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# CHANGES OF SERUM UREA VALUES IN RATS WITH A PORTO-CAVAL SHUNT

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*The study was aimed at an investigation of the role of insulin and glucagon on serum urea concentrations in rats with chronic hyperammoniemia (porto-caval shut) in the period of 14 weeks after surgery. Serum urea levels of these animals were not significantly changed ($p>0.05$), although the progressive elevation of ammonia levels was highly significant ($p<0.01$).*

*Elevation of basal insulin concetrations was also highly significant ($p<0.01$), but the ratio between urea and insulin concentrations was not statisticaly significant ($p>0.05$).*

*Alterations of basal glucagon levels were highly significant ($p<0.01$) and there was a close positive correlation between urea levels and glucagonemia ($p<0.01$).*

*Key words: porto-caval shunt, urea, insulin, glucagon, ammonia*

## INTRODUCTION

Insulin plays an important role in metabolic regulation of carbohydrates, fats and proteins in the liver. The effect of hormones (insulin and glucagon) has been studied on isolated perfused rat liver (Vom Dahl et al., 1991). It was found that physiological concentrations of these hormones change the volume of the liver cells which is responsible for modulation of their function. Insulin induces to cellular edema (increasing intracellular water space) with uptake of hepatic potassium and inhibition of proteolysis. These findings have confirmed the hypothesis (Vom Dahl et. al., 1991) that cellular volume changes may be the "secondary messenger" of hormonal action.

The metabolic action of insulin on proteins is based on increased mRNA synthesis in the liver, as well as on an increased quantity of available amino acids and reduction of gluconeogenesis (Fehlmann et al., 1979). Owing to the correlation of gluconeogenesis and ureogenesis, ureogenesis is also inhibited. It has been suggested that insulin inhibitis intrahepatic metabolism of alanine before its uptake by the liver. Moreover, insulin reduces the release of endogenous amino acids from the peripheral tissues and diminishes synthesis of urea (Hansen et al., 1986, Mondon and Mortimore, 1967).

However, in *in vitro* conditions (primary culture of rat hepatocytes) it has been found that insulin fails to influence enzymes of the urea cycle (Gebhardt and Mecke, 1984).

Nevertheless, there was a correlation of insulin concentration with the capacity for urea synthesis (CUNS) with alanine as the source of nitrogen (Hansen et al., 1986). CUNS is defined as accumulation of urea from which intestinal hydrolysis was subtracted at a constant concentration of amino acids (7.3 - 11.6 mmol/l). Study of the insulin effect necessitates maintenance of euglycaemia with feed-back controlled glucose infusion (euglycaemic clamp technique). The endogenous hormonal response was controlled with a somatostatin infusion which does not influence CUNS. Insulin is administered until different levels of hyperinsulinemia have been reached. CUNS is reduced linearly with insulin levels of up to 200 mU/l (normal fasting values are 15 mU/l). CUNS is not reduced with higher insulin concentrations. Reduction of CUNS is insulin dose dependant and independant of glucose. The effect is momentary, i. e. it does not cause changes in the quantity of urea cycle enzymes (Vilstrup et al., 1988).

Glucagon plays an important role in amino acid metabolism, since amino acid concentration is increased in the absence of glucagon (after pancreatotomy) and reduced with a surplus of glucagon (by glucagon) (Roth et al., 1987). It has been proposed that reduced blood amino acid cencentrations result from increased haeptic conversion of amino acids into urea under the influence of glucagon. Amino acids which reach the liver are used for protein synthesis in the liver or for conversion into non-nitrogen metabolites such as glucose and lactates and into urea (Almdal and Vilstrup, 1988). Thus, glucagon increases hepatic conversion of amino acids into urea and glucose, i. e. stimulates ureogenesis and gluconeogenesis (Smith et al., 1991. Takada et al., 1991, Aoki et al., 1974).

