

## Changes in quantitative measures of hepatic function after 90% hepatectomy in rats

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**Abstract.** Quantitative measures of liver functions were investigated in rats up to 360 h after 90% hepatectomy and related to total hepatic DNA. Galactose elimination capacity (cytosolic phosphorylation of carbohydrate), p-nitro-anisole demethylase activity (endoplasmic drug hydroxylation) and prothrombin index (protein synthesis at rough endoplasmic membranes) were initially reduced as much as the liver weight, but recovered differently. During hepatic regeneration prothrombin index and galactose elimination were back to control values after an interval of 360 h, while p-nitro-anisole demethylase activity was about 0.4 times control value after that interval. The correlation between total hepatic DNA and liver weight, and between total hepatic protein and liver weight was 0.92 in both cases, indicating their close relationship during hepatic regeneration. Compared to earlier studies on 70% hepatectomy the recovery of metabolic functions after 90% hepatectomy is delayed, as compared to regeneration of total hepatic DNA. The compensatory hyperfunction observed after 70% hepatectomy was not found after 90% hepatectomy, indicating a lost ability to hyperfunction when hepatic function is reduced close to the minimal residual function. This is suggested to be important for the sudden onset of hepatic insufficiency.

**Key words.** Hepatic regeneration, galactose elimination, drug metabolism, prothrombin, liver function.

### Introduction

The liver is considered to possess a considerable functional reserve capacity. The magnitude of this is not known, but it is known that 70% of the liver can be removed with only slight and transient signs of liver insufficiency and without mortality. In that situation, i.e. after 70% hepatectomy, hepatic functions are reduced. In the case of some functions a reduction according to the reduction in liver mass appears and other functions show a compensatory hyperfunction [1].

It has been suggested that a minimum hepatic residual function exists [2] below which survival is not

possible. The exact limit is not known. Recently, it has been demonstrated that in rats 90% of the liver can be removed with survival of about 90% of the animals [3]. In this model residual liver function must be very close to the minimum. In the present paper hepatic functions were studied, that can be related to different subcellular hepatocyte structures in this model of severe hepatic insufficiency, for comparison to our earlier studies on less severe hepatic insufficiency.

### Materials and Methods

Female Wistar rats weighing about 190 g were fed Rostock rat pellets and tap water *ad libitum*. They were housed in individual cages under constant temperature and humidity conditions. Light was controlled to a 12 h light/12 h dark cycle. Animals were divided into groups of four, and were examined in random order immediately or 6, 18, 24, 52, 72, 120, 168, 216 or 360 h after 90% hepatectomy, performed between 0800 and 2300 h, as previously described [3]. Animals that died before the time of investigation were replaced to maintain a group size of four. No more than one animal had to be replaced in any group. Until the time of surgery the animals had access to food and water, for the next 72 h food was withdrawn and 20% glucose was added to the drinking water, thereafter the glucose was removed and the animals again had free access to tap water and Rostock pellets. Sham operations were performed in four rats, the liver being exteriorized through a midline abdominal incision, manipulated and replaced.

In one series the galactose elimination capacity was determined [1, 4, 5]. Subsequently the liver was removed for estimation of total hepatic DNA [6]. Another series of animals, treated similarly, were anesthetized with diethyl ether and bled from a 19 gauge needle inserted in the aorta. Blood was diluted 1:1 with isotonic saline for estimation of prothrombin index [7]. The liver was removed, blotted on filter paper, weighed, and stored in liquid nitrogen for 1-12 h. After thawing the liver was homogenized in a Potter-Elvehjem glass teflon homogenizer with 10 ml of isotonic KCl ( $153 \text{ mmol l}^{-1}$ ) for determination of protein [8], cytochrome P450 [9], and for preparation of 100 000 G microsomes [10]. This subcellular fraction

