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Changes in urinary taurine and hypotaurine excretion after two-thirds hepatectomy in the rat

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Summary. This study followed the time course of urinary taurine and hypotaurine excretion after two-thirds hepatectomy in rats. The excretion of both taurine and hypotaurine was elevated during 18h following the hepatectomy, with maximal excretion during the first 6h. Twelve and 24h after partial hepatectomy, the hepatic hypotaurine concentration was increased but liver taurine did not differ significantly from controls. No changes were observed in hypotaurine and taurine concentrations of heart, kidney, lung, muscle tissue and spleen. We postulate that partial hepatectomy induces a rapid increase of hepatic (hypo)taurine synthesis from precursor amino acids. The increased (hypo)taurine concentrations spill over into urine.

Keywords: Amino acids – Hepatectomy – Hypotaurine – Liver – Taurine – Urine

Introduction

Taurine (2-aminoethane sulphonic acid) constitutes the major free amino acid of many mammalian. Taurine is not incorporated into protein but remains free in the intracellular cytosol tissues (Chesney, 1985).

In the rat, taurine is derived either from the diet or de novo synthesized. Synthesis of taurine from methionine or cysteine via cysteic acid or hypotaurine was reported for liver, brain, lung and muscle tissue (Pasantes-Morales et al., 1980; Sturman and Fellman, 1983; Tamura et al., 1984; Garcia and Stipanuk, 1992; Ensunsa et al., 1993; Sharma et al., 1995). In the liver, taurine is involved in many important physiological processes such as conjugation of bile acids and xenobiotics, calcium mobilisation and osmoregulation (Chesney, 1985; Wright et al., 1986; Gaull, 1989; Huxtable, 1992; Brand et al., 1994, 1995b). The rat liver shows a diurnal variation in taurine concentration, which may be related to food intake of the animals (Waterfield et al., 1991).

Administration of several hepatotoxic compounds (like carbon tetrachloride, thioacetamide and galactosamine which all caused hepatic necrosis)

resulted in elevated urinary taurine levels in rats (Sanins et al., 1990; Timbrell et al., 1995; Timbrell and Waterfield, 1996; Waterfield et al., 1991, 1993a). Originally, it has been suggested that this increase resulted from leakage of taurine from damaged hepatocytes, and that the changes in urinary taurine would be useful as a non-invasive indicator of liver damage (Sanins et al., 1990; Waterfield 1991, 1993c). However, the loss in liver taurine after exposure to most hepatotoxic compounds accounted only for a part of the increase in taurine in urine over the same time (Waterfield, 1991, 1993b), which suggests that simple leakage from damaged tissue is not the sole cause of the increased levels. Therefore, it has been suggested that the inhibition of protein synthesis by the hepatotoxicants raises the intracellular pool of cysteine and increases taurine synthesis, which would then overspill into the urine (Waterfield, 1993a; Timbrell et al., 1995; Timbrell and Waterfield, 1996). This hypothesis is supported by the observation that inhibition of protein synthesis by cycloheximide increases urinary taurine excretion in rats (Waterfield et al., 1993b, 1996).

In rats, surgical removal of the median and left lateral lobe induces a rapid regenerative process in the remaining liver. Within two weeks, the liver has regained its original size and the regeneration is terminated (Higgins and Anderson, 1931). Two-thirds hepatectomy is a strong anabolic signal and during liver regeneration hepatic protein synthesis is increased (Scornik, 1974; Luk, 1986; Murawaki et al., 1992; Okano et al., 1997). Because of the assumption that leakage of taurine from damaged hepatocytes after partial hepatectomy will contribute minimally to urinary excretion, since the liver tissue is surgically removed, we hypothesized that urinary taurine excretion will be reduced after two-thirds hepatectomy. However, by comparing urinary excretion of taurine and hypotaurine of rats during the first 24 h after two-thirds hepatectomy with sham-operated animals we observed, unexpectedly, a significant increase in (hypo)taurine excretion. An alternative hypothesis for the increased urinary (hypo)taurine excretion will be brought forward.

Material and methods

Animals

Experiments were performed on male Wistar rats (HSD, Zeist, The Netherlands) weighing 317 ± 4 g at the time of surgery were housed in individual metabolic cages, starting 7 days before the study to allow adaptation to this environment. Animals were maintained in a constant-temperature room (21 ± 2°C) with a regular 12-h light-dark cycle (light on at 7:00 a.m., off at 7:00 p.m.). Unless otherwise stated, rats had free access to water and standard diet (RMH 1410, Hope Farms, Woerden, The Netherlands); food and water intake water were determined.