In 1965 McLean and Novello reported that pharmacological doses of glucagon increase levels of carbamoy 1-phosphate-synthetase (CPS), arginosuccinate synthetase (ASS) and argininosuccinase (AS), but not ornithine transcarbamoylase and arginase. However, subsequent experiments (Snodgrass et al., 1978) suggest that glucagon induces the whole urea cycle in rat liver and that physiological plasma glucagon concentrations stimulate activity of all five enzymes involved in the cycle. Recent experimental data (Snodgrass, 1991, Sigsgaard et al., 1988) suggest that glucocorticoids potentiate glucagon action on the induction of five enzymes of the urea cycle in rat hepatocyte culture.

It has been experimentally shown that glucagon administration to healthy rats causes numerous secondary effects such as release of insulin and STH, elevation of blood glucose levels, increased amino acid uptake in the liver and induction of enzymes participating in carbohydrate and amino acid metabolism. These data suggest that induction of the urea cycle *in vivo* cannot be the result of a direct effect of glucagon on hepatocytes,but that other substances must also be involved (Snodgrass et al., 1978).

In *in vivo* conditions glucagon treatment of rats increases the capacity for urea synthesis (Vilstrup et al., 1988, Petersen et al., 1987). Glucagon increases the activity or quantity of urea cycle enzymes in a time-dependant manner which

has been demonstrated in both *in vivo* and *in vitro* studies (Snodgrass et al., 1978, Petersen et al., 1987).

## MATERIAL AND METHODS

Male Wistar rats, of body weight 200-300 g were used in the study. The animals were kept in separate cages, with free access to standard pelleted food and water. Optimum temperature and atmospehric moisture were maintained in the room.

The animals were divided into two groups: non-operated animals (controls N=10) and animals with a surgical porto-caval shunt (N=18).

The surgical technique of end-to-side porto-caval anastomosis was first described in 1961 (Lee and Fisher, 1961.) and modified in 1963. (Bismith et al., 1963). It was used as a model of hepatic insufficiency.

After introduction of general anaesthesia with ether narcosis and opening of the abdominal cavity, vena cava inferior and vena porta were exposed and clamped. "S" shaped Halsted's curved hemostat was used for clamping of vena cava inferior. It was placed along the blood vessel at the level of confluence with the right renal vein. The part of the clamped venous wall was dissected. Vena porta was distally clamped with a small bull-dog clamp, proximally ligated with N/2 silk suture and subsequently cut above the gastroduodenal vein stump. Two edge sutures were placed on the upper and lower angles of the anastomosis using N 7/0 or N 8/0 silk suture. Thus, the venous vessels were approximated and a continuous suture was placed on the posterior wall from the lower angle on the interior side of the anastomosis up to the angle at which the needle pierced the wall of the vena cava inferior. A suture was placed behind the edge stich and the anterior wall was sutured on the external side of the anastomosis.

Immediate survival depends on the duration of cassation of circulation into the vena porta which should not last longer than 15 minutes.

At determined intervals (before the operation, and 2, 6, 10 and 14 weeks after the operation) blood samples were taken from the animals to determine insulin, glucagon, urea and ammonia concentrations.

Insulin and glucagon levels were determined by radioimmunoassay (RIA), ammonia by an enzymatic UV test, and urea by Berthelot's method.

## RESULTS AND DISCUSSION

Serum urea levels in the rats with chronic hyperammoniemia (porto-caval shunt) did not change significantly during the period of 14 weeks after the operation ($p>0.05$; Figure 1).

Ammonia concentrations increased significantly ($p<0.01$; Figure 2).

The elevation of basal insulin levels was also highly significant ($p<0.01$; Figure 3) while changes in the ratio of urea and insulin concentrations were not significant ($p>0.05$).

