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INFLUENCE OF AGE-RELATED CHANGES IN RODENT LIVER MORPHOLOGY AND PHYSIOLOGY ON DRUG METABOLISM – A REVIEW

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SUMMARY

Age-related changes in weight, morphology and physiology of the rodent liver influencing hepatic drug metabolism are reviewed. Next to the changes in liver weight/body weight ratio with age, the spontaneous occurrence of neoplastic and non-neoplastic lesions may be of particular importance. In addition, the decrease in liver blood blow with age diminishes the biotransformation capacity of the total liver. However, the albumin concentration in plasma and drug uptake do not play important roles, since they are unchanged or only slightly lower in old rats or mice.

Drugs are generally metabolized by the liver in two phases: the so-called phase I and phase II metabolism. For most drugs, the phase I reaction is an oxidation. This reaction is catalyzed by cytochrome P-450, cytochrome b_5 and NADPH-cytochrome *c* reductase. In microsomes, a decrease with age is generally observed in the cytochrome P-450 concentration and the NADPH-cytochrome *c* reductase activity, while there is no change in cytochrome b_5. In addition, most microsomal drug-metabolizing enzymes decrease with age in male rats but not in females. The changes in enzyme activities in the male and female mouse are more complex. In fact, increases, decreases and no changes were found. Important phase II reactions are glutathione conjugation and glucuronidation; changes in both reactions with age seem to be of minor importance.

Studies with hepatocytes isolated from male rats of different ages reveal that the monooxygenase system mediated metabolism of digitoxin and aflatoxin B1 decreases with age.

It can be concluded that the observed decrease in the functional capacity of the monooxygenase system greatly determines the decrease in drug metabolism with age. However, it should always be kept in mind that, among others, the age-related changes in drug metabolism in rats are strongly sex dependent, which is not the case in man. Therefore, caution should be exercised in transferring these data to the human situation.

Key words: Age; Drug metabolism; Liver; Rodents

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1. INTRODUCTION

Individual variation in response to drugs with important consequences for drug therapy is a hallmark of human pharmacology. This interindividual variation in drug response might be due to great differences in drug elimination depending on both the drug and the heterogeneity within the population selected for investigation.

A clearer understanding of the causes of great differences in the rates of drug elimination is necessary. Over the past several years, many such causes have been studied in laboratory animals. Based on the results of these animal studies, possible causes can be subdivided into external environmental, internal environmental and pharmacological ones [1]. External environmental variables include diet, smoking, alcohol intake, *etc.* Among the pharmacological variables are, for example, the route of drug elimination and drug interactions. To the internal environmental variables belong, among others, genetic constitution, disease, sex, liver function and age. This review will deal with some of the internal environmental causes – namely, liver morphology and physiology in relation to aging.

Literature data on the effect of age on rat and mouse liver morphology and physiology relevant to *in vitro* drug metabolism will be reviewed to indicate the significance of these age-related changes in *in vivo* studies on drug metabolism with age.

2. AGE-RELATED CHANGES IN THE LIVER WEIGHT/BODY WEIGHT RATIO AND LIVER MORPHOLOGY

2.1 Liver weight/body weight ratio

Changes in liver weight with age play an important role in the capacity of the liver to metabolize drugs. Since the dosage of a drug is mostly based on the body weight, the liver weight/body weight ratio is most relevant. The liver weight/body weight ratio ranges from 2.2% to 4.4% depending on the strain, sex and age of the rats used. Changes in the overall liver weight/body weight ratio with age are shown in Table I.

On the basis of these observations, it can be concluded that age-related changes in the rat liver weight/body weight ratio are strain and sex dependent. Therefore, when studies are undertaken to investigate the influence of age on the elimination of drugs by the rat liver *in vivo,* the changes in liver weight/body weight ratio with age should be determined for the rat strain and sex employed, since these changes will greatly affect the drug disposition.

