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Age dependence of rat liver function measurements

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Summary

Changes in the galactose elimination capacity, the capacity of urea-N synthesis and antipyrine clearance were studied in male Wistar rats at the age of 8, 20 and 44 weeks. Further, liver tissue concentrations of microsomal cytochrome *P*-450, microsomal protein and glutathione were measured. All liver function measurements increased from the age of 8 to 44 weeks when expressed in absolute values. In relation to body weight, these function measurements were unchanged or reduced from week 8 to week 20. At week 44, galactose elimination capacity and capacity of urea-N synthesis related to body weight were increased by 10% and 36%, respectively, and antipyrine plasma clearance was reduced to 50%. Liver tissue concentrations of microsomal cytochrome *P*-450 and microsomal protein increased with age when expressed in absolute values, but were unchanged per g liver, i.e., closely related to liver weight in the age range studied. Glutathione showed an increase of 35% from 8 to 44 weeks of age expressed per g liver. Careful age matching of control animals is important for experimental rat studies.

Introduction

Quantitative liver function measurements in rats are often used in experimental hepatology, pharmacology and toxicology. It is of current interest to obtain information about possible changes in these values during the life span of the animals. Several studies concerning the liver weight/body weight ratio have been made, reporting either a decrease [1,2] or almost no change [3–5] with age, but to our knowledge, no reports have been published concerning possible changes of quantitative liver function measurements with age in rats.

The aim of this work was to study age dependence of some commonly used liver function measurements in male rats during the age span most often used, i.e.,

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from 2 to 10 months. The liver function measurements studied were the galactose elimination capacity as a measure of the cytosolic function, the capacity of urea-N synthesis as a mixed cytosolic and mitochondrial function and the antipyrine plasma clearance as a microsomal function. In addition, liver tissue concentrations of the important detoxifying tripeptide glutathione and of the microsomal drug-metabolizing enzyme cytochrome *P*-450 were measured, being of importance for the detoxification of a variety of reactive metabolites and xenobiotics, respectively.

Material and Methods

Experimental design

Twenty-eight male Wistar rats were kept under constant temperature and humidity, a 12 h controlled dark/light cycle and fed stock pellets and tap water ad libitum.

Quantitative liver function measurements, liver biopsies for estimation of cytochrome *P*-450 and protein in raw homogenates and 100 000 × *g* microsomal fraction, and total glutathione concentration in liver biopsies were carried out when the rats were 8, 20 and 44 weeks old. The experiments were performed between 9 a.m. and 1 p.m. Liver function measurements took about 1.5 h, all functions were estimated on all animals as was liver biopsy, with the exceptions identifiable in Table 1 from the number of measurements.

Liver function measurements

During penthothal anaesthesia (87.5 mg/kg body weight), thracheotomy and retroperitoneal nephrectomy was performed to avoid extrahepatic galactose and urea elimination during the procedure. Polyethylene catheters were placed in the left internal jugular vein for infusion and into the right common carotid artery for blood sampling [6].

Galactose elimination capacity (GEC). Galactose (Kabi, Sweden) was given as an injection of 0.15–0.25 ml of a 50% solution, followed by an infusion of 1.2 ml/h of a 2% solution.

Blood samples (100 μl) were taken in duplicate after 20 min of equilibration at intervals of 10 min for determination of galactose concentration by the galactose dehydrogenase method [7]. The galactose elimination capacity was calculated as

$$GEC = I - (dc/dt \cdot 0.40\ b.w.),$$

where I is the galactose infusion rate, dc/dt is the linear slope of galactose blood concentration-time curve, b.w. is the body weight (0.40 b.w. is the volume of distribution for galactose [8]).

Capacity of urea-N synthesis (CUNS). Alanine was administered as an intravenous injection of 0.6–1.8 ml of a 10% w/v solution in water, followed by a constant infusion for 70 min of 2–9 ml/h of a 2% solution to a steady state amino acid concentration between 7.3 and 11.6 mmol/l [9] adjusted from rapid analysis of total α-amino-N. Blood samples (150 μl) were taken after an equilibration period of 20 min at intervals of 10 min for determination of urea and total α-amino-N.