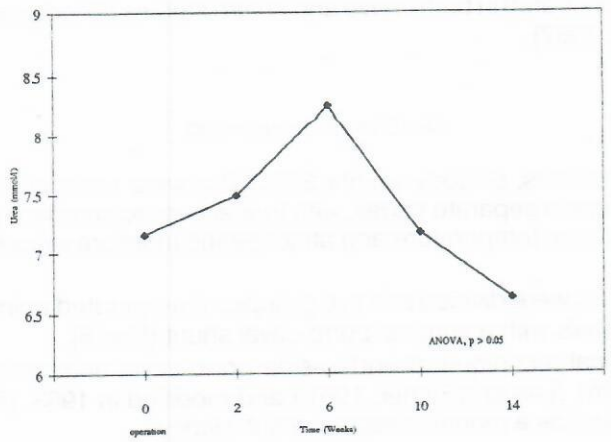


Figure 1. Serum urea levels in rats with a porto-caval shunt.

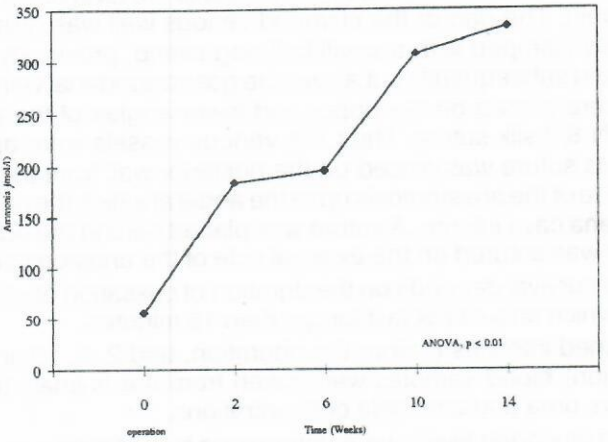


Figure 2. Serum ammonia levels in rats with a porto-caval shunt.

Changes of basal glucagon levels were highly significant ($p<0.01$; Figure 4), and the positive correlation between urea levels and glucagonemia was also highly significant ($p<0.01$; Figure 5).

Thus, investigation of serum urea values in rats with a porto-caval shunt (POS) for 14 weeks showed that the changes were not significant (ANOVA, $p>0.05$).

Hickman et al. 1974, failed to obtain significant changes in serum urea concentrations in pigs, which is similar to our results. Conversely, Reichle et al. 1977., reported reduced synthesis of urea in rats and dogs.

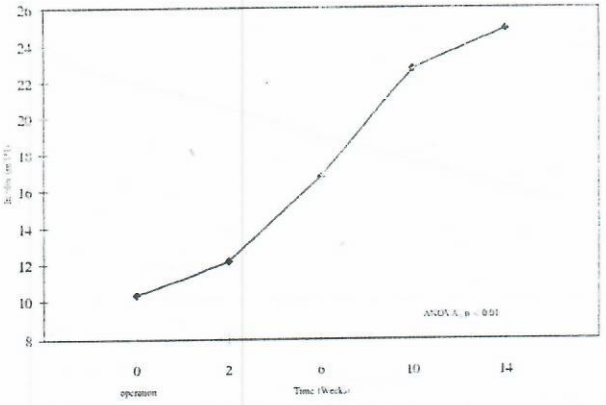


Figure 3. Blood insulin serum levels in rats with a porto-caval shunt.

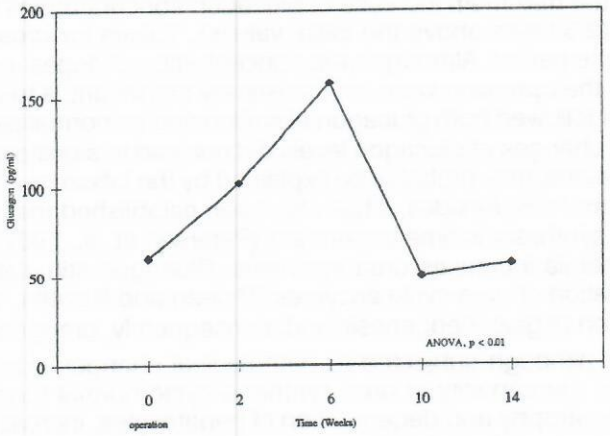


Figure 4. Serum glucagon changes in rats with a porto-caval shunt.