2.2 Ploidy status

A pronounced age-related change in liver morphology is the shift of the hepatocytes to a higher ploidy. This increase in the polyploidy of rat hepatocytes with age is strain dependent. Although the pattern of the shift is mostly the same – viz. a shift from mononuclear diploid via binuclear diploid to mononuclear tetraploid and via binuclear tetraploid to mononuclear octaploid – the times of the beginning and end of the ploidy

TABLE I

CHANGES[a] IN LIVER WEIGHT/BODY WEIGHT RATIO WITH AGE

Species	*Strain*	*Sex*	*Age (months)*	*Liver weight/ body weight ratio (%)*	*Reference*
Rat	Wistar	Female	3 vs 20	4.2 → 3.0	2
Rat	Wistar	Male	3 vs 20	4.1 → 3.0	2
Rat	Wistar	Male	3 vs 24	3.9 → 3.5	3
Rat	Wistar	Male	3 vs 30	↓	4
Rat	Wistar	Male	7 vs 31	2.7 → 2.6	5
Rat	RU	Female	3 vs 27	3.1 → 3.2	6
Rat	WAG/Rij	Female	3 vs 36	4.3 → 4.4	7
Rat	WAG/Rij	Female	3 vs 30	3.3 → 2.7	8
Rat	WAG/Rij	Male	3 vs 30	3.2 → 2.4	8
Rat	BN/Bi	Female	3 vs 12	2.7 → 2.2	9
Rat	BN/Bi	Female	3 vs 24	2.7 → 2.7	9
Rat	Fischer 344	Male	1 vs 27	2.9 → 2.7	10
Rat	Sprague-Dawley	Male	2 vs 16	4.5 → 3.4	11
Mouse	NIH White Swiss	Male	–	6.6 → 6.2	12
Mouse	NIH White Swiss	Female	–	6.4 → 7.2	12

[a] ↓ = decrease with age.

changes differ from strain to strain [13]. In general, the most pronounced changes in ploidy state occur within the first year of life. Thereafter, only relatively small changes occur. It can be generally stated that changes in the ploidy state of the hepatocytes in mice are more spectacular. They continue to the age of at least 28 months and ploidy values of 16 and 32 N are quite common [14].

The question can be raised of whether changes in the metabolism of drugs by the liver *in vivo* are at least partly attributable to age-related changes in the ploidy state of the hepatocytes. Assuming that a mononuclear diploid cell has half the volume of a binuclear diploid or a mononuclear tetraploid cell (which has half the volume of a binuclear tetraploid or a mononuclear octaploid cells), the number of hepatocytes per unit liver volume will also decrease with age. Since drugs are transported across the hepatocyte membrane and the sinusoidal membrane area involved in drug uptake may be relatively decreased, the rate of uptake may be influenced by the observed increase in polyploidy of the hepatocytes. In section 3.4.2, evidence will be presented that the decrease in the hepatic uptake with age does not play an important role.

2.3 Smooth endoplasmic reticulum

The smooth endoplasmic reticulum (SER) is the intracellular structure directly responsible for the hepatic capacity to metabolize drugs. Data on changes in the amount of SER with age in rats are quite conflicting. Pieri *et al.* [15] observed an increase in the cytoplasmic volume occupied by the SER at between 12 and 27 months. However, Schmucker

et al. [16] found a decrease in the amount of SER at between 10 and 30 months. These discrepancies might be due to differences in the methods and the strain and sex of the rats employed by the two groups. Pieri *et al.* used the immersion fixation method and female Wistar rats. Schmucker *et al.* used the preferable perfusion fixation method and male Fischer 344 rats. A study conducted by Meihuizen and Blansjaar [17] using the perfusion fixation method and female WAG/Rij rats revealed an increase in the cytoplasmic volume occupied by the SER at between 3 and 35 months of age. Pieri *et al.* [18] also studied age-related changes in the SER in mouse liver: no significant changes at between 12 and 24 months of age were observed. These data indicate that changes in the SER with age may be species, strain and sex dependent. Therefore, it is difficult to generalize about the influence of changes in the SER with age on the capacity of the liver to metabolize drugs.

TABLE II

COMMON SPONTANEOUS NON-NEOPLASTIC AGE-RELATED LIVER LESIONS IN VARIOUS RAT STRAINS

Only those lesions occurring with a prevalence of 20% or more are included. "Frequency in %" means the percentage of rats in which the lesions have been observed.