Total blood α-amino-N concentration was measured by the dinitrofluorobenzene method [6], blood urea concentration was determined by the urease-Berthelot method [10].

The capacity of urea-N synthesis was calculated as

$$CUNS = dc/dt \cdot 0.63\ b.w. \cdot 1.25$$

where dc/dt is the slope of the linear regression of arterial blood urea concentration on time during α-amino-N steady-state (defined as less than 10% change during a period of 50 min or longer), 0.63 b.w. is the volume of distribution for urea [11], and 1.25 is a correction for intestinal urea hydrolysis [12].

Antipyrine plasma clearance (APC). Antipyrine (2 mg/100 g b.w.) was given intragastrically 4–6 h before the start of the GEC and CUNS determination.

A blood sample of 1 ml was taken as soon as the arterial catheter was in place, i.e., before the start of the infusions described above.

The antipyrine concentration in plasma was measured by high-pressure liquid chromatography [13]. The antipyrine plasma clearance was calculated ac-

cording to the one-sample method as

$$APC = 0.66\,b.w.\cdot(\ln(D/0.66\,b.w.) - \ln(c_t))/t$$

where D is the antipyrine dose, 0.66 b.w. is the antipyrine apparent volume of distribution [13] and c_t is the concentration at the sampling time t [13].

Liver biopsy measurements

Immediately following the above procedures, the animals were exsanguinated by puncture of the aorta, the liver was quickly excised, blotted on filter paper, sliced with scissors in isotonic KCl and homogenized in a Potter–Elvehjelm homogenizer with 10 ml of isotonic chilled KCl. The liver homogenate was assayed for cytochrome *P*-450 [14], total glutathione concentration [15], and protein [16]. Microsomes [17] were assayed for protein [16], and cytochrome *P*-450 [14] was assayed by the methods indicated. There was no difference in the recovery of protein and cytochrome *P*-450 in homogenate vs. microsomes for any of the age groups and as a consequence, only microsomal data are given.

Due to the abundant biological sampling and the demand for immediate analysis on liver biopsies, analysis was maximally performed on six animals; due to technical difficulties, urea synthesis of only five animals was estimated at the age of 44 weeks. In addition, a few technical failures occurred, identifiable in Table 1 where the initial number of animals can be read in the body weight row.

Statistical analysis

The three age groups were tested with a one-way analysis of variance using Bartel's test for variance homogeneity by conventional parametric methods, P values less than 0.05 were considered statistically significant.

Results

Table 1 shows means and S.E. of galactose elimination capacity, capacity of urea-N synthesis, antipyrine clearance and liver biopsy measurements in rats of 8, 20 and 44 weeks. Body weight increased twofold during the first 12 weeks (i.e., age 8 to 20 weeks) with a further increase of only 20% during the following 24 weeks. The increase in liver weight was also slower from 20 to 44 weeks of age, bringing about a reduction in the relative liver weight from 4 to 2.7% of body weight (Fig. 1a).

There was an almost linear rise in galactose elimination capacity (Fig. 1b) and capacity of urea-N synthesis (Fig. 1c), which at week 44 was 300 and 400%, respectively, of the initial value, i.e., at week 8, whereas antipyrine plasma clearance (Fig. 1d) only increased 50% throughout the entire period.

TABLE 1

AGE DEPENDENCE OF GALACTOSE ELIMINATION CAPACITY (GEC), CAPACITY OF UREA-N SYNTHESIS (CUNS), ANTIPYRINE CLEARANCE (APC) AND LIVER BIOPSY MEASUREMENTS IN MALE RATS

Values are given as mean ± S.E., the number of animals are given in brackets.