The following mechanisms have been proposed as those which maintain normal urea synthesis without major fluctuations:

- increased circulation through the hepatic artery,
- compensatory hepatic hyperfunction.

POS not only reduces the total hepatic circulation, but the liver receives portal blood indirectly through recirculation via the hepatic artery (McLean and Novello, 1965). Liver function is probably maintained by the increased blood flow. Oxygen is provided in this way, as well as hormones and substrates, although in smaller amounts than when the liver obtains the same substances directly via vena porta.

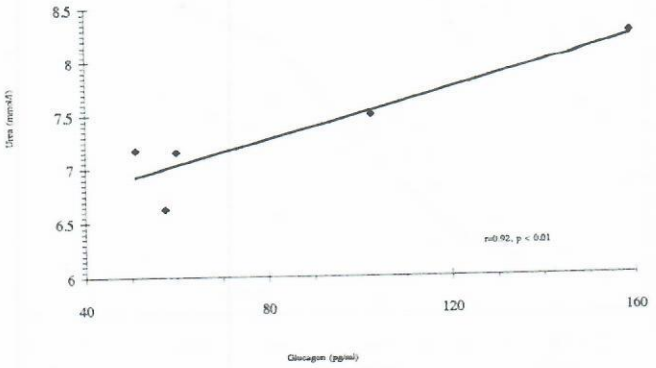


Figure 5. Correlation between serum urea levels and glucagonemia in rats with PCS.

A highly significant correlation between glucagon and urea concentrations has been shown in our experiments ($r=0.92$, $<0.01$). Analysis of the obtained results shows the maximum increase of glucagon concentrations 6 weeks after the operation (2.5 times above the initial values). Values for urea reached the peak in the same period. Although urea concentration changes in the period of 14 weeks after the operation were not statistically significant, a trend was noted that urea levels followed both glucagon elevation and its normalization. The fact that significant changes of glucagon levels did not lead to significant changes in urea concentrations, may probably be explained by the influence of other factors which result from POS. Besides, it has also been established that the glucagon effect on urea synthesis is time-dependant (Petersen et al., 1987) i. e. chronic hyperglucagonemia increases urea synthesis. Glucagon stimulates urea synthesis via activation of urea cycle enzymes (Mclean and Novello, 1965), as well as via stimulation of gluconeogenesis and, consequently, ureogenesis.

The liver, although subjected to pathological changes occurring due to POS, preserves the capacity of urea synthesis cycle normal functioning. As a response to the atrophy and degeneration of hepatocytes, increased regeneration and hyperfuncion of unaffected hepatocytes occur.

In 1986 Hansen and Poulsen monitored changes in hepatic capacity for urea synthesis (CUNS) in partially hepatectomized rats (70%).Immediately after hepatectomy CUNS was reduced, but six weeks later an increase ensued due to compensatory hyperfunction of the remaining part of the liver. Since urea synthesis is an essential liver function (Haussinger and Gerok, 1985) it recovers before others, eg galactose elimination. However, Aldmal et al. 1989., also studied partially hepatectomized rats (85%) and noted a significant reduction of ureogenesis which did not significantly disturb the acid-base balance.

In addition to this experimental evidence of regeneration and compensatory hyperfunction of the lesioned functional mass of the liver (POS, hepatectomy), clinical studies show that impaired synthesis of serum urea is pertinent only to severe liver diseases. However, since urea level is dependant more on renal

function and the degree of protein catabolism, it is not an appropriate parameter for evaluation of liver function. In partients with liver cirrhosis, elevation of urea levels suggests an initial hepatorenal syndrome and it is a bad prognostic sign (Teodorović et al., 1991).