Lesion	*Strain*	*Sex*	*Age (months)*	*Frequency (%)*	*Reference*
Foci or areas of cellular alteration	WAG/Rij	Male	24[b]	36	19
Foci or areas of cellular alteration	WAG/Rij	Female	32[b]	84	19
Foci or areas of cellular alteration	Fischer	Female	24[a]	23	20
Altered cell foci	Wistar	Male	31–36[a]	84	5
Bile duct hyperplasia	Fischer	Male	>25[a]	98	21
Bile duct cysts	BN/Bi	Male	32[b]	26	19
Bile duct cysts	BN/Bi	Female	33[b]	55	19
Dilated common bile ducts	WAG/Rij	Male	30[b]	>20	8
Hepatic telangiectasis	WI	Female	18[a]	50	22
Hepatic telangiectasis	WI	Male	18[a]	60	22
Fatty change	Sprague-Dawley	Male		26	23
Eosinophilic foci	Sprague-Dawley	Male		21	23
Focal chronic hepatitis	Fischer	Male	32[a]	47	21
Periportal inflammation	BN	Male	13–36[a]	28	24
Periportal inflammation	BN	Female	13–36[a]	38	24
Periportal inflammation	BN	Male	24[a]	24	20
Periportal inflammation	Sprague-Dawley	Male		68	23
Sinusoidal dilation	Sprague-Dawley	Male		25	23

[a]Age at interim kill.
[b]Mean age when killed moribund.

TABLE III

COMMON SPONTANEOUS NEOPLASTIC AND NON-NEOPLASTIC AGE-RELATED LIVER LESIONS IN VARIOUS MOUSE STRAINS

Lesion	*Strain*	*Sex*	*Age (months)*	*Frequency (%)*	*Reference*
Hepatomas	C3HeB/De	Female	24[a]	58	25
Hepatomas	CBA/J	Male		65	26
Hepatomas	CF-1	Male	30[a]	34	27
Hepatomas	C3Hf	Male	14[a]	31	28
Hepatomas	CBA	Male	30[a]	100	29
Hepatomas	CF-1	Male	28[a]	27	30
Hepatomas	SWJ/Jac	Male	21[a]	63	31
Liver cell neoplasms	C3H-A^{vy}fB	Male	15[a]	83	32
Hepatocellular neoplasm type A	CBA	Male	28[a]	23	33
Hepatic neoplasm (basophilic)	B6C3F1	Male	18[a]	25	34
Amyloidosis	C57Bl/Ka	Male	23[b]	83	33
Amyloidosis	C57Bl/Ka	Female	20[b]	73	33
Ischemic liver cell necrosis	NZB	Male	17[b]	26	33
Ischemic liver cell necrosis	NZB	Female	14[b]	33	33

[a,b] See Table II.

2.4. Histopathology

In addition to general aging changes in liver morphology, specific histopathological changes occur in the livers of old rats. Spontaneous lesions observed in the liver of various rat strains are shown in Table II. Such liver lesions occurring in mice are shown in Table III. Only those lesions found with a prevalence of 20% or more are included.

It is clear from the histopathological data presented in these tables that rat and mouse strains differ considerably in the type and frequency of liver lesions observed. These lesions varying with age will certainly influence the capacity of the liver to metabolize drugs. An attempt was recently made to relate certain spontaneously occurring age-related pathological changes in the liver in female and male WAG/Rij and BN/BiRij rats to specific functional disturbances. By determining the bromsulfophthalein (BSP) retention and serum glutamic-pyruvic transaminase (SGPT) and glutamic-oxalacetic transaminase (SGOT) levels, it was found that no such relationship could be demonstrated [35]. The assumption that some rat strains such as, for example, WAG/Rij rats may be less suitable subjects for liver aging research because of their high incidence of foci and areas of cellular change apparently does not hold true for studies on age-related changes in BSP retention and SGOT and SGPT serum levels. However, these two histopathological changes in the liver might be relevant in other specific tests for liver function. Therefore, baseline data on pathological lesions should be collected and taken into account when

studying the changes in the capacity of the liver to metabolize drugs with age in any specific strain of mouse or rat.

3. AGE-RELATED CHANGES IN LIVER PHYSIOLOGY

3.1. Possible contribution of non-parenchymal liver cells to drug metabolism

The mammalian liver consists of various cell types which can be divided into two large groups: parenchymal cells (hepatocytes) and non-parenchymal cells. The latter population consists mainly of endothelial, Kupffer and fat-storing cells and these types can be considered as major components of the reticuloendothelial system. The functional capacity of the reticuloendothelial system as determined by the clearance rate of particulate material from the bloodstream was reported to decline with age in both mice [36,37] and rats [38]. However, most of these studies did not include truly senescent animals and, therefore, little is known about the clearance function of the reticuloendothelial system in senescent animals [39]. Moreover, other functions of the reticuloendothelial liver cells were not studied during aging [39].