Animal welfare was in accordance with institutional guidelines of the University of Amsterdam.

Experimental groups

The animals were randomly assigned to three experimental groups. The first group (NORM, n = 8) consisted of rats without previous surgery, in which plasma and tissues

were sampled after an overnight fast. In the rats of the second group (HP), a two-third hepatectomy was performed after an overnight fast (Higgins and Anderson, 1931). The median and left lateral lobes were removed under diethyl ether anaesthesia between 8.30 and 9.30 a.m.. The abdominal cavity was closed after it was ensured that no bleeding occurred. After 12(HP 12, n = 8) or 24h (HP 24, n = 8), the hepatectomized rats were sacrificed.

The third group were pair-fed, sham-operated rats (SHAM-PF 12, n = 8; SHAM-PF 24, n = 8). Sham operations were performed in a similar fashion by externalising the same lobes and returning them to the abdominal cavity after a brief period of manipulation.

Following surgery, water was provided ad libitum. Food intake in the individual HP animals was determined and matched with individual SHAM-PF animals. Consecutive 6-h urine collections were made over ice throughout the study period and urine volumes were determined gravimetrically. Urine samples were centrifuged (8,500g, 4°C, 10min) to remove hair and food debris, and frozen (−70°C) in aliquots until analysis.

Post mortem procedure and tissue processing

Animals were anaesthetized with diethyl ether and exsanguinated from the abdominal aorta. Blood samples were collected in heparinized tubes (Becton Dickinson, Franklin Lakes NJ, USA) and centrifuged within 10min (8,500g, 4°C, 10min). One hundred microliters of plasma was deproteinized with 4mg sulfosalicylic acid (BDH, Poole, United Kingdom) and stored at −70°C.

Immediately after blood sampling liver, spleen, left kidney, heart, lungs and the right gastrocnemius muscle were rapidly excised, weighed, freeze-clamped with Wollenberger tongs (Wollenberger et al., 1960), put into liquid nitrogen (total procedure less than 2min) and stored at −70°C until further analysis.

Frozen tissue specimens were ground under liquid nitrogen with a porcelain mortar and pestle. Approximately 100mg of tissue powder was added to pre-weighed vials containing 400μl of 5% sulfosalicylic acid containing 500μM norvaline internal standard (BDH, Poole, United Kingdom). The tissues fragments were further homogenized at −10°C with an Ultra Turrax (IKA-labortechnik, Staufen, Germany) (Dejong et al., 1992; Heeneman and Deutz, 1993). The homogenate was centrifuged (8,500rpm, 4°C, 10min) and the supernatant was used for amino acid analysis.

Biochemical analysis

Taurine and hypotaurine were measured in deproteinized plasma, tissue homogenates and diluted urine samples (1:4 with UHQ water) using a HPLC system after pre-column-derivatization with ortho-phthaldialdehyde (Sigma, St. Louis MO, USA) (van Eijk, 1988). Creatinine was measured in diluted urine samples (1:10 with UHQ water) in a Hitachi/BM 747 automatic analyzer using the appropriate kit (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany). Urine taurine and hypotaurine concentrations were expressed per mmol of creatinine, since creatinine clearance is identical in HP and SHAM-PF animals (Ohno et al., 1991; Lorenzi et al., 1993).

Statistical analysis

Data are presented as means ± S.E.M. Statistical analyses were performed using the SPSS/PC+ Statistical Software Package, version 5.0 (SPSS Inc, Chicago, USA).

ANOVA (two-way procedure) was used for comparison of HP and SHAM-PF groups. Comparison of data from HP and SHAM-PF animals at individual time points was performed by Mann-Whitney U non-parametric tests. ANOVA (one-way procedure) was used for analysis of time effects within groups, followed by the Scheffé procedure for individual group comparison where appropriate. A p value of <0.05 was considered statistically significant.

