

Changes in the hepatic capacity of urea-N synthesis, galactose elimination and antipyrine clearance following 70% hepatectomy in the rat

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The capacity of urea-N synthesis (CUNS), galactose elimination capacity (GEC), and antipyrine clearance (APC) was investigated in rats 0, 3, 6, 24, 96, and 240 h after 70% hepatectomy. Sham-operated animals were used as controls. The CUNS was assessed during alanine infusion as urea accumulation in total body water, corrected for intestinal hydrolysis, GEC was measured during constant galactose infusion, and APC by the one-sample method. Immediately after the 70% hepatectomy, CUNS was reduced from 8.9 ± 2.4 to $3.9 \pm 1.1 \mu\text{mol} (\text{min} \cdot 100 \text{ g body wt})^{-1}$ (mean \pm SD, $p < 0.05$), that is, 0.43 times the control value. The corresponding reduction in liver weight was 0.36. After 6 h CUNS rose to 0.62 times the control values versus 0.36 for the liver weight. The recovery of GEC and APC was slower than the recovery of the liver weight, and was only restored to 0.81 and 0.59 times the control value ($p < 0.05$), respectively, after 240 h. This study demonstrates that after partial hepatectomy the capacity of urea-N synthesis rate is increased, that is, a compensatory hyperfunction of the remnant liver, and that this function is restored to normal more rapidly than other measures of functional liver mass.

Key words: hepatic regeneration; liver function; urea synthesis

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Assessment of 'the functional liver mass' and its sequential changes during hepatic regeneration is of importance for the evaluation of therapy or recovery from hepatic injury such as fulminant hepatic failure. Previous animal studies have demonstrated that some liver functions are dissociated in time following 70% hepatectomy, and that the galactose elimination capacity (GEC) is restored before the liver weight [1], suggesting a 'compensatory hyperfunction' of the remaining hepatocytes. This did not occur

following 90% hepatectomy [2], implying that dividing hepatocytes metabolically behave according to the functional demand of the organism, and that a different regulation of intracellular metabolic processes occurs during moderate and intense hepatic regeneration.

The present study was performed to see if urea synthesis, assumed to be a more 'essential' liver function than galactose elimination, behaves similarly.

MATERIALS AND METHODS

Experimental design. The estimations of the capacity of urea-N synthesis (CUNS), the galactose elimination capacity (GEC), and the antipyrine clearance (APC) were made simultaneously in each animal. For technical reasons APC (determination of APC lasts 5 h) in the sham-operated and in the 0 h groups was estimated simultaneously in a litter mate. Female Wistar rats ($n=35$), with an average body weight of 205 g (range 193–239) were investigated.

The animals were fed rat pellets and tap water ad libitum. A 70% partial hepatectomy was performed according to Higgins & Anderson [3] under ether anaesthesia. The control animals had an identical laparotomy during which the liver was manipulated and replaced. The CUNS, GEC and APC (APC not at 3 h) were estimated 0, 3, 6, 24, 96, and 240 h after 70% hepatectomy in groups of five animals. Five control animals were examined immediately after sham operation. The animals were anaesthetized by intraperitoneal injection of thiopental, 100 mg per kg body wt. Polyethylene catheters were inserted into both jugular veins for infusion, and into the right common carotid artery for blood sampling, immediately after tracheotomy and intubation. The kidney vessels were ligated after exteriorization by dorsal route. Alanine was administrated as a priming dose of 0.5–0.7 ml of a 1.09 mol/l solution in sterile water, followed by a constant infusion for 70 min at 0.65–3.50 ml/h of a 224 mmol/l solution by means of a roller pump (Perfusor Secura). Steady-state amino acid concentration was defined as a less than 10% change during a period of 50 min or longer. Galactose (Kabi, Sweden) was given as an intravenous priming dose (200 μ mol/100 g body wt) followed by a continuous infusion (1.0–1.5 μ mol/min). After an equilibration period of 20 min blood was sampled (150 μ l) at intervals of 10 min for determination of urea, total alpha-amino-N, and galactose concentration. Antipyrine, 1 ml (4 mg/ml), was given by gastic tube about 5 h before the above described procedures [4]. Blood samples were taken and heparinized immediately before administration of alanine and galactose and the plasma was stored at -20°C until analysis for antipyrine concentration. Other blood samples

were drawn into chilled tubes containing aprotinin and EDTA and stored at -20°C until analysis of insulin and glucagon concentration.