Only recently were aging studies on the functional capacity of purified Kupffer cells in maintenance culture performed at our Institute. Preliminary results indicate that the capacity of these cells to adsorptively endocytose heat-aggregated colloidal albumin declines with age, while the intracellular degradation of the substrate does not appear to be affected by the age of the donor rat [39]. Biochemical studies focused mainly on lysosomal enzymes indicated no general age-related decline in enzyme activities in Kupffer and endothelial cells [40,41].

Only a few reports in the literature indicate a direct role of sinusoidal liver cells in hepatic drug metabolism [42]. *N*-Acetyltransferase, an enzyme which catalyses the acetylation of certain drugs such as isoniazid, serotonin and sulphametazine, was shown to be almost exclusively localized in the sinusoidal cells of the rat liver [43]. Gamma-glutamyl transferase (γGT) is another enzyme implicated in the metabolism of certain drugs. This enzyme was detected in sinusoidal liver cells with an activity twice as high as found in the parenchymal cells (44). An age-related increase in this enzyme was observed (see Table X).

In summary, little is known about the functional changes in sinusoidal liver cells in relation to drug metabolism during aging and their possible contribution to changes in the biotransformation capacity of the whole liver is difficult to estimate.

3.2 Metabolic activities

Functions performed by hepatocytes can be classified into three main categories: metabolic activities, excretory activities (see section 3.3) and biotransformation (see section 3.4).

The liver plays an important role in carbohydrate, protein and lipid metabolism. Changes with age in these functions of the hepatocytes were reviewed earlier: carbohydrate metabolism by Sanadi [45] and Hansford [46], and protein metabolism by Van

Bezooijen [13], Richardson [47] and Rothstein [48]. Changes in lipid metabolism were described in Session 5 of the volume *Liver and Aging – 1978* [49]. Specific age-related changes in the functions mentioned above in relation to drug metabolism are reviewed below.

3.2.1 Protein and albumin metabolism. Changes in serum protein binding capacity for drugs may influence drug biotransformation (see section 3.4.1). These changes may be due to changes in serum protein concentrations which are the results of protein synthesis and elimination. Recent studies on age-related changes in protein synthesis in isolated rat hepatocytes reveal that the changes are strain and sex dependent. A decrease for female WAG/Rij [50], female Sprague-Dawley [51] and male Fischer rats [52] was observed during the first year of life. However, no change in protein synthesis was found for male Sprague-Dawley [53], male WAG/Rij and female BN/Bi rats at between 3 and 12 months of age [54]. At an age between 12 and 18–24 months, no change in protein synthesis by isolated hepatocytes was observed for male and female WAG/Rij, female BN/Bi [50,54], female Sprague-Dawley [51] or male Fischer rats [52]. However, in male Sprague-Dawley rats, a decrease in protein synthesis was found at these ages [53]. In advanced age, a sharp increase in the protein synthesizing capacity was observed for female WAG/Rij [50], male Fischer [52] and male WAG/Rij and female BN/Bi rats [54]. These results make it likely that the sharp increase in protein synthesis in hepatocytes isolated from old rats is independent of strain and sex.

Albumin is important as a transport protein for, among others, anions, fatty acids and drugs. The influence of age on the capacity of isolated hepatocytes to synthesize albumin was studied in female WAG/Rij rats [55,56]. A decrease in albumin synthesis was observed at between 3 and 24 months of age; this was followed by a sharp increase in advanced age.

This increase in advanced age may be in agreement with *in vitro* studies with liver microsomes isolated from male Fischer rats, which showed an increase in albumin synthesis between 15- and 24-month-old rats [57]. *In vivo* studies on age-related changes in albumin synthesis were performed by Beauchene *et al.* [58], Salatka *et al.* [59] and Ove *et al.* [60]. They all found an increase in albumin synthesis with age among the age groups investigated: 11- and 28-month-old male and female Wistar rats [58], 15- and 20-month-old Fischer rats [59] and 1- and 20-month-old female Fischer rats [60]. Recent studies [61] on albumin elimination in 3-, 12-, 24- and 36-month-old female WAG/Rij rats revealed no change in elimination half-life with age. However, as there was an increase in the whole body albumin pool, an concomitant increase in albumin clearance was observed at between 12 and 36 months of age. The age-related changes in albumin clearance were thought not to be caused by changes in albumin excretion via the urine or via the gastrointestinal tract [61].

