6 h before the determination ($P > 0.05$), the values were 2.11 ± 0.10 and 2.43 ± 0.21 $\mu\text{mol}/\text{min}$ (mean \pm SEM, $N = 5, 6$), respectively.

The time course of liver weight, total hepatic DNA content, and total hepatic protein content was identical (Table 1) as judged from the residual variance ($s_e^2 = 0.25$) of the multiple regression of liver weight on hepatic protein and DNA content of the liver residues ($r^2 = 0.92$).

Extrahepatic galactose elimination can be assessed as follows: if GEC is the total galactose elimination (measured), GE_h is the elimination in the liver and GE_o is the elimination outside the liver then $\text{GEC} = \text{GE}_h + \text{GE}_o$. Immediately after 70% hepatectomy GE_h is reduced to $0.3 \times \text{GE}_h$ giving $\text{GEC} = 0.3 \times \text{GE}_h + \text{GE}_o$. Entering the data from the control group in the first equation and the data of the group studied immediately after hepatectomy into the latter leads to twelve equations with two variables. Solving these with linear regression analysis gives $\text{GE}_o = 0.71$ $\mu\text{mol}/\text{min}$ ($\text{SE} = 0.25$) or 27% of control value, which is significantly different from zero ($P < 0.05$).

Discussion

This study confirms that the galactose elimination capacity (GEC) and the prothrombin index (PP) are dissociated in time following partial hepatectomy, and that the GEC is restored more rapidly than the PP. These findings are in agreement with our earlier

Table 1. The values of liver weight, total hepatic DNA and total hepatic protein, mean \pm SEM are indicated; the number of animals in each group is stated under Materials and Methods

	Control	6 h	12 h	18 h	24 h	48 h	72 h
Liver weight (g)	7.42 ± 0.27	2.72 ± 0.43	2.59 ± 0.18	3.29 ± 0.31	3.50 ± 0.21	4.29 ± 0.05	4.96 ± 0.21
Total hepatic DNA (μ g)	23.7 ± 0.90	6.19 ± 0.76	6.64 ± 0.57	7.64 ± 0.47	7.60 ± 0.24	11.1 ± 0.32	15.0 ± 0.85
Total hepatic protein (mg)	1362 ± 77	546 ± 101	463 ± 59	650 ± 67	649 ± 83	748 ± 35	844 ± 29

communication on liver functions after acute liver damage due to paracetamol [1].

Galactose is mainly eliminated by metabolism in the liver and excretion in the urine [4]. Renal excretion was eliminated by nephrectomy.

The observed reduction in GEC after removal of 70% of the liver is 50%. This deviation may be due to an overestimation of GEC, since it is restored within the first 6 h and the time required to estimate GEC is 1 h. Another possible or contributing factor is extra-hepatic galactose elimination, which may be as high as 20–30% but probably is smaller. Since surgery in itself, as shown by the control experiment, does not stimulate GEC, extrahepatic galactose elimination is probably constant and the rapid rise in GEC after hepatectomy therefore is attributed to a two- to three-fold increase in the capacity of the remaining hepatocytes to metabolize galactose.

The rate limiting step in hepatic galactose metabolism is probably the galactokinase step [9], which as the other reactions in galactose metabolism is a bisubstrate reaction. Whether the increase 6 h after hepatectomy in the capacity of the remaining liver cells to metabolize galactose is due to a rapid *de novo* synthesis of galactose metabolizing enzymes is unknown. A change in cosubstrate concentration, elicited by the hepatectomy [10], or a change in enzyme configuration could also explain the increase in the capacity per gram liver.

The increase in GEC cannot be attributed to liver cell regeneration, since DNA synthesis starts 12–18 h after the hepatectomy [11].

The prothrombin index is at a nadir 12 h after the hepatectomy. The rapid fall can be caused by decreased hepatic synthesis of coagulation factors or by peripheral degradation, or both. The nadir 12 h after the hepatectomy reflects the time where the production rate of coagulation factors is identical with the degradation rate, the slope of the initial fall in prothrombin index probably reflects the half life of prothrombin factors rather than the decrease in hepatic synthesis rate. The peripheral degradation is assumed to be constant, as demonstrated in a study on partially hepatectomized dogs [12]. The course of the prothrombin index during the first 12 h thus indicates a prompt decrease in the production rate of coagulation factors, concomitantly with the hepatectomy.

The rise in prothrombin index from 12 h and onwards mainly reflects an increasing production rate of prothrombin factors by the liver. The increase correlates with the increase in liver weight, total liver protein and DNA.

The time course of GEC and prothrombin index is similar to that reported after toxic liver injury [1], except that a rapid decrease and rise in GEC may have been overlooked in their experiment due to the difficulty in establishing the exact time for the injury.

In conclusion this study demonstrates that liver functions located to different organelles in the hepatocyte are dissociated following a simple reduction in liver mass in that they recover with different velocity.

Whether this is the case for other functions remains to be investigated.

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