	Rat age		
	8 weeks	20 weeks	44 weeks
Body weight (g)	200 ± 5.22 (6)	448 ± 10.8 (10)	542 ± 11.0 (12)
Liver weight (g)	8.03 ± 0.512 (6)	13.4 ± 0.590 (10)	14.6 ± 0.464 (12)
Quantitative liver function			
GEC (μmol/min)	2.33 ± 0.231 (5)	4.37 ± 0.356 (9)	6.83 ± 0.318 (10)
CUNS (μmol/min)	14.3 ± 1.88 (5)	31.4 ± 2.60 (8)	55.1 ± 5.41 (5)
APC (ml/min)	1.28 ± 0.112 (5)	1.58 ± 0.073 (6)	1.90 ± 0.102 (10)
Liver biopsy measurements			
Microsomal protein (mg)	335 ± 21.8 (5)	581 ± 46.5 (5)	596 ± 26.8 (6)
Microsomal cyt. *P*-450 (nmol)	234 ± 6.19 (5)	434 ± 30.2 (5)	411 ± 30.0 (6)
Glutathione (μmol)	36.5 ± 16.3 (5)	64.8 ± 7.21 (6)	85.1 ± 6.13 (6)

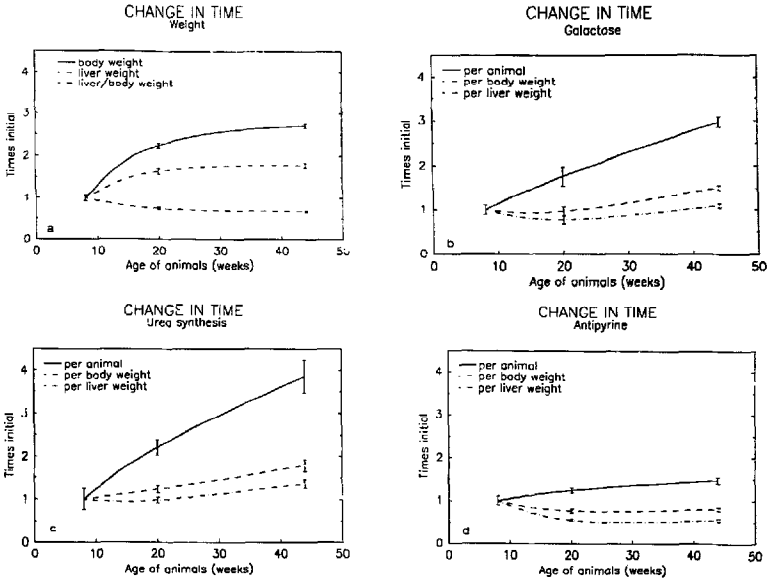

Fig. 1. Increase in liver weight (g), body weight (g) and liver weight/body weight ratio related to age in male rats (panel a). Panels b, c and d depict the age-related changes in the galactose elimination capacity (μmol/min), the capacity of urea-N synthesis (μmol/min) and the antipyrine clearance (ml/min), respectively. All changes shown in the figures are statistically significant ($P < 0.05$) from the age of 8 to 44 weeks.

In relation to body weight, these function measurements were unchanged or reduced from week 8 to week 20. At week 44, galactose elimination and the capacity of urea-N synthesis relative to body weight were increased by 10 and 36%, respectively, and antipyrine plasma clearance was reduced to about 50%. The same pattern was found when related to liver weight, only the level was higher because of the smaller increase in liver weight.

In the liver biopsies, microsomal protein, microsomal cytochrome *P*-450 and glutathione were found in almost identical concentrations at all ages, i.e., the amount is closely related to liver weight, with a 35% glutathione increase as the only exception (Table 1).

TABLE 2

GALACTOSE ELIMINATION CAPACITY (GEC), CAPACITY OF UREA-N SYNTHESIS (CUNS) AND ANTIPYRINE CLEARANCE (APC) EXPRESSED IN TERMS GIVING THE LOWEST *F*-RATIO IN ANALYSIS OF VARIANCE

Values are given as mean ± S.D.

	Rat age		
	8 weeks	20 weeks	44 weeks
GEC (μmol/min/100 g b.w.)	1.18±0.244	0.963±0.194	1.25±0.207
CUNS (μmol/min/100 g b.w.)	7.21±2.03	7.08±1.25	9.79±1.65
APC (ml/min)	1.28±0.251	1.58±0.178	1.90±0.323