The observed increase in albumin and protein synthesis in advanced age are thought to be a compensation by the liver for the observed increased elimination of albumin due to, for example, increased proteolytic activity, increased fluid phase endocytotic clearance or the occurrence of altered, malfunctional proteins, among which is albumin. This

TABLE IV

CHANGES[a] IN LIVER MICROSOMAL PHOSPHOLIPID AND CHOLESTEROL COMPOSITION WITH AGE IN MALE RATS AND MICE

Species	*Strain*	*Age (months)*	*Cholesterol (A)*	*Total phospholipid (B)*	*A/B*	*Phospholipids*[b]					*Reference*
						PC	*SPH*	*PS*	*PE*	*PI*	
Rat	Sprague-Dawley	6 vs 24	← →	↓	↑	← →			← →		62
Rat	Sprague-Dawley	3 vs 16				↑		← →	↓	↑	63
Rat	CFN	3,12,27		↓							64
Rat	Fischer 344	4,14,24		↓							65
Rat	Fischer 344	3,16,27	↑	← →	↑	← →			← →		10
Mouse	C57Bl/6J	3,12,26		← →							66
Mouse	C57	3,8,10,14,16, 19,22		↓		↓	↓	↓	← →	← →	67
Mouse		3,8,10,14,16, 19,22		↓		↓	↓	← →			68

[a] ↓ = decrease with age, ← → = no change with age, ↑ = increase with age.

[b] PC = phosphatidylcholine, SPH = sphingomyelin, PS = phosphatidylserine, PE = phosphatidylethanolamine, PI = phosphatidylinositol.

TABLE V

CHANGES[a] IN FATTY ACID COMPOSITION OF LIVER MICROSOMES WITH AGE IN MALE RATS AND MICE

Species	*Strain*	*Age (months)*	*Fatty acids*						*Reference*
			16:0	*18:0*	*18:1*	*18:2*	*20:4*	*22:6*	
Rat	Sprague-Dawley	6 vs 24	↓	←→	←→	↓	←→	↑	62
Rat	Fischer 344	4,14,24	←→	↑	↓	↓	←→	↓	65
Rat	Fischer 344	3,16,27				↓	↓	↑	10
Mouse	C57	3,8,10,14, 16,19,24	↓	↓	↓	↓	↓	↓	67

[a] ↓ = decrease with age, ↑ = increase with age, ←→ = no change with age.

supposed compensational capacity may enable the liver to keep the serum concentration of albumin constant with age (see section 3.4.1).

3.2.2 Lipid metabolism. There is evidence that the lipid composition of liver microsomal membranes influences the activity of the hepatic microsomal monooxygenase system. About 80% of membrane lipids are phospholipids. The molar ratio of cholesterol/phospholipid is of considerable importance for the physicochemical properties of membranes. An increase in this ratio would result in a decrease in the permeability and fluidity of the membranes and in the activity of certain membrane-bound enzymes such as the monooxygenases. In addition, the fatty acid composition of the phospholipids influences the fluidity of the membranes. In general, increased saturation of fatty acids increases the membrane fluidity at body temperature. Changes with age in liver microsomal phospholipid and cholesterol (Table IV) and fatty acid composition (Table V) are found. An age-related decrease in total phospholipids, an increase in cholesterol and, consequently, an increase in the molar ratio cholesterol/phospholipid are observed by most authors. However, no consistent changes with age in the proportions of different phospholipids in the hepatic microsomal membranes have been revealed (Table IV). The decrease in total phospholipid content may account for the age-related declines in some monooxygenase activities (see section 4.3).

There is little doubt that the fatty acid composition of phospholipids of rat and mouse liver microsomes undergoes an age-related change. Oleic acid (18 : 1) and especially linoleic acid (18:2) decrease in proportion. There appear to be no consistent changes in the proportions of arachidonic (20:4), stearic (18:0), palmitic (16:0) and the minor decosohexaenoic (22:6) acids with age. The functional significance of these changes is difficult to deduce.

