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The Capacity of Urea-N Synthesis as a Quantitative Measure of the Liver Mass in Rats

Bent Adel Hansen and Henrik Enghusen Poulsen

Department of Medicine A, Division of Hepatology and Laboratories of Experimental Pathology, Rigshospitalet, Copenhagen (Denmark)

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Summary

The capacity of urea-N synthesis (CUNS), the galactose elimination capacity (GEC) and the antipyrine clearance (APC) were measured in rats immediately after 30, 70 and 90% partial hepatectomy and after sham operation. CUNS was assessed during alanine infusion as urea accumulation in total body water, corrected for intestinal hydrolysis, and GEC was measured during constant galactose infusion in the same animals. APC was determined by the one-sample method in a separate group of animals, treated similarily. CUNS, GEC and APC were all correlated to the liver weight with correlation coefficients (r) above 0.8 and the correlation coefficients (r) between CUNS, GEC and APC were all above 0.7.

It is concluded that CUNS is a quantitative measure of the functional liver mass in the rat.

Introduction

In mammals excess amino-nitrogen is eliminated from the body by urea synthesis in the mitochondria and cytosol of the hepatocytes and may be considered an essential liver function. Therefore the capacity for urea synthesis may be a relevant

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Address correspondence to: Bent Adel Hansen, M.D., Rigshospitalet A 2152, DK-2100 Copenhagen, Denmark.

measure of the 'functional liver mass'.

Established measures of the 'functional liver mass' are the galactose elimination capacity (GEC) reflecting cytosolic liver function [1], and the antipyrine clearance (APC) reflecting hepatic microsomal function [2].

The purpose of the present work was to validate the reliability of CUNS as a measure of the functional liver mass by comparing it with GEC and APC in rats subjected to different degrees of partial hepatectomy.

Materials and Methods

Experimental design

Measurement of the capacity of urea-N synthesis (CUNS) and the galactose elimination capacity (GEC) was made simultaneously in each animal, whereas for technical reasons antipyrine clearance (APC) was estimated in litter mates. The material consists of 2 × 20 female Wistar rats with an average body weight of 208 g (range 192–230).

The animals were fed rat pellets and kept at constant ambient temperature with a fixed 12-h controlled light/dark cycle. For determination of CUNS and GEC anesthesia was induced by intraperitoneal injection of thiopenthal, 100 mg/kg body weight. Sham operation consisted of laparotomy and manipulation of the liver, followed by replacement. A 90% partial hepatectomy was performed according to Gaub and Iversen [3], 70% and 30% hepatectomy according to Higgens and Anderson [4], with 5 animals in each group. 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 exteriorisation via the dorsal route. Alanine was administered as a single injection of 0.3–0.6 ml of a 1.09 mol/l solution in sterile water, followed by a constant infusion for 70 min at 0.4–3.0 ml/h of a 224 μmol/l solution by means of a roller pump (Perfusor Secura). Steady-state amino acid concentrations were defined as less than 10% change during a period of 50 min or longer. Galactose (Kabi, Sweden) was given as an intravenous priming dose (200 μmol/100 g body weight) followed by a constant infusion (1.0–1.5 μmol/min). Samples (150 μl) were taken after an equilibration period of 20 min, at intervals of 10 min, for determination of urea and total alpha-amino-N and galactose concentrations. For determination of APC, the animals were sham-operated or partially hepatectomized under diethyl ether anesthesia, as described above. Immediately after the operation the animals received antipyrine 1 ml (4 mg/ml) by gastric tube, 5 h later blood samples were taken from the aorta and heparinezed plasma stored at –20 °C until analysis for antipyrine [5].

Calculations

CUNS μmol (min·100 g BW)$^{-1}$ was calculated as

$$CUNS = dc^u/dt \cdot 0.63\ BW \cdot 1.25,$$

where dc^u/dt is the slope of the linear regression of arterial blood urea nitrogen concentration on time during steady state, 0.63 BW is the estimated volume of distribu-

tion of urea [6], and 1.25 is a correction for intestinal hydrolysis of urea [7]. GEC μmol (min·100 g BW)$^{-1}$ was calculated as

$$GEC = I-(dc/dt \cdot 0.40\ BW)$$

where I is the infusion rate, dc/dt is the linear slope of the galactose blood concentration-time curve, and 0.40 BW is the volume of distribution of galactose [8]. APC ml(min·100 g BW)$^{-1}$ was calculated as

$$APC = (\ln(D/0.66\ BW)-\ln(c_t))/t \cdot 0.66\ BW$$

where D is the antipyrine dose given, 0.66 BW is the antipyrine volume of distribution [5] and c_t is the concentration corresponding to the sampling time t [5].

Immediately following determination of CUNS and GEC or APC the liver was removed, blotted on filter paper, and weighed.

Analysis

Total blood alpha-amino-N concentration was measured by the dinitrofluorobenzene method [9], blood urea concentration by the urease-Berthelot method [10], blood galactose concentration by the galactose dehydrogenase method [11], and antipyrine by HPLC [5].