Table 1. Water consumption, urine production and concentrations of taurine and hypotaurine in urine of rats during the first 24h after two-thirds hepatectomy (HP) and sham-operated pair-fed controls (SHAM-PF)

	HP				SHAM-PF			
	0–6	6–12	12–18	18–24	0–6	6–12	12–18	18–24
Water consumption	3.3 ± 0.7	4.5 ± 1.1	6.4 ± 1.7	3.2 ± 0.7	1.0 ± 0.4	1.9 ± 0.4	6.3 ± 1.5¶&	2.3 ± 0.7#†
Urine production	1.4 ± 0.4	4.0 ± 0.7	5.9 ± 2.1¶	2.1 ± 0.7#†	0.6 ± 0.3	1.2 ± 0.3	4.6 ± 1.0¶&	1.6 ± 0.7#†
Hypotaurine*	49.5 ± 7.0$	37.4 ± 5.3$	7.7 ± 2.0¶&	4.9 ± 2.5#¶&	5.3 ± 2.3	5.6 ± 1.4	3.5 ± 1.8	1.3 ± 0.9
Taurine*	1,947 ± 287$	1,740 ± 174$	668 ± 97$¶&	314 ± 69#¶&	339 ± 136	359 ± 67	235 ± 61	202 ± 43
Hypotaurine/ taurine ratio*	0.031 ± 0.004$	0.022 ± 0.002$	0.010 ± 0.002¶&	0.006 ± 0.002¶&	0.012 ± 0.008	0.011 ± 0.004	0.017 ± 0.011	0.006 ± 0.004

Values are total ml during each period of 6 hours or μmol/mmol creatinine (mean ± SEM, N = 8–16). ANOVA for group differences HP vs SHAM-PF: *$p < 0.05$. Mann Whitney-U for differences between HP and SHAM-PF: \$$p < 0.05$. ONEWAY procedure for time effects within groups: #$p < 0.05$. Scheffe comparison for differences within groups: vs 0–6: ¶$p < 0.05$, vs 6–12: &$p < 0.05$, vs 12–18: †$p < 0.05$.

Results

Urinary taurine and hypotaurine excretion following two-thirds hepatectomy are compared with those in sham-operated pair-fed animals at each time point. The use of sham-treated animals is essential to act as controls for diurnal variations and for the effects of anaesthesia and surgery, independent of partial hepatectomy. Water intake and urine volumes were not significantly different between HP and SHAM-PF rats.

The effect of partial hepatectomy on urinary taurine and hypotaurine levels, measured at 6-hourly intervals, is presented in Table 1. Partial hepatectomy induced a significant increase in taurine excretion, which is maximal during the first 6 h after hepatectomy (+474% compared to SHAM-PF). The urinary taurine excretion remained elevated between 6–12 h and 12–18 h after hepatectomy(+385% and +184%, respectively).

Two-thirds hepatectomy has an even more pronounced effect on the urinary excretion of hypotaurine: during the 0–6 h and 6–12 h the hypotaurine excretion increased +834 and +568%, respectively.

Partial hepatectomy reduced the amount of liver tissue to 44% and 52% after 12 and 24 h, respectively. No significant effects were observed on the weights of heart, kidney, lungs or spleen (Table 2).

Taurine plasma concentrations showed no significant changes (Table 3). Two-thirds hepatectomy has no significant effect on taurine concentrations in liver, heart, kidney, lung, spleen and muscle tissue (Table 3). The non-significant reduction in taurine concentration of the remaining liver tissue 12 h after partial hepatectomy, accounted for only a small proportion (10%) of the increased urinary taurine excretion between 0 and 12 h. The changes in hepatic taurine concentrations in sham-operated animals may represent diurnal variations.

Significant increases of the intrahepatic hypotaurine concentration were observed 12 and 24 h after partial hepatectomy (+389 and +255% vs. SHAM-PF, respectively) (Table 4). Partial hepatectomy had no significant effect on hypotaurine concentrations of heart, kidney, lung, spleen and muscle.

Table 2. Weight of tissues of normal rats after an overnight fast (NORM) and rats after two-thirds hepatectomy (HP) and sham-operated pair-fed controls (SHAM-PF)

	NORM	HP12	HP24	SHAM-PF 12	SHAM-PF 24
Liver*	2.86 ± 0.07	1.39 ± 0.05$¶	1.52 ± 0.04$#¶	3.17 ± 0.05¶	2.91 ± 0.06$&
Heart	0.39 ± 0.01	0.39 ± 0.02	0.45 ± 0.02$#¶	0.40 ± 0.02	0.39 ± 0.01
Kidney	0.40 ± 0.01	0.43 ± 0.02	0.40 ± 0.01	0.41 ± 0.01	0.39 ± 0.01
Lung	0.46 ± 0.02	0.41 ± 0.02	0.47 ± 0.01#¶	0.43 ± 0.03	0.46 ± 0.01
Spleen	0.21 ± 0.02	0.20 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.20 ± 0.01

