Starvation Induced Changes in Quantitative Measures of Liver Function in the Rat

B. A. Hansen* and H. E. Poulsen†

*Department of Medicine A, Division of Hepatology and Laboratories of Experimental Pathology, Rigshospitalet A 2152, DK-2100 Copenhagen, Denmark.
†Department of Pharmacology, University of Copenhagen, DK-2100 Copenhagen, Denmark.
(Reprint requests to B.A.H.)

ABSTRACT Liver function was estimated quantitatively as the capacity of urea-N synthesis (CUNS), the galactose elimination capacity (GEC) and the antipyrene plasma clearance (APC) in rats following 24, 48, and 96 h of food deprivation. CUNS rose to a maximum following 24 h of starvation, then decreased to the control value at 48, 72, and 96 h (p = 0.02). GEC decreased following starvation, significant so at 48 h (p = 0.02). APC decreased at 48, and 72 h (p = 0.01), but rose to the control value at 96 h. It is concluded that a precise standardisation of starvation is important for interpretation of minor changes in CUNS, GEC, and APC.

INTRODUCTION

The capacity of urea-N synthesis (CUNS), the galactose elimination capacity (GEC), and the antipyrene plasma clearance (APC) are all established measures of 'the functional liver mass' [1].

Urea cycle enzymes have increased activity during states when protein catabolism is increased, such as starvation [2]. Hepatic antipyrine metabolism is found to be influenced by malnutrition [3], while the effect of starvation on galactose elimination to our knowledge has only been investigated following 18 h of food deprivation [4].

According to this the liver function tests could be changed following various degrees of food deprivation, meaning that a precise standardisation with regard to nutritional status should be carefully taken into consideration. The aim of the present study was to investigate the effect of starvation for various periods on liver function tests in the rat.

MATERIAL AND METHODS

Experimental design

Measurement of the capacity of urea-N synthesis (CUNS), the galactose elimination capacity (GEC), and the antipyrene plasma clearance (APC) was made simultaneously in 25 female Wistar rats.

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, GEC, and APC anesthesia was induced by intraperitoneal injection of thiopentonal, 100 mg per kg body weight. Estimation of CUNS, GEC and APC was made in fed rats and following 24, 48, 72, and 96 h of starvation. Five animals were examined 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. Alane was administered as a single injection of 0.40-0.75 ml of a 1.09 mol/l solution in sterile water, followed by a constant infusion for 70 min at 1.5-3.0 ml/h of 224 mmol/l solution by means of a roller pump (Per fusor Secura). Steady-state amino acid concentration was defined as less than 10% change during a period of 50 min or longer. Simultaneously 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).

Immediately before administration of alanine and galactose blood samples were drawn into chilled tubes containing aprotinin and EDTA and stored at -20°C until analysis of insulin and glucagon concentration by RIA. Glucagon was extracted by ethanol precipitation according to Heding [5]. Samples (150 μl) were taken

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Body weight (BW)g, and liver weight (LW)g at various intervals of starvation. Values are mean ± SEM of five animals</th>
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<tbody>
<tr>
<td>Food deprivation for</td>
<td>Fed</td>
</tr>
<tr>
<td>BW</td>
<td>222 ± 8</td>
</tr>
<tr>
<td>LW</td>
<td>7.7 ± 0.2</td>
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after an equilibration period of 20 min, at intervals of 10 min, for determination of urea [6], total alpha-amino-N [7] and galactose concentrations [8]. For determination of APC, the animals received antipyrine 1 ml (4 mg/ml) by gastric tube 5 h prior to the procedures described above. At the time of investigation blood samples were taken and heparinised plasma stored at −20°C until analysis for antipyrine by HPLC [9].