Statistical analysis

Comparisons between mean values were performed by *t*-tests. The correlations between CUNS on liver weight, GEC, APC was performed by the least squares method. *P*-values less than 0.05 were considered statistically significant.

Results

Liver weight was reduced on the average to 71, 36 and 16% of control values following the different partial hepatectomies. The corresponding reductions of CUNS were 63, 42, and 25%, of GEC 53, 33 and 20%, and of APC 84, 35, and 11%. The absolute values are given in Table 1.

TABLE 1

LIVER WEIGHT, CAPACITY OF UREA-N SYNTHESIS (CUNS) GALACTOSE ELIMINATION CAPACITY (GEC), AND ANTIPYRINE CLEARANCE (APC) IN RELATION TO SHAM OPERATION, 30, 70, 90% PARTIAL HEPATECTOMY (pHx)

Mean ± SEM, n = 5.

Hepatectomy	Sham operation	30% pHx	70% pHx	90% pHx
Liver weight (g)	7.59 ± 0.18	5.38 ± 0.17	2.77 ± 0.15	1.20 ± 0.14
Liver weight (g) (for APC)	7.62 ± 0.32	6.27 ± 0.34	2.92 ± 0.11	1.31 ± 0.04
CUNS μmol (min·100 g)$^{-1}$	8.89 ± 1.05	5.64 ± 0.59	3.66 ± 0.59	2.20 ± 0.58
GEC μmol (min·100 g)$^{-1}$	1.49 ± 0.10	0.77 ± 0.08	0.53 ± 0.04	0.35 ± 0.23
APC ml (min·100 g)$^{-1}$	0.371 ± 0.058	0.309 ± 0.037	0.134 ± 0.061	0.044 ± 0.013

CUNS, GEC, and APC correlated with the liver weight with correlation coefficients (r) above 0.8. The correlation coefficient (r) between CUNS, GEC and APC were all above 0.7. The coefficients are given in Table 2, and individual values are plotted in Figs. 1 and 2.

TABLE 2

CORRELATIONS (r) BETWEEN THE LIVER WEIGHT (LW), THE CAPACITY OF UREA-N SYNTHESIS (CUNS), THE GALACTOSE ELIMINATION CAPACITY (GEC), AND THE ANTIPYRINE CLEARANCE (APC)

	CUNS	GEC	APC
LW	0.87	0.79	0.96
CUNS	1	0.77	0.79[a]
GEC	–	1	0.72[a]

[a] The correlation originate from estimations on litter mates performed on the same day.