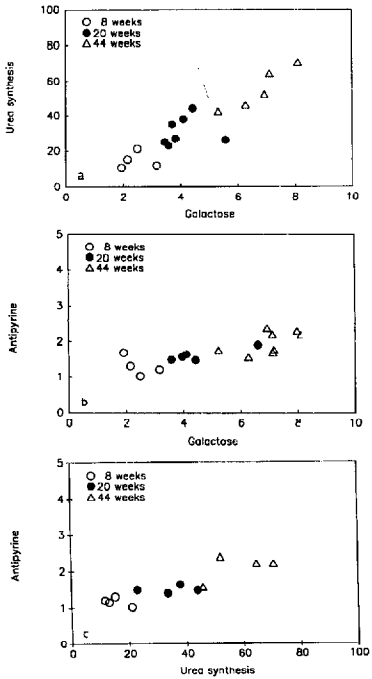

Fig. 2. The relation between the galactose elimination capacity (μmol/min), the capacity of urea-N synthesis (μmol/min) and the antipyrine clearance (ml/min) in male rats at 8, 20 and 44 weeks of age. Only experiments where both the respective functions were estimated in the same animal are included in this figure, therefore the number of animals does not correspond to Table 1.

All function measurements (galactose elimination capacity, capacity of urea-N synthesis and antipyrine plasma clearance) showed a significant dependence on age when expressed as absolute values as well as related to body weight or liver weight (*F*-ratio by one-way analysis of variance from 33.5 (galactose elimination capacity) to 4.2 (antipyrine plasma clearance per liver weight)). The lowest *F*-ratios for galactose elimination capacity and capacity of urea-N synthesis were found when related to body weight (6.5 and 5.0, respectively) and for antipyrine clearance when not related to body or liver weight (8.7) (Table 2).

Fig. 2a–c shows the relations between the three quantitative liver function measurements.

Discussion

In this study we found age-dependent changes related to liver function in rats in the time span from adolescence to adult age. All liver function measurements increased approximately linearly when expressed in absolute values contrasting the non-linear increase in body and liver weight. Expressed relative to body or liver weight, both the galactose elimination capacity and the capacity of urea-N synthesis increased from week 8 to week 44, whereas antipyrine plasma clearance decreased. The hepatocellular concentrations of microsomal protein and microsomal cytochrome *P*-450 were closely related to liver weight, whereas glutathione increased with age when expressed per g liver.

The decrease with age in liver weight/body weight ratio in this study (Fig. 1a) is similar to that seen in other studies [1,2,18,19], although it varies with sex and rat strain [18]. Kato and Takanaka [1] found in male Wistar rats, as in the present study, a decrease in the ratio from 4.1 to 3.0 g/100 g in the age range of 3–20 months, which is about the same as we found (4.0 to 3.0 g/100 g) from 2 to 4.5 months. In the period of 4.5–10 months we only found a minor decrease (3.0 to 2.7 g/100 g). This indicates that changes in the liver weight/body weight ratio with age mainly takes place during the first 5 months of life in rats (male, Wistar).

The galactose elimination capacity and the capacity of urea-N synthesis increase during life when expressed per 100 g body weight. The galactose elimination may be taken to reflect cytosolic liver function [8], and urea synthesis to represent partly mitochon-

drial and partly cytosolic function [9]. The results may accordingly be interpretated to indicate that there may be an increase in the 'requirement' of the animal's metabolism for the hepatic metabolic capacity for cytosolic functions relative to weight with increasing age. Other cytosolic functions may also change during life as reported from Rikans and Moore [20], who found an increasing activity in cytosolic enzyme alcohol dehydrogenase from the age of 4 to 25 months in male Fischer-344 rats.

The procedure used in the present study of measuring galactose elimination capacity and urea synthesis capacity in the same measurement period could introduce bias in either of the two measurements. It has been shown, however, that there is no measurable interaction with the procedure being confined to alanine concentrations between 7.3 and 11.6 mmol/l [9] and galactose concentrations higher than 2 mmol/l [8] (Hansen; unpublished data).

In contrast to galactose elimination and urea synthesis, the clearance of antipyrine, relative to body weight, interpretated as a measure of some of the liver microsomal functions, decreased with age, indicating a lower 'requirement' for these functions during life span. Interestingly, there was a continued decrease, when related to body weight, from week 20 to week 44 in microsomal cytochrome *P*-450 (from 95 to 75 nmol/100 g b.w.) and protein (from 127 to 108 mg/100 g b.w.) with no concomitant decrease of the antipyrine clearance (Table 2), suggestive of a differential metabolization by the different cytochrome *P*-450 isoenzymes with age.