Values are g wet weight/100 gram body weight (mean ± SEM, N = 8). ANOVA for group differences HP vs SHAM-PF: *$p < 0.05$. Mann-Whitney U for differences between HP and SHAM-PF: $$p < 0.05$. ONEWAY procedure for time effects within groups: #$p < 0.05$. Scheffé comparison for differences within groups vs NORM: ¶$p < 0.05$, vs T = 12: &$p < 0.05$.

Table 3. Taurine concentration in plasma and tissues of normal rats after an overnight fast (NORM) and rats after two-thirds hepatectomy (HP) and sham-operated pair-fed controls (SHAM-PF)

	NORM	HP12	HP24	SHAM-PF 12	SHAM-PF 24
Plasma	204 ± 20	193 ± 28	177 ± 22	145 ± 20	182 ± 21
Liver	2.85 ± 0.41	3.91 ± 0.52	3.68 ± 0.90	5.46 ± 0.88¶	2.66 ± 0.62#&
Heart	26.69 ± 0.86	26.53 ± 1.45	26.97 ± 0.95	25.61 ± 1.09	29.76 ± 1.10#&
Kidney	9.35 ± 0.44	8.72 ± 0.24	11.67 ± 0.44#¶&	9.81 ± 0.35	10.89 ± 0.74
Lung	15.37 ± 0.50	14.36 ± 0.45	13.33 ± 0.46	10.71 ± 0.52	14.22 ± 0.45
Muscle	17.80 ± 0.56	17.11 ± 0.82	16.77 ± 0.45	15.37 ± 0.96	16.87 ± 0.69
Spleen	16.33 ± 0.56	17.58 ± 0.33	17.46 ± 0.76	16.44 ± 0.77	16.91 ± 0.60

Values are μmol/L or μmol/g wet weight tissue (mean ± SEM, N = 8). For significance symbols, see Table 2.

Table 4. Hypotaurine concentration in tissues of normal rats after an overnight fast (NORM) and rats after two-thirds hepatectomy (HP) and sham-operated pair-fed controls (SHAM-PF)

	NORM	HP12	HP24	SHAM-PF 12	SHAM-PF 24
Liver*	0.11 ± 0.01	0.39 ± 0.11	0.39 ± 0.07$#	0.08 ± 0.01	0.11 ± 0.01
Heart	0.24 ± 0.03	0.22 ± 0.02	0.21 ± 0.02	0.27 ± 0.02	0.22 ± 0.02
Kidney	0.22 ± 0.03	0.14 ± 0.01¶	0.13 ± 0.01#¶	0.17 ± 0.01	0.15 ± 0.01
Lung	0.15 ± 0.03	0.17 ± 0.01	0.11 ± 0.01#¶	0.11 ± 0.01	0.12 ± 0.01#¶
Muscle	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.00	0.07 ± 0.00	0.05 ± 0.01
Spleen	0.29 ± 0.04	0.33 ± 0.02	0.24 ± 0.02	0.30 ± 0.01	0.25 ± 0.02

Values are μmol/g wet weight tissue (mean ± SEM, N = 8). For significance symbols, see Table 2.

Table 5. Methionine concentration in tissues of normal rats after an overnight fast (NORM) and rats after two-thirds hepatectomy (HP) and sham-operated pair-fed controls (SHAM-PF)

	NORM	HP12	HP24	SHAM-PF 12	SHAM-PF 24
Liver*	0.05 ± 0.00	0.03 ± 0.00$¶	0.05 ± 0.00#&	0.05 ± 0.00	0.05 ± 0.00
Heart	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.00
Kidney	0.04 ± 0.00	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.00	0.05 ± 0.00
Lung	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Muscle	0.03 ± 0.00	0.03 ± 0.00	0.05 ± 0.01	0.03 ± 0.00	0.03 ± 0.01
Spleen	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01

Values are μmol/g wet weight tissue (mean ± SEM, N = 8). For significance symbols, see Table 2.

A significant decrease in intrahepatic methionine concentration was observed 12 h after two-thirds hepatectomy (Table 5). No changes were observed in the methionine concentration of heart, kidney, lung, spleen and muscle tissue.