3.2.3 Carbohydrate metabolism. Changes in carbohydrate metabolism with age can influence the capacity of the liver to metabolize drugs. For example, the generation of NADPH may be rate controlling in drug metabolism, since the supply of this reduced cofactor is most important in the maintenance of the oxidation–reduction state of the NADP:NADPH couple which plays an important role in the formation of reduced gluta-

thione (GSH) from oxidized glutathione (GSSG). NADPH is generated by the pentose phosphate pathway in a series of reactions starting with glucose-6-phosphate and involving the enzymes glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH). NADPH is also generated by a malate shunt which involves the production of NADPH from malate via the malic enzyme. Results of studies in rats on changes in G6PDH activity with age are contradictory: no change [69,70] as well as an increase or a decrease [71] have been reported. An increase in G6PDH activity with age has been observed in mice [69,72]. No change [69] or a decrease [73] with age was observed in the activity of the malic enzyme.

These data suggest that there may be no age-related decrease in NADPH production due to a decrease in energy supply via the pentose phosphate pathway but there might be a decrease in energy supply via the malate shunt.

3.3 Excretory functions

The liver excretes bilirubin and other bile pigments as well as exogenous substances such as eosin, bromsulfophthalein (BSP) and indocyanine green (ICG), which are removed from the blood and excreted in the bile.

A decrease in the biliary excretion of eosin was found during the first year of life in rats [74]. An increase in BSP retention with age, especially during the first twelve months, was also found in rats [6,7]. The increased BSP retention in the serum of old rats was ascribed to a decrease in the maximal excretion capacity (T_m), whereas the relative storage capacity (S) of the liver for BSP remained unchanged [8,75].

Kitani [76] also determined the removal of ICG from the serum with age and observed a decrease in maximal removal rate.

To investigate whether the age-related increase in BSP retention was due to a decreased storage capacity of individual hepatocytes, these cells were isolated from rats of various ages. A sharp decrease in the amount of BSP stored by the hepatocytes was observed during the first twelve months of age. Thereafter, a less pronounced decrease was found up to 36 months [7]. Comparison of *in vivo* and *in vitro* data leads to the conclusion that the decrease in the capacity of the liver to remove foreign substances is at least partly due to an age-related decline in the storage capacity of the individual hepatocytes for those substances [7].

Kitani *et al.* [3] observed that the biliary excretion of ouabain, a cardiac glycoside which is not biotransformed by the liver before it is excreted in the bile, decreased progressively with age in rats.

It can be concluded that compounds such as BSP, ICG and ouabain which are not metabolized by the liver prior to biliary excretion clearly show age-dependent changes in blood removal rates.

3.4 Biotransformation and factors influencing it

In addition to possible age-related changes in the capacity of the individual hepatocytes to metabolize drugs, changes in liver blood flow, the serum protein-binding capacity

and in hepatic uptake with age may influence the biotransformation capacity of the total liver.

3.4.1 Liver blood flow and serum protein-binding capacity. Changes in liver blood flow with age are of especial importance where drugs with a high extraction ratio are concerned. Examples of such drugs are chloromethiazole, labetol. lignocaine, morphine, nortryptiline, pentazocine, pethidine, propoxyphene and propranolol. Changes in blood flow with age are of great importance for the age-related changes in the clearance of these drugs by the liver, while changes in protein binding with age are of minor importance. Hepatic blood flow in rats decreases with age, especially during the first 12 months [74]. Therefore, age-related changes in the clearance of drugs by the liver may also be attributable to changes in liver blood flow.

For drugs that are poorly extracted by the liver, changes in protein-binding capacity will result in changes in hepatic clearance. The protein binding of drugs in the blood is mainly determined by the serum albumin concentration. The albumin concentration in plasma is unchanged in old rats [6,61] and slightly lower in old mice [77]. Consequently, changes in the hepatic clearance of drugs with age observed in rats are seldom due to age-related changes in serum albumin content. However, indications for structural changes in the albumin molecule have been found [61]. These might influence the drug-binding capacity of albumin in advanced age.

3.4.2 Hepatic drug uptake. Using perfused rat liver, Kroker *et al.* [78] observed that in old rats the hepatic uptake of bile acids was decreased to a lesser extent than was their secretion. Thus, for bile acids, the hepatic uptake is not a rate-limiting step in their removal from the blood by the liver.

3.4.3 Drug metabolism. Drugs are generally metabolized by the liver in two phases. The phase I metabolism comprises those reactions in which lipophilic drugs are transformed by oxidation, hydrolysis and reduction. Phase II metabolism involves those reactions in which drugs or their phase I metabolites are transformed to less lipophilic metabolites by conjugation with small endogenous molecules such as glucuronic acid.