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**Table 1.** Galactose elimination capacity (GEC,  $\mu\text{mol min}^{-1}$ ), total hepatic DNA (TDNA, mg) and liver weight (LW, g) various intervals after 90% hepatectomy

|      | Time interval (h) |           |           |           |           |           |           |           |           |           |           |           |
|------|-------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|      | 0                 | 6         | 18        | 24        | 36        | 52        | 72        | 120       | 168       | 216       | 360       |           |
| GEC  | 3.37±0.23         | 1.15±0.11 | 0.71±0.24 | 0.30±0.23 | 0.72±0.29 | 0.90±0.42 | 1.12±0.14 | 0.55±0.27 | 1.54±0.31 | 1.23±0.21 | 2.46±0.23 | 2.87±0.53 |
| TDNA | 22.3±1.4          | 3.3±0.4   | 2.5±0.6   | 1.80±0.2  | 3.0±0.4   | 7.30±1.6  | 5.0±0.8   | 6.6±0.6   | 9.9±1.1   | 14.7±0.21 | 26.1±3.9  | 32.1±4.0  |
| LW   | 8.18±0.31         | 1.12±0.06 | 0.77±0.06 | 1.36±0.09 | 1.36±0.19 | 2.40±0.12 | 1.70±0.12 | 1.98±0.17 | 2.72±0.23 | 3.69±0.25 | 5.58±0.42 | 8.03±0.30 |

Values are mean  $\pm$  SEM of four animals. The reductions in GEC, TDNA and LW are statistically significant ( $P < 0.01$ , one way analysis of variance).

**Table 2.** Total hepatic protein (Tprot, g), microsomal p-nitro-anisole demethylase activity (PNA,  $\text{nmol min}^{-1}$ ) and prothrombin index (PPI, arbitrary units) various intervals after 90% hepatectomy

|       | Time interval (h) |           |           |           |           |           |           |           |           |           |           |           |
|-------|-------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|       | 0                 | 6         | 18        | 24        | 36        | 52        | 72        | 120       | 168       | 216       | 360       |           |
| Tprot | 1.74±0.10         | 0.21±0.02 | n.d.      | 0.21±0.01 | 0.25±0.01 | 0.28±0.02 | 0.38±0.03 | 0.55±0.04 | 0.75±0.06 | 0.99±0.10 | 1.12±0.11 |           |
| PNA   | 329±87            | 29±5      | n.d.      | 16±3      | 12±3      | 16±3      | 30±9      | 47±17     | 69±22     | 109±19    | 142±14    |           |
| PPI   | 0.82±0.05         | 0.90±0.09 | 0.36±0.03 | 0.09±0.02 | 0.05±0.01 | 0.08±0.02 | 0.11±0.02 | 0.18±0.01 | 0.75±0.14 | 0.68±0.20 | 0.70±0.10 | 0.91±0.25 |

Values are mean  $\pm$  SEM of four animals. The reductions in Tprot, PNA and PPI are statistically significant ( $P < 0.01$ , one way analysis of variance). n.d. = Not determined.

**Table 3.** Galactose elimination capacity per gram liver after 70% and 90% hepatectomy ( $\mu\text{mol min}^{-1} \text{g}^{-1}$  liver)

| % Hepatectomy | Control     | 6 h         | 18 h        | 24 h        |              |
|---------------|-------------|-------------|-------------|-------------|--------------|
| 70% resection | 0.34 ± 0.06 | 1.19 ± 0.34 | 0.62 ± 0.11 | 0.35 ± 0.06 | $P < 0.05^*$ |
| 90% resection | 0.42 ± 0.04 | 0.90 ± 0.30 | 0.48 ± 0.35 | 0.48 ± 0.16 | $P > 0.05^*$ |

\* Indicates  $P$  value for one way analysis of variance.

was analysed for protein [8], cytochrome P450 [9] and p-nitro-anisole demethylase activity [11], with exception of the 6 and 18 h groups. Recovery of microsomes, determined in each animal as (P450 in microsomes from 1 g liver)/(P450 in liver homogenate from 1 g liver), varied from 0.4 to 0.6, probably due to varying lipid content. Each individual value of total hepatic p-nitro-anisole demethylase activity was corrected for recovery of microsomes.

## Results

The change in liver weight (LW), total hepatic DNA (TDNA) and galactose elimination capacity (GEC) at various intervals after 90% hepatectomy is given in Table 1 as the mean  $\pm$  SEM of groups of four animals.