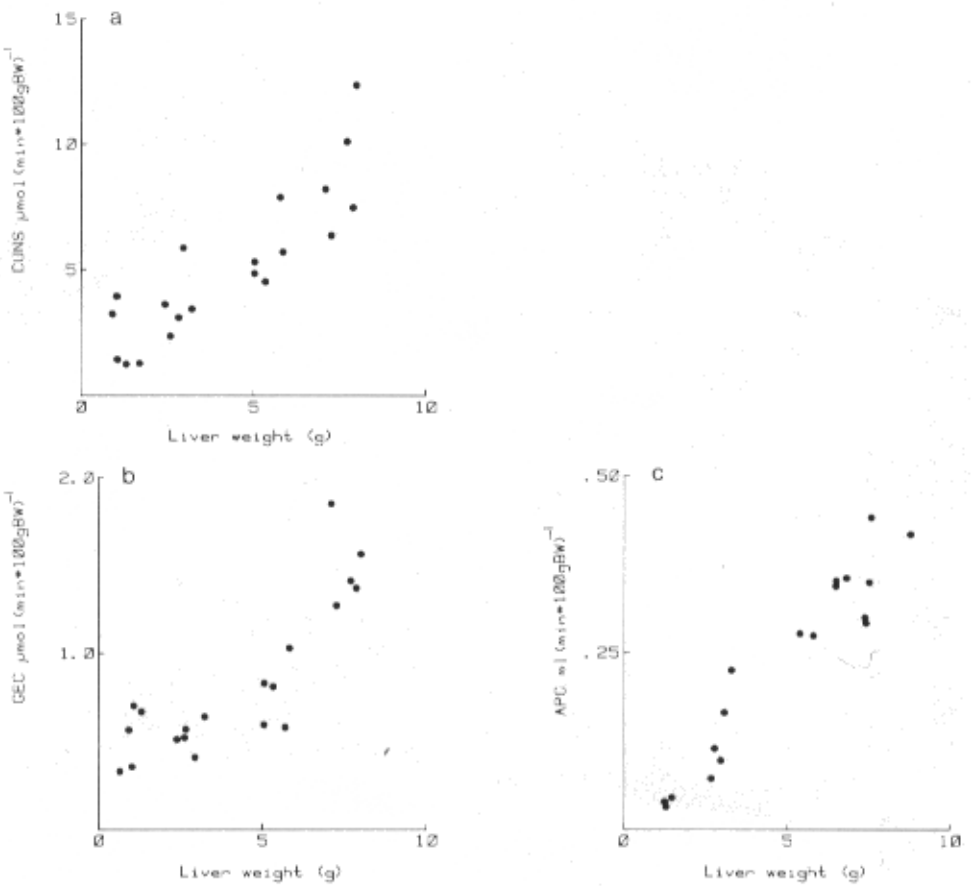

Fig. 1. *a*: The capacity of urea synthesis (CUNS) in relation to liver weight after partial hepatectomy.
b: Galactose elimination capacity (GEC) in relation to liver weight after partial hepatectomy.
c: Antipyrine clearance (APC) in relation to liver weight after partial hepatectomy.
The correlation coefficients are given in Table 1.

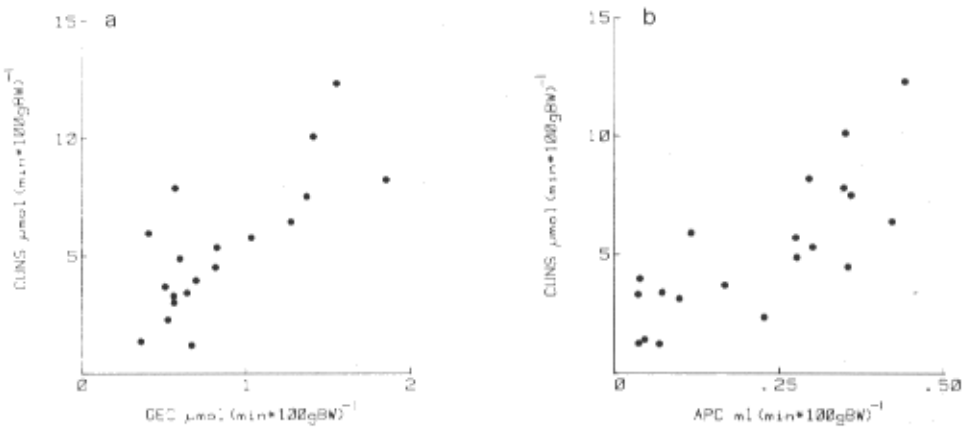

Fig. 2. *a*: The capacity of urea-N synthesis (CUNS) in relation to galactose elimination capacity (GEC). Each point represents simultaneous estimates in one animal.
b: The capacity of urea-N synthesis (CUNS) in relation to antipyrine clearance (APC) in litter mates, each pair studied simultaneously.

Discussion

In order to reduce the 'functional liver mass' to different degrees, various proportions of the liver were removed by partial hepatectomy. The weight of the liver tissue left in the animals was correlated with the results of the functional tests performed. A high degree of correlation was also found between CUNS and the established quantitative measures of liver function, the plasma clearance of antipyrine (APC) and the galactose elimination capacity (GEC). Thus, decreased CUNS can be taken to reflect reduction in the 'functional liver mass'.

Estimation of urea synthesis rate for assessment of liver function in patients has only been partly successful in previous studies [12,13], possibly due to lack of standardization of the experimental conditions. The rate of urea synthesis is dependent on the plasma concentration of alpha-amino-N. In man it appears to increase linearly with amino acid concentrations, and ratio between this concentration and urea synthesis rate the 'functional hepatic nitrogen clearance' (FHNC), is correlated with both GEC and APC [13]. In the rat the urea synthesis rate is at maximum at amino acid concentrations between 7 and 11 mmol/l [7]. This was utilized to standardize the conditions for estimation of the urea synthesis rate in the present study.

Antipyrine clearance quantitates the function of the microsomal enzyme system, but may be influenced by environmental factors, especially by xenobiotics, which can induce or inhibit antipyrine metabolism [14]. Furthermore, the amount of antipyrine excreted in the kidneys, in control rats about 10% of the dose, may be increased by hepatectomy [15].

GEC quantitates cytosolic liver function. It has been claimed that galactose to some extent is eliminated by extrahepatic organs [16]. In the present study renal excretion of galactose was excluded because the animals were nephrectomized. The regression line between liver weight and GEC has no significant intercept, indicat-

ing that in this model extrahepatic galactose elimination must be small. The extrapolated GEC corresponding liver weight zero is 0.1 μmol (min. 100 g BW)$^{-1}$, or 7% of GEC in control animals.

The capacity of urea-N synthesis from alanine quantitates cytosolic and mitochondrial functions. However, like other measures of functional hepatic mass, it may be influenced by other factors, e.g. by hormonal changes [17]. Unpublished studies from this laboratory, indicate that insulin decreases CUNS, and glucagon has a time-dependent stimulatory effect.

It is concluded that CUNS may be used as a quantitative measure of functional liver mass. Estimation of CUNS together with other measures of functional liver mass, related to different subcellular structures of the hepatocytes, may be useful for the study of the pathophysiology of hepatic diseases.

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