All liver function measurements showed significant changes during the life span. The lowest *F*-ratios of the one-way analysis of variance in relation to age were found for the galactose elimination capacity and the capacity of urea-N synthesis when related to body weight; for antipyrine plasma clearance the lowest *F*-ratio was found when expressed in absolute terms. This means that in studies where the effect of an experimental intervention on some of these liver function measurements is studied, the inevitable effect of age is minimized by expressing the galactose elimination capacity and the capacity of urea-N synthesis relative to body weight and antipyrine plasma clearance in absolute terms. As the liver weight will not be available in clinical studies, the *F*-ratios related to this have not been taken into consideration here.

In an earlier study [9], we found a close linear relationship between each of the three quantitative functions, without any intercept, when rats of identical ages underwent various degrees of hepatectomy. In the present study, where liver weight varied by age, a positive intercept of regression of the antipyrine plasma clearance on the galactose elimination capacity or on the capacity of urea-N synthesis appeared. The reason for the difference is not clear from the present data, and an intercept was not found in a human study where the age span was limited [21].

Cytochrome *P*-450 has been reported to decrease with age [1,3,22,23]. It is important here, however, to note that the values may be expressed in different ways, e.g., as microsomal cytochrome *P*-450/mg protein [3,22], showing a decrease with age, or as in the present study in absolute values showing an increase from 234 to 411 nmol (Table 1), as cytochrome *P*-450 per g liver remaining constant at approx. 30 nmol/g liver (data from Table 1), or as cytochrome *P*-450 per 100 g body weight showing a decrease from 112 to 75 nmol/100 g body weight (data from Table 1). Also there is an influence of the age range studied on the results. In the present study there was no change in the cytochrome *P*-450 content per g liver from 2 to 10 months, which is consistent with the results from Kitahara et al. [23], who found no change at 12 months of age, but a reduction to about half from 12 to 24 months.

Glutathione plays an important role in the hepatic detoxification of nucleophilic metabolites, e.g., generated by cytochrome *P*-450 metabolism. In contrast to microsomal protein and cytochrome *P*-450, its concentration increased with age when expressed per animal and per g liver, whereas no changes were observed per 100 g body weight. Other studies report either no changes or changes in either direction [17,23,24], for which we have no possible explanation.

In man, quantitative liver function in the elderly (70–90 years of age) shows a reduction to 78–81% in the galactose elimination capacity related to body

weight [25,26] and a reduction to 65–78% in microsomal liver function [25]. This suggests, concerning the latter, an influence of age in man similar to that reported for rats in the present study. In contrast, the galactose elimination capacity decreases with age in man, whereas in rats we found a slight increase with age. This could, however, be explained by the difference in the age range studied.

The study demonstrates age-related changes in the liver functions measured and in liver glutathione concentrations. The influence of age is less pronounced when the galactose elimination capacity and the capacity of urea-N synthesis are expressed relative to body weight, and for antipyrine plasma clearance when expressed in absolute terms. In any case, however, this study stresses the importance of carefully age-matched control animals in experimental rat liver studies.