Discussion

During the last decade, several studies have demonstrated that administration of hepatotoxic compounds to rats resulted in elevated urinary taurine levels (Sanins et al., 1990; Timbrell et al., 1995; Timbrell and Waterfield 1996; Waterfield et al., 1991, 1993a). After administration of carbon tetrachloride, only a small proportion (8%) of the increase in taurine lost in the urine could be accounted for by loss of liver taurine (Waterfield et al., 1991). However, several hepatotoxic compounds may adversely affect renal tissue and therefore kidney damage may contribute to the increased urinary taurine excretion (Ogawa et al., 1992; Fleck et al., 1988; Zimmermann et al., 1983).

The data from the present study demonstrate that urinary taurine excretion also increased during the first 18 h after partial hepatectomy (Table 1). After hepatectomy, a similar small proportion (10%) of the increase in urinary taurine excretion during the first 12 h could be due to the decrease in taurine concentration of the remnant liver tissue over the same time. This loss of hepatic taurine may result from simple leakage from damaged hepatocytes due to the surgical procedure and/or the induced cell swelling after partial hepatectomy. The volume of hepatocytes increases rapidly after partial hepatectomy (Grisham et al., 1975) and it has been shown that isolated rat livers release taurine during periods of cell swelling (Brand et al., 1994, 1995b).

In the rat, most tissues contain high levels of taurine (Chesney, 1985). Potentially, release of taurine from extrahepatic sources may contribute to the increase in urinary taurine after two-thirds hepatectomy. However, the taurine concentration in the organs investigated was not decreased by partial hepatectomy (Table 3). In addition, the concentration of taurine in the brain, which also contains high levels of taurine, was unaltered after partial hepatectomy (Sturman and Fellman, 1982). This suggests that urinary excreted taurine is not just merely released from these tissue sources after partial hepatectomy, but de novo synthesised.

In rats, the liver is the major site of taurine biosynthesis (Garcia and Stipanuk, 1992; Ensunsa et al., 1993). Although synthesis of taurine has also been reported for brain, lung and muscle tissue of the rat (Pasantes-Morales et al., 1980; Sturman and Fellman, 1983; Sharma et al., 1995), the taurine synthetic capacity of liver tissue is more than 10 times as great as the synthetic capacity of the whole body skeletal muscle tissue (Ensunsa et al., 1993).

We observed an increase in the concentration of hypotaurine in the remnant liver tissue at 12 h after partial hepatectomy (Table 4). Previous studies have shown that the hepatic hypotaurine concentration increases within 4 h after hepatectomy (Sturman, 1980; Sturman and Fellman, 1982, 1983). The increase in hypotaurine concentration and the concomitant decrease in methionine concentration are specific for liver tissue and not observed in other tissues (Table 4). This suggests that the excreted (hypo)taurine originates from the liver. However, we can not completely eliminiate a role of extrahepatic tissues as potential sources of the increased urinary taurine excretion.

Recently, it has been suggested that administration of hepatotoxic compounds may inhibit protein synthesis, which will result in an accumulation of amino acids, including cysteine. The excess cysteine may be metabolised to taurine and excreted in urine (Waterfield 1993a, 1993b, 1996; Timbrell et al., 1995; Timbrell and Waterfield, 1996). However, during the first 24 h after two-thirds hepatectomy the hepatic protein synthesis is increased (Scornik, 1974; Luk, 1986; Murawaki et al., 1992; Okano et al., 1997).

Other possible explanations for increased levels of taurine precursors after partial hepatectomy, like a reduced synthesis of glutathione, a reduction in the synthesis of cysteine-rich proteins or a reduced production of taurine-conjugated bile-acids are also very unlikely. The hepatic glutathione concentration level is increased after partial hepatectomy (Cockerill et al., 1983; Tsuboi et al., 1992; Teshigawara et al., 1995; Akaza et al., 1996) and the synthesis of metallothioneins, a group of cysteine-rich proteins, in the remnant liver is dramatically increased (Tohyama et al., 1993; Margeli et al., 1994; Tsujikawa et al., 1994). The production of bile is increased during the first hour after partial hepatectomy and is normalized after 6 h (Perez-Barriocanal et al., 1990; Sainz et al., 1997). In addition, bile collected from hepatectomized rats during this period contained an increased percentage of taurine-conjugated bile acids (Garcia-Marin et al., 1990).