The hepatectomy reduced LW to 14% on the average, TDNA to 15%, while GEC was reduced to 34% immediately after surgery. However, after an interval of 18 h GEC was reduced to 9% ( $P = 0.02$ ,  $t$ -test, control *vs* 18 h value). After 360 h GEC had returned to control values ( $P > 0.05$ ,  $t$ -test, control *vs* 360 h value). TDNA was restored after 216 h, and after the 360 h interval there was an overshoot (144% of control value,  $P < 0.05$ ,  $t$ -test control *vs* 360 h value), while liver weight returned to initial value, indicating a higher DNA concentration in the liver after that interval.

Table 2 shows that total hepatic protein was reduced to 12% on average immediately after the hepatectomy. After an interval of 360 h it was 64% of initial value ( $P < 0.01$ ,  $t$ -test control *vs* 360 h value). Microsomal drug hydroxylation, p-nitro-anisole demethylation (PNA), was reduced to 11% immediately after the hepatectomy, it recovered slowly being 43% of initial value after the 360 h interval ( $P < 0.05$ ,  $t$ -test control *vs* 360 h value).

Prothrombin index (PPI) was initially unchanged, but after an interval of 18 h, corresponding to 4–6 half lives of prothrombin in plasma [12], it was reduced to 9% of initial value (Table 2), and after the 360 h interval PPI returned to control values.

The GEC/g LW after 90% hepatectomy (Table 3) showed an increase 6 h after the hepatectomy which was not statistically significant. For comparison corresponding data from our earlier study on 70% hepatectomy [1] are given, demonstrating a statistically significant three-fold increase in GEC/g LW in that situation.

**Table 4.** Squared correlation coefficients ( $r^2$ ) between liver weight (LW), total hepatic protein (Tprot), total hepatic DNA (TDNA) and galactose elimination capacity (GEC), microsomal p-nitro-anisole demethylation (PNA), prothrombin index (PPI)

|       | GEC  | PNA  | PPI  | LW   |
|-------|------|------|------|------|
| LW    | 0.71 | 0.66 | 0.25 | *    |
| Tprot | *    | 0.74 | 0.26 | 0.85 |
| TDNA  | 0.67 | *    | *    | 0.85 |

\* Indicates that the correlation coefficient cannot be calculated since the data in Tables 1 and 2 originate from estimations on different animals.

In Table 4 the correlation coefficients between the various measures of liver size and liver function are given.

## Discussion

The liver function tests used in this study are established quantitative measures of metabolic functions in the liver associated with different subcellular structures: (i) galactose elimination capacity, GEC (cytosolic phosphorylation of carbohydrate); (ii) p-nitro-anisole demethylase activity, PNA (drug hydroxylation at the smooth endoplasmic membranes); (iii) prothrombin index, PPI, (protein synthesis at the rough endoplasmic membranes). The concept of a 'functional hepatic mass' implies that the value of these measures should be diminished proportionally to the reduction in liver mass. This was confirmed concerning the period immediately after 90% hepatectomy, but during regeneration PPI recovered more rapidly and PNA more slowly than GEC.

The use of total hepatic DNA content as a reference throughout this work is based on the fact that changes in liver DNA reflect changes in the number of parenchymal liver cells, with minor reservations. Firstly, the DNA synthesis (S phase) in non-parenchymal cells lags about 12 h behind that of liver cells [13]; secondly, polyploidy occurs during and after liver cell proliferation. However, apart from the first, fairly synchronous wave of DNA synthesis, mean hepatocellular DNA changes little, since a tendency to form polyploid nuclei is counteracted by a reduction in the proportion of binucleated cells [14, 15]. With these

reservations, the expression of hepatic contents and functions per weight unit DNA designates the content or functional capacity of the average parenchymal liver cell.

Immediately after 90% hepatectomy GEC was reduced from  $3.37 \mu\text{mol min}^{-1}$  to  $1.15$ , i.e. to  $0.34$  times the control value. The corresponding reduction in liver weight was to  $0.14$  times control value, and therefore similar reduction in GEC was expected. This expected reduction was, however, found 18 h after the hepatectomy. After this 18 h period the hepatocytes enter cell division [3], indicating important cell-biological changes in that situation, in accordance with our findings after 70% hepatectomy [1].