Calculation. CUNS \( \mu \text{mol/(min 100 g BW)}^{-1} \) was calculated as

\[
\text{CUNS} = (d_{c}/dt) \cdot 0.63 \text{ BW}^{-1.25}
\]

where \( d_{c}/dt \) 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 distribution of urea [10], and 1.25 is a correction for intestinal hydrolysis of urea [11]. GEC \( \mu \text{mol/(min 100 g BW)}^{-1} \) was calculated as

\[
\text{GEC} = I - (dc/dt \cdot 0.40 \text{ BW})
\]

where \( I \) is the infusion rate of galactose, dc/dt is the linear slope of the galactose blood concentration-time curve, and 0.40 BW is the volume of distribution of galactose [4].

APC \( \text{ml/(min 100 g BW)}^{-1} \) was calculated as

\[
\text{APC} = (\ln(D/0.66 \text{ BW}) - (\ln(c_{i})))/t
\]

where \( D \) is the antipyrine dose given, 0.66 BW is the antipyrine volume of distribution [9], and \( c_{i} \) is the concentration corresponding to the sampling time \( t \) [9].

The period of 5 h between antipyrine dose and the determination of the plasma concentration corresponds to the optimal time for sampling [9]. The liver was removed, blotted on filter paper, and weighed.

Statistical calculations. A two-sided \( t \)-test was performed between each of the treatment group versus the control group, resulting in \( p < 0.05 \) as level of significance.

**RESULTS**

Table 1 shows the decrease in body weight and liver during 96 h of starvation (\( p = 0.001 \), \( p = 0.001 \), respectively).

Table 2 and Figure 1 show the sequential changes in CUNS, GEC, and APC at various intervals of starvation. CUNS rose to a maximum following 24 h of starvation, then decreased to the control value at 48, 72, and 96 h (\( p = 0.02 \)). GEC decreased following starvation, significant so at 48 h (\( p = 0.02 \)). APC decreased at 48 and 72 h (\( p = 0.01 \)), but rose to the control value at 96 h.

Table 3 shows the sequential changes in plasma concentration of glucose, glucagon and insulin at various intervals of starvation. The glucose concentration decreased during starvation, and was nearly half the control value after 96 h (\( p = 0.001 \)). The glucagon response to starvation was at maximum at 24 h (\( p = 0.01 \)), then decreased to the control value after 48, 72, and 96 h. The insulin concentration was only half the control value at 24, 48, 72, and 96 h (\( p = 0.01 \)).

**DISCUSSION**

The present study demonstrates that both the capacity of urea-N synthesis (CUNS), the galactose elimination capacity (GEC), and the antipyrine plasma clearance
increase in CUNS found at 24 h of starvation, is in parallel with gluconeogenesis after this interval. We found that starvation for longer than 48 h decreased CUNS, in accordance with inhibited protein breakdown after prolonged starvation [14]. In vitro, Schimke found the urea cycle enzyme activity increased following starvation for 4 and 7 days [2]. As urea excretion in that situation was decreased, it apparently has no significance in vivo, or might be due to regulation by circulating humoral factors, not present in the in vitro system.

Recently it has been demonstrated that within short periods CUNS is regulated by insulin [15] whereas glucose has a time dependent stimulating effect [16]. The increase in CUNS in the 24-48 h fasted rats can therefore be attributed to the decreased circulating plasma insulin and increased glucagon concentrations.

In accordance with the findings of Keiding [4], there was no change in GEC during starvation for 24 h. Following starvation for 48 h we found a decrease in GEC, meaning that the rate limiting enzyme in galactose metabolism, galactokinase [4], could be under influence of the metabolic alteration induced by food deprivation.

APC is decreased considerably in protein-caloric malnutrition and is reestablished after nutritional rehabilitation [3]. In the present study, APC was decreased following 48 and 72 h of starvation. At 96 h APC was not different from that of the control. This could be interpreted as an adaptation to starvation condition, but the mechanism is not clear.

It is concluded that it is important also in human studies to use a precise standardisation with regard to starvation during determination of urea synthesis rate, galactose elimination capacity, and antipyrine clearance, in order to make a correct interpretation of minor changes in these measurements.

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