In the plasma membrane of hepatocytes, the activity of the amino acid transporter system A and system ASC increased extensively within 6 h after hepatectomy (Le Cam et al., 1979; Fowler et al., 1992; Martinez-Mas et al., 1993). Since these two amino acid transporters are capable of transporting methionine and cysteine, respectively (Meijer et al., 1990), this may increase the concentration of these precursors of taurine. Measurement *in vivo* of the hepatic amino acid-fluxes after two-thirds hepatectomy supports this suggestion. Two-thirds hepatectomy induces a specific two- to threefold increased net hepatic uptake of the amino acids alanine and glycine, which both can be transported by the amino acid transport system A/ASC (Brand et al., 1995a).

Another possible source of amino acid precursors is endocytosis of serum albumin. Within 30 minutes after two-thirds hepatectomy, serum albumin is actively taken up into the liver cells by endocytosis. In the lysosomal compartment, the albumin is rapidly digested to yield free amino acids (Ryoo et al., 1997).

In summary, these data suggest that the increased urinary excretion of hypotaurine and taurine after partial hepatectomy is associated with increased synthesis of these amino acids, probably in the remnant liver tissue. These changes in urinary excretion seem not to be related to a reduced hepatic protein synthesis. Therefore, we postulate that this increase is due to activated import of precursor amino acids into the remnant liver, needed for regenerative protein synthesis. This hypothesis might also be an alternative explanation for the increased taurine excretion after damage of liver cells by hepatotoxic compounds (Sanins et al., 1990; Timbrell et al., 1995; Timbrell and Waterfield, 1996; Waterfield et al., 1991, 1993a), as such damage is another strong trigger for liver regeneration (e.g. Holtta et al., 1973; Nakata et al., 1985; Sasaki et al., 1989; Clawson et al., 1991).

Therefore, measuring urinary taurine excretion does not seem to be a specific (non-invasive) method for monitoring alterations in protein synthesis (Waterfield et al., 1996).

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References

Akaza K, Nonami T, Kurokawa T, et al (1996) Doxorubicin-induced disturbance of the energy metabolism after hepatectomy. J Surg Res 61: 454–458

Brand HS, Meijer AJ, Gustafson LA, et al (1994) Cell-swelling-induced taurine release from isolated perfused rat liver. Biochem Cell Biol 72: 8–11

Brand HS, Deutz NEP, Meijer AJ, Jörning GGA, Chamuleau RAFM (1995a) In vivo amino acid fluxes in regenerating liver after two-thirds hepatectomy in the rat. J Hepatol 23: 333–340

Brand HS, Meijer AJ, Gustafson LA, Chamuleau RAFM (1995b) Hypoosmotic cell-swelling induced taurine release from rat liver. In: Capocaccia L, Merli M, Riggio O(eds) Advances in hepatic encephalopathy and metabolic nitrogen exchange. CRC Press, Boca Raton, pp 500–506

Le Cam A, Rey J-F, Fehlmann M, Kitabgi P, Freychet P (1979) Amino acid transport in isolated hepatocytes after partial hepatectomy in the rat. Am J Physiol 236: E594–E602

Chesney RW (1985) Taurine: its biological role and clinical implications. Adv Pediatr 32: 1–42

Clawson GA, Madsen KR, Blankenship LJ, Hatem CL (1991) Alterations in nuclear scaffold constituents during carbon tetrachloride-induced liver regeneration. Hepatology 13: 515–522

Cockerill MJ, Player TJ, Horton AA (1983) Studies on lipid peroxidation in regenerating rat liver. Biochim Biophys Acta 750: 208–213

Dejong CHC, Kampman MT, Deutz NEP, Soeters PB (1992) Altered glutamine metabolism in rat portal drained viscera and hindquarter during hyperammonemia. Gastroenterology 102: 936–948

Van Eijk HMH, van der Heijden MAH, van Berlo CLH, Soeters PB (1988) Fully automated liquid-chromatographic determination of amino acids. Clin Chem 34: 2510–2513

Ensunsa JL, Hirschberger LL, Stipanuk MH (1993) Catabolism of cystein, cystine, cysteinesulfinate and OTC by isolated perfused rat hindquarter. Am J Physiol 264: E782–E789

Fleck C, Zimmermann T, Franke H, Braunlich H, Dargel R (1988) Relation between renal and hepatic excretion of drugs: VII. Hepatic and renal excretion of phenol red in thioacetamide-induced acute and chronic liver damage. Exp Pathol 33: 47–54