The GEC rose slowly compared to the rise in total hepatic DNA. This is in contrast to earlier findings after 70% hepatectomy [1, 16]. Six h after the 70% hepatectomy GEC was identical to the control value [1] in accordance with the findings of increased activities of the galactose metabolizing enzymes [16]. One main difference between the two situations, i.e. 70% and 90% partial hepatectomy, is the low proportion of dividing hepatocytes after 70% partial hepatectomy and the high proportion of dividing hepatocytes after 90% hepatectomy [3, 17]. This may be indicative of loss of compensatory hyperfunction when hepatocytes divide. However, it appears that after the first wave of regeneration has disappeared this ability is still missing. The average hepatocyte eliminates  $0.09 \mu\text{mol galactose min}^{-1} \text{mg}^{-1} \text{DNA}$  360 h after 90% partial hepatectomy vs  $0.15$  in control animals ( $P=0.028$ , *t*-test, control value vs 360 h value).

These arguments rest on the assumption that extrahepatic galactose elimination is minimal and constant in the two situations. In our earlier study on 70% hepatectomy [1] we estimated that extrahepatic galactose elimination was below  $0.71 \mu\text{mol min}^{-1}$ , and probably much smaller. In the present study we found a GEC of  $0.30 \mu\text{mol min}^{-1}$ . Assuming a reduction in GEC proportional to the reduction in total hepatic DNA (or liver weight or total hepatic protein) the expected GEC is  $3.37$  times  $1.80/22.3$ , amounting to  $0.27 \mu\text{mol min}^{-1}$ . This leaves very little room for extrahepatic galactose elimination and indicates that in the rat model extrahepatic galactose elimination is negligible.

PNA was restored to only about 0.4 times mean control value after 90% partial hepatectomy. We did not measure PNA after 70% partial hepatectomy, but other drug metabolizing activities rise faster after 70% partial hepatectomy [18]. This indicates that for a considerable time period, during and after hepatocyte division, restoration of drug metabolizing activity is delayed. The high correlation to the other measures of hepatic function indicate that the restoration of drug metabolizing capacity is proportional to the liver growth.

However, it should be noted that even after 15 days the regenerative process does not seem to have come to conclusion, since TDNA is at  $1.44$  and PNA at  $0.34$

times the initial values. This can be interpreted as a distortion in cell composition, each hepatocyte containing less than 50% of its normal content of drug metabolizing capacity. This should be taken into account when interpreting the correlations in Table 4.

Twenty-four h after 90% hepatectomy PPI was at a nadir. The fall can be caused by decreased hepatic synthesis or by increased peripheral degradation, or both. The peripheral degradation is assumed to be constant, as demonstrated in a study on partially hepatectomized dogs [19]. The initial course of the PPI-time curve following 90% hepatectomy reflects the PPI half life of a few hours [12]. The nadir after 24 h corresponds to the liver mass left after the 90% hepatectomy, and the subsequent rise of PPI reflects an increased synthesis of clotting factors. The missing correlation to total hepatic DNA mainly originates from the delay in PPI decrease due to the half life in plasma during the first hours. After the later intervals PPI follows total hepatic DNA, in accordance with our findings after 70% hepatectomy [1]. This indicates that hepatocyte division does not influence the synthesis of proteins for coagulation.

The squared correlation coefficient between liver weight and total hepatic protein, and between liver weight and total hepatic DNA is high,  $0.85$ , indicating a close relationship between these three measures of hepatic size. This is in agreement with our findings in 70% hepatectomized animals [1]. The functional changes found in the present work can be related to either of these three measures. However, the conclusions drawn are identical regardless of which one is chosen as reference, because of the high correlations.

From this study, it appears that the 70% and the 90% hepatectomized rat models for hepatic regeneration differ substantially with regard to recovery of metabolic functions. The increased galactose elimination capacity per gram liver, or per mg of DNA, observed after 70% hepatectomy is not present after 90% hepatectomy. The functional reduction immediately after the resection corresponds to the reduction in hepatic mass, but the recovery of some functions are delayed compared to regeneration of total hepatic DNA. This indicates that when hepatic functions are reduced close to the minimal residual function compatible with survival, the hepatocytes lose the ability to hyperfunction. This may be of importance for the sudden onset of hepatic insufficiency. The 90% hepatectomized rat is a promising model for further pathophysiological studies on hepatic insufficiency.

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