The second group of results show reduced liver synthesis of urea associated with POS (Reichle et. al., 1977, Starzl et al., 1983). Due to the liver bypass the total hepatic blood flow is reduced, numerous hepatotrophic substances are missing (Starzl etal., 1975, Starzl et. al., 1976) as a consequence of hepatofugal blood flow (Teodorović et al., 1991).

The most important hepatotrophic factors in portal blood for maintenance of liver structure and function are pancreatic hormones, especially insulin, and glucagon (Ozawa et al., 1974, Starzl et al., 1983, Starzl et al., 1978. Starzl et al., 1975, Starzl et al., 1973).

Qualitative and quantitative reduction of granulated endoplasmatic reticulum (GER) and polyribosomes is a relatively specific effect of POS. Since GER is a cellular "factory", numerous biosynthetic processes are reduced.

In 1977 Rechle et al. demonstrated that urea synthesis (Krebs-Henseleit's cycle) is impaired in conditions of end-to-side POS in rats and dogs. According to these authors reduced urea synthesis occurs due to lackof insulin in the liver. Due to the hepatofugal blood flow, insulin may reach the liver only via arteria hepatica. Its increased circulation cannot provide normal liver insulin perfusion, so that insulin exerts a major influence on the peripheral tissues and only a minor influence on the liver (McLean and Novello, 1965). It has been shown that the destruction of GER characteristic of POS occurs during alloxan provoked diabetes, and may be recovered with insulin supplementation.

In addition to structural changes, it has been shown that insulin deficincy in the liver leads to reduced activity of arginase. This has been confirmed in diabetic rats, while insulin therapy promotes activity of this enzyme.

During the porto-caval shunt, hepatofugal blood flow leads to disturbance of hepatic circulation, causing progressive impairment of hepatic function. Measurement of the respiratory control ratio of hepatic mitochiondria is the most convenient criterion of the integrity of cellular function. The interaction of portal blood and phosphorylating capacity of mitochondria of the liver takes an important place in the homeostasis of energy production. It has been shown that insulin, as a portal factor, plays an important regulatory role in the stimulation of the phosphorylating capacity of liver mitochondria (Ozawa et al., 1974). Since hepatocytes are "bathing" in portal blood, liver mitochondria can respond to changes in the insulin level. Normally, a quantitative relationship betwen hepatocyte count and insulin level in the portal blood is necessary for maintenance of normal mitochondrial function. If hepatocytes are devoid of portal blood (POS), reduction of insulin availability leads to depression of phosphohrylating capacity of mitochondria which is necessary for maintenance, of hepatocyte integrity, causing reduction of some important functions and leading to liver atrophy. Since mainenance of biological function depends on a continuous supply of high energy phosphates produced in mitochondria, reduction

of the insulin level below hepatocyte requirements due to the detour of portal blood away from the liver causes severe metabolic and physiologic liver dysfunction, and is the principal cause of hepatic failure (Ozawa et al., 1974., Lambert and Wright, 1984).

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## PROMENA VREDNOSTI UREJE U SERUMU PACOVA SA HRONIČNOM HIPERAMONIJEMIJOM

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SADRŽAJ

Istraživanje je vršeno da bi se utvrdila uloga insulina i glukagona na vrednosti ureje u serumu pacova sa hroničnom hiperamonijemojom, (portokavni šant) u periodu od 14 nedelja nakon operacije. Promena vrednosti ureje u serumu životinja se nije značajno promenila ($p>0.05$).

Porast vrednosti amonijaka je visoko statistički značajan ($p<0.01$).

Porast vrednosti bazalnog nivoa insulina je visoko statistički značajan ($p<0.01$), dok odnos koncentracija ureja i insulina nije statistički značajan ($p>0.05$).

Promene bazalnog nivoa glukagona su statistički visoko značajne ($p<0.01$) a između nivoa ureje i glukagona postoji pozitivna korelacija koja je visoko statistički značajna ($p<0.01$).