Fowler FC, Banks RK, Mailliard ME (1992) Characterization of sodium-dependent amino acid transport activity during liver regeneration. Hepatology 16: 1187–1194

Garcia RAG, Stipanuk MH (1992) The splanchnic organs, liver and kidney have unique roles in the metabolism of sulfur amino acids and their metabolites in rats. J Nutr 122: 1693–1701

Garcia-Marin JJ, Regueiro P, Perez-Antona JC, Villanueva GR, Perez-Barriocanal (1990) Pre-replicative phase-related changes in bile acid-induced chloleresis in the regenerating rat liver. Clin Sci 78: 55–62

Gaull GE (1989) Taurine in pediatric nutrition. Review and update. Pediatrics 83: 433–442

Grisham JW, Tillman RL, Nägel AEH, Compagno J (1975) Ultrastructure of the proliferating hepatocyte: sinusoidal surfaces and endoplasmic reticulum. In: Lesch R, Reutter W (eds) Liver regeneration after experimental injury. Stratton, New York, pp 6–23

Heeneman S, Deutz NEP (1993) Effects of decreased glutamine supply on gut and liver metabolism in vivo in rats. Clin Sci 85: 437–444

Higgins GM, Anderson RM (1931) Restoration of the liver of white rat following partial surgical removal. Arch Pathol 12: 186–202

Holtta E, Sinervirta R, Janne J (1973) Synthesis and accumulation of polyamines in rat liver regenerating after treatment with carbon tetrachloride. Biochem Biophys Res Comm 54: 350–357

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72: 101–163

Lorenzi M, De Martino A, Carlucci F, et al (1993) Nitrogen metabolism during liver regeneration. Biochim Biophys Acta 1157: 9–14

Luk GD (1986) Essential role of polyamine metabolism in hepatic regeneration. Inhibition of deoxyribonucleic acid and protein synthesis and tissue regeneration by difluoromethylornithine in the rat. Gastroenterology 90: 1261–1267

Margeli AP, Theocharis SE, Yannacou NN, Spiliopoulou C (1994) Metallothionein expression during liver regeneration after partial hepatectomy in cadmium-pretreated rats. Arch Toxicol 68: 637–642

Martinez-Mas J-V, Ruiz-Montasell B, Felipe A, Casado J, Pastor-Anglada M (1993) Up-regulation of system A activity in the regenerating rat liver. FEBS Lett 329: 189–193

Meijer AJ, Lamers WH, Chamuleau RAFM (1990) Nitrogen metabolism and ornithine cycle function. Physiol Rec 70: 701–748

Murawaki Y, Ikuta Y, Yamamoto H, Kawasaki H (1992) Serum amino acid levels and hepatic protein synthesis during liver regeneration after partial hepatectomy in rats. Res Comm Chem Pathol Pharmacol 77: 43–54

Nakata R, Tsukamoto I, Miyoshi M, Kojo S (1985) Liver regeneration after carbon tetrachloride intoxication in the rat. Biochem Pharmacol 34: 586–588

Ogawa M, Mori T, Mori Y, et al (1992) Study on chronic renal injuries induced by carbon tetrachloride: selective inhibition of the nephrotoxicity by irradiation. Nephron 60: 68–73

Ohno T, Sabra R, Branch RA (1991) Sodium retention and hepatic function after two-thirds hepatectomy in the rat. Hepatology 14: 511–517

Okano K, Tsubouchi T, Yamashita Y, et al (1997) Hepatic protein synthesis in the regenerating rat liver after hepatectopancreatectomy. Surg Today Jpn J Surg 27: 511–517

Pasantes-Morales H, Chatagner F, Mandel P (1980) Synthesis of taurine in rat liver and brain in vivo. Neurochem Res 5: 441–451

Perez-Barriocanal F, Perez-Antona JC, Regueiro P, Villanueva GR, Marin JJG (1990) Biliary lipid secretion during the prereplicative phase of rat liver regeneration. J Exp Path 71: 63–68

Ryoo HY, Taga M, Sassa T, Oka T, Natori Y (1997) Endocytosis of serum albumin in regenerating liver. Proc Soc Exp Med Biol 215: 179–185

Sainz GR, Monte MJ, Barbero ER, Herrera MC, Marin JJG (1997) Bile secretion by the rat liver during synchronized regeneration. Int J Exp Path 78: 109–116