The blood urea concentration was measured by the urease-Berthelot method [4], and total blood alpha-amino-N concentration by the dinitrofluorobenzene method [5]. The blood galactose concentration was determined by the

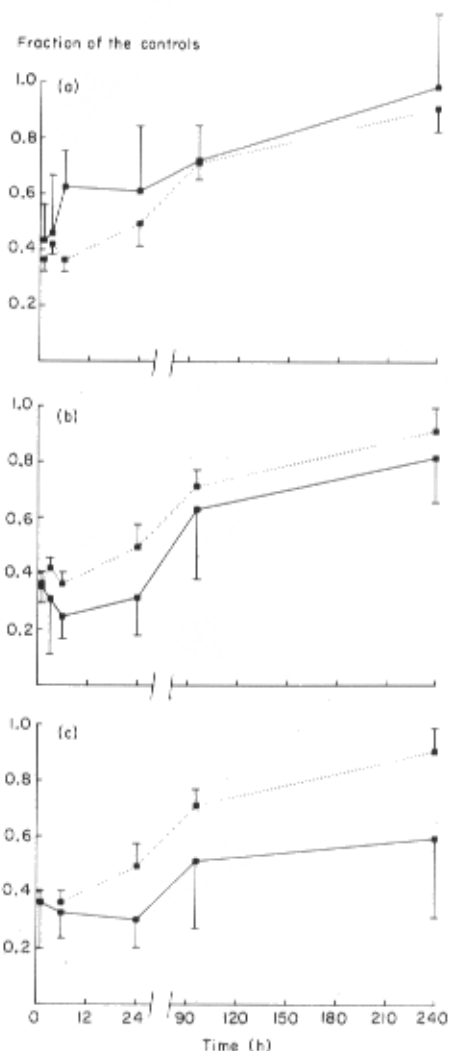


FIG. 1. The relative (in relation to mean value of basal control group) capacity of urea-N synthesis (a), galactose elimination capacity (b) and antipyrine clearance (c) at various intervals after 70% hepatectomy (unbroken lines). The relation to liver weight is given as dotted lines. Symbols indicate mean, bars SD of five animals.

TABLE I. Liver weight (LW), the capacity of urea-N synthesis (CUNS), galactose elimination capacity (GEC) and antipyrine clearance (APC) at various intervals after 70% hepatectomy

	Controls	0 h	3 h	6 h	24 h	96 h	240 h
LW (g)	7.6±0.4	2.8±0.3	3.2±0.3	2.8±0.3	3.8±0.6	5.4±0.5	6.9±0.6
CUNS*	8.89±2.35	3.86±1.14	4.08±1.85	5.56±1.16	5.43±2.08	6.42±1.11	8.76±2.33
GEC*	1.49±0.13	0.53±0.09	0.46±0.30	0.37±0.12	0.47±0.20	0.94±0.37	1.22±0.24
APC†	0.37±0.06	0.13±0.06	n.d.	0.12±0.03	0.11±0.04	0.19±0.09	0.22±0.11

*Unit= $\mu\text{mol}(\text{min}\cdot 100\text{ g body wt})^{-1}$.

†Unit= $\text{ml}(\text{min}\cdot 100\text{ g body wt})^{-1}$.

Not determined=n.d.

Values are mean±SD of five animals.

TABLE II. Glucagon (ng/l) and insulin (mU/l) at various intervals after 70% hepatectomy

	Controls	0 h	3 h	6 h	24 h	96 h	240 h
Glucagon (ng/l)	45±30	243±55	168±64	430±263	292±77	191±39	171±80
Insulin (mU/l)	58±8	38±30	33±18	44±33	87±8	41±10	28±15

Values are mean±SD of five animals.

galactose dehydrogenase method (6), and antipyrine by HPLC [7]. The plasma insulin and glucagon concentrations were measured by RIA. Glucagon was extracted by ethanol precipitation according to Heding [8].