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References

1 Kato R, Takanaka A. Metabolism of drugs in old rats (1). Activities of NADPH-linked electron transport and drug-metabolizing enzyme systems in liver microsomes of old rats. Jpn J Pharmacol 1968; 18: 381–388.
2 Kitani K, Zurcher C, Van Bezooijen CFA. The effect of aging on the hepatic metabolism of sulfobromophthalein in BN/Bi rats and WAG/Rij male and female rats. Mech Ageing Dev 1981; 17: 381–393.
3 McMartin DN, O'Connor JA, Fasco MJ, Kaminsky LS. Influence of aging and induction on rat liver and kidney microsomal mixed function oxidase systems. Toxicol Appl Pharmacol 1980; 54: 411–419.
4 Van Bezooijen CFA, Knook DL. A comparison of age-related changes in bromosulfophthalein metabolism of the liver and isolated hepatocytes. In: Kitani K, ed. Liver and Aging – 1978. Amsterdam: Elsevier/North-Holland Biomedical Press, 1979; 131–141.
5 Schmucker DL, Wang RK, Kwong P. Age-dependent alterations in rat liver microsomal NADPH cytochrome *c* (*P*-450) reductase. In: Kitani K, ed. Liver and Aging – 1982, Liver and Drugs. Amsterdam: Elsevier Biomedical Press, 1982; 75–96.
6 Goodwin JF. Spectrophotometric quantitation of plasma and urinary amino nitrogen with fluorodinitrobenzene. Stand Methods Clin Chem 1970; 6: 89–98.
7 Kurz G, Wallenfals K. D-Galactose. UV-test mit galactose-dehydrogenase. In: Bergmeyer HU, ed. Methoden der Enzymatischen Analyse. Weinheim/Bergstr: Verlag Chemie, 1970; 1241–1244.
8 Keiding S. Galactose elimination capacity in the rat. Scand J CLin Lab Invest 1973; 31: 319–325.
9 Hansen BA, Poulsen HE. The capacity of urea-N synthesis as a quantitative measure of the liver mass in rats. J Hepatol 1986; 2: 468–474.
10 Fawcett JK, Scott JE. A rapid and precise method for determination of urea. J Clin Pharmacol 1960; 13: 156–159.
11 Foy J, Schneiden H. Estimation of total body water (virtual tritium space) in the rat, cat, rabbit, guinea pig and man, and biological halflife of tritium in man. J Physiol 1960; 154: 169–176.
12 Hansen BA, Vilstrup H. A method for determination of the capacity of urea synthesis in the rat. Scand J Clin Lab Invest 1985; 45: 346–350.
13 Pilsgaard H, Poulsen HE. A one-sample method for antipyrine clearance determination in rats. Pharmacology 1984; 29: 110–116.
14 Omura T, Sato R. The carbon monoxide binding of liver microsomes. J Chem Biol 1964; 239: 2570–2578.
15 Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Analyt Biochem 1969; 27: 502–522.
16 Groves WE, Davis FC Jr, Sells BH. Spectrophotometric determination of microgram quantities of protein without nucleic acid interference. Analyt Biochem 1968; 22: 195–210.
17 Andreasen PB, Bremmelgård A, Larsen BK. Interaction between antipyrine and the microsomal oxidation in rat liver. Pharmacology 1974; 12: 244–250.
18 Van Bezooijen CFA. Influence of age-related changes rodent liver morphology and physiology on drug metabolism – a review. Mech Ageing Dev 1984; 25: 1–22.
19 Kato R, Takanaka A. Effect of phenobarbital on electron transport system, oxidation and reduction of drugs in liver microsomes of rats of different age. J Biochem 1968; 63: 406–408.
20 Rikans LE, Moore DR. Effect of age and sex on allyl hepatotoxicity in rats: role of liver alcohol and aldehyde dehydrogenase activities. J Pharmacol Exp Ther 1987; 243: 20–26.
21 Andreasen PB, Ranek L, Statland BE, Tygstrup N. Clearance of antipyrine – dependent on quantitative liver function. Eur J Clin Invest 1974; 4: 129–134.
22 Stier A, Finch SAE, Greinert R, Hohne M, Muller R. Membrane structure and function of the hepatic microso-

mal cytochrome P-450 system. In: Kitani K, ed. Liver and Aging – 1982, Liver and Drugs. Amsterdam: Elsevier Biomedical Press, 1982; 3–14.
23 Kitahara A, Ebina T, Ishikawa T, Yasushi S, Kiyomi S, Kanai S. Changes in activities and molecular forms of rat hepatic drug metabolizing enzymes during aging. In Kitani K, ed. Liver and Aging – 1982, Liver and Drugs. Amsterdam: Elsevier Biomedical Press, 1982; 135–142.
24 Birnbaum LS, Baird MB. Senescent changes in rodent hepatic epoxide metabolism. Chem Biol Interact 1979; 26: 245–256.
25 Schnegg M, Lauterburg BH. Quantitative liver function in the elderly assessed by galactose elimination capacity, aminopyrine demethylation and caffeine clearance. J Hepatol 1986; 3: 164–171.
26 Marchesini G, Bua V, Brunori A, et al. Galactose elimination capacity and liver volume in aging man. Hepatology 1988; 8: 1079–1083.