Most drugs undergo an oxidation as a phase I reaction. This is catalyzed by the mixed-function oxidase system. This system has as important components a series of hemoproteins collectively known as cytochrome P-450, cytochrome b_5 and a flavoprotein referred to as NADPH-cytochrome *c* reductase. These components are localized in the smooth endoplasmic reticulum (SER) of the hepatocyte and after fractionation of the cells appear in the microsomal fraction. Morphometric age-related changes in the SER have been discussed in section 2.3. Age-related changes in cytochrome P-450, cytochrome b_5 and NADPH-cytochrome *c* reductase activities with age are recorded in Table VI. Most studies report a decrease in the cytochrome P-450 concentration and the NADPH-cytochrome *c* reductase activity with age. However, some authors have reported no age-related differences. With respect to cytochrome b_5, no change with age was observed in males, while both an increase and a decrease were found in females. The specific activity of purified NADPH-cytochrome *c* reductase isolated from young rats was recently found to be apparently two-fold higher than of that obtained from senescent rats [94].

TABLE VI

THE EFFECT[a] OF AGE ON MIXED-FUNCTION OXIDASE ACTIVITIES

Species	*Strain*	*Sex*	*Age (months)*	*Cytochrome P-450*	*Cytochrome b_5*	*NADPH-cytochrome c reductase*	*Reference*
Rat	Wistar	Male	1,3,10,20	↓		↓	79
Rat	Wistar	Male	7 vs 31	↓	←→	↓	5
Rat	Wistar	Male/female	3,16,24	←→			80
Rat			2 vs 24			←→	81
Rat	Sprague-Dawley	Male	6 vs 24			↓	82
Rat	Sprague-Dawley	Male	3 vs 16	↓			63
Rat	CFN	Male	1,2,3,12, 20,30	←→		↓	83
Rat	CFN	Male	3,12,27	←→		←→	64
Rat	CFN	Male	3 vs 29	↓	←→	←→	84
Rat		Female	3 vs 24	↓	↓		85
Rat	Fischer 344	Male	16 vs 27	↓		↓	86
Rat	Fischer 344	Male	3 vs 23	↓		↓	87
Rat	Fischer 344	Male	4 vs 24			↓	88
Rat	Fischer 344	Female	3,6,12,24, 28	↓	↑	←→	89
Rat	Fischer 344	Male	3,6,12,24, 28	↓	←→	↓	89
Rat	Fischer	Male	4,12,24	↓			90
Rat	Fischer		3 vs 24			↓	91
Mouse	Swiss-Webster	Male	3,6,9,12, 15,18	↓			92
Mouse	C57Bl/6J	Male	3,12,26	←→	←→	↓	66
Mouse	C57A	Male	3 vs 10	↑	↑		
			10 vs 20	↑	↑		93

[a] ↓ = decrease with age, ←→ = no change with age, ↑ = increase with age.

In addition, the "old" enzyme exhibited an increased thermostability profile in comparison with the "young" enzyme [94]. These data suggest possible molecular alterations to be responsible for the age-dependent decline in the functional capacity of the rat liver microsomal mixed-function oxidase system.

Many investigators have studied the influence of age on liver microsomal drug-metabolizing enzymes. The data for rats are summarized in Table VII and for mice in Table VIII. Most enzyme activities in male rats decrease with age. In contrast, an increase in activity was observed for epoxide hydrase and nitroanisole *O*-demethylase. In female rats, most enzyme activities do not change with age, with the exception of an increase in aminopyrine *N*-demethylase [91] and a decrease in 7-ethoxycoumarin *O*-deethylase [89] and aminopyrine *N*-demethylase [85].

TABLE VII

CHANGES IN DRUG-METABOLIZING ENZYME ACTIVITIES WITH AGE IN RATS

Strain	*Sex*	*Age (months)*	*Enzyme*	*Change*[a] *with age*	*Reference*
Wistar	Male	1,3,10,20	Hexobarbital hydroxylase	↓	2,79
Fischer 344	Male	3,6,12,24,28	Hexobarbital hydroxylase	↓	89
Fischer 344	Female	3,6,12,24,28	Hexobarbital hydroxylase	←→	89
Wistar	Male	1,3,10,20	Aminopyrine *N*-demethylase	↓	2,79
	Female	3 vs 24	Aminopyrine *N*-demethylase	↓	85
Fischer	Male	3,6,12,24,28	Aminopyrine *N*-demethylase	↓	89