Calculations. CUNS ($\mu\text{mol}(\text{min}\cdot 100\text{ g body wt})^{-1}$) was calculated as:

$$\text{CUNS} = \text{dc}^u/\text{dt} \times 0.63 \text{ body wt} \times 1.25,$$

where dc^u/dt is the slope of the linear regression of arterial blood urea nitrogen concentration on time during steady state, 0.63 body wt is the volume of distribution of urea [9], and 1.25 a correction for intestinal hydrolysis of urea [10]. The GEC ($\mu\text{mol}(\text{min}\cdot 100\text{ g body wt})^{-1}$) was calculated as:

$$\text{GEC} = I - (\text{dc}/\text{dt} \times 0.40 \text{ body wt}),$$

where I is the infusion rate, dc/dt is the linear slope of the galactose blood concentration time curve, and 0.40 body wt is the volume of distribution of galactose [11]. The APC ($\text{ml}(\text{min}\cdot 100\text{ g body wt})^{-1}$) was calculated as:

$$\text{APC} = (\ln(D/aVd) - \ln(c_t)) / t \times aVd,$$

where D is the antipyrine dose given, aVd is the antipyrine apparent volume of distribution calculated as 0.66 body wt [7], and c_t is the concentration corresponding to the sampling

time t [7]. The liver was removed, blotted on filter paper, and weighed.

RESULTS

Figure 1 shows the sequential posthepatectomy changes in liver weight, CUNS, GEC, and APC, in relation to control values. At all times the relative CUNS is higher than the relative liver weight, significantly so at 6 h ($p < 0.05$, t-test). The GEC is below the time curve of liver weight. This phenomenon is more pronounced for APC that is 0.6 times the control value after 10 days despite the normal liver weight (Table I).

The glucagon response to the hepatectomy was at maximum after 6–24 h. Insulin reached the control value after 240 h, whereas glucagon remained increased (Table II).

DISCUSSION

The liver function tests used in this study reflect the function of different subcellular structures. The capacity of urea-N synthesis (CUNS) is located to mitochondria and cytosol [10], the galactose elimination capacity (GEC) reflects the cytosolic liver function [11,12], and the antipyrine clearance the microsomal function [13]. The concept of a 'functional liver mass'

implies that these measures should be decreased proportionally to the reduction in liver mass. This was confirmed in a previous study, where CUNS, GEC, and APC correlated with each other and with the liver weight [13].

Immediately after 70% hepatectomy, CUNS was reduced to 0.43 times the control value, and the liver weight was reduced to 0.36. After 6 h, CUNS was 0.62 times the control value, whereas the liver weight was still 0.36. This indicates a relative hyperfunction, similar to that described for GEC [1]. In the present study GEC was reduced from 1.5 to 0.5 μmol ($\text{min}\cdot 100\text{ g body wt}^{-1}$) after the hepatectomy, that is, 0.31 times the control value corresponding to our earlier observation [1]. However, in contrast to that study, this study showed no compensatory hyperfunction concerning GEC. The time course of the relative GEC was below that of the liver weight, and it was still only 0.81 times the control value after 240 h. The only difference between our earlier [1] and the present study is the high alanine load in order to estimate CUNS simultaneously with the determination of GEC.

High alanine concentrations stimulate glucagon blood concentrations but in the present experimental situation this effect may be overridden by the effect of hepatectomy [14], since hormonal changes were identical with those found after hepatectomy without alanine infusion (B.A. Hansen, K.F. Petersen and H.E. Poulsen, unpublished observations). Glucagon increases CUNS, but only after a considerably longer period of time (K.F. Petersen, B.A. Hansen, H. Vilstrup, unpublished observations).

The high urea synthesis increases the demand for ATP production [15]. Galactose conversion to glucose is also energy demanding, this phosphorylation, however, being based on UTP rather than on ATP [11]. Competition for activation of phosphate may explain the lower capacity for galactose elimination after hepatectomy in this study and indicates that urea production has a higher 'priority' than carbohydrate phosphorylation.

A direct effect of alanine or urea cycle intermediates is a less likely explanation, since the GEC of the present control animals is very similar to that previously found in animals which did not receive alanine [1].

From this study it appears that liver functions

related to different subcellular structures in the hepatocytes have considerable influence on each other during hepatic regeneration. We suggest that this influence is due to competition for activation of phosphate.

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