Sanins SM, Nicholson JK, Elcombe C, Timbrell JA (1990) Hepatotoxin-induced hypertaurinuria: a proton NMR study. Arch Toxicol 64: 407–411

Sasaki Y, Hayashi N, Ito T, Fusamoto H, Sato N, Kamada T (1989) Heterogeneous activation of protein kinase C during rat liver regeneration induced by carbon tetrachloride administration. FEBS Lett 254: 59–65

Scornik OA (1974) In vivo rate of translation by ribosomes of normal and regenerating liver. J Biol Chem 249: 3876–3883

Sharma R, Kodavanti UP, Smith LL, Mehendale HM (1995) The uptake and metabolism of cytamine and taurine by isolated perfused rat and rabbit lungs. Int J Biochem Cell Biol 27: 655–664

Sturman JA (1980) Formation and accumulation of hypotaurine in rat liver after partial hepatectomy. Life Sci 26: 267–272

Sturman JA, Fellman JH (1982) Taurine metabolism in the rat: effect of partial hepatectomy. Int J Biochem 14: 1055–1060

Sturman JA, Fellman JH (1983) Methionine metabolism in the rat: accumulation of hypotaurine after partial hepatectomy. Progr Clin Biol Res 125: 435–447

Tamura J, Ohkuma S, Ida S, Zuo PP, Kuriyama K (1984) Cysteine uptake and taurine biosynthesis in freshly isolated and primary cultured rat hepatocytes. Cell Biochem Funct 2: 195–200

Teshigawara M, Matsumoto S, Tsuboi S, Ohmori S (1995) Changes in levels of glutathione and related compunds and activities of glutathione-related enzymes during rat liver regeneration. Res Exp Med 195: 55–60

Timbrell JA, Waterfield CJ (1996) Changes in taurine as an indicator of hepatic dysfunction and biochemical perturbations. Adv Exp Med Biol 403: 125–134

Timbrell JA, Waterfield CJ, Draper RP (1995) Use of urinary taurine and creatine as biomarkers of organ dysfunction and metabolic perturbations. Comp Haematol Int 5: 112–119

Tohyama C, Suzuki JS, Hemelraad J, Nishimura N, Nishimura H (1993) Induction of metallothionein and its localization in the nucleus of rat hepatocytes after partial hepatectomy. Hepatology 18: 1193–1201

Tsuboi S, Miyazaki M, Kondo Y, et al (1992) Increase of S-(1,2-dicarboxyethyl)glutathione in regenerating rat liver. Res Exp Med 192: 281–286

Tsujikawa K, Suzuki N, Sagawa K, et al (1994) Induction and subcellular localization of metallothionein in regenerating rat liver. Eur J Cell Biol 63: 240–246

Waterfield CJ, Turton JA, Scales MDC, Timbrell JA (1991) Taurine, a possible urinary marker of liver damage: a study of taurien excretion in carbon tetrachloride-treated rats. Arch Toxicol 65: 548–555

Waterfield CJ, Turton JA, Scales MDC, Timbrell JA (1993a) Investigations into the effects of various hepatotoxic compounds on urinary and liver taurine levels in rats. Arch Toxicol 67: 244–254

Waterfield CJ, Turton JA, Scales MD, Timbrell JA (1993b) Effect of various non-hepatotoxic compounds on urinary and liver taurine levels in rats. Arch Toxicol 67: 538–546

Waterfield CJ, Turton JA, Scales MDC, Timbrell JA (1993c) Reduction of liver taurine in rats by ß-alanine treatmment increases carbon tetrachloride toxicity. Toxicology 77: 7–20

Waterfield CJ, Asker DS, Timbrell JA (1996) Does urinary taurine reflect changes in protein metabolism? a study with cycloheximide in rats. Biomarkers 1: 107–114

Wollenberger A, Ristau O, Schoffa G (1960) Eine einfache Technik der extrem schnellen Abkühlung grösserer Gewebestücke. Pflügers Arch 270: 399–412

Wright CE, Tallan HH, Lin YY, Gaul GE (1986) Taurine – biological update. Annu Rev Biochem 55: 427–453

Zimmermann SW, Norback DH, Powers K (1983) Carbon tetrachloride nephrotoxicity in rats with reduced renal mass. Arch Pathol Lab Med 107: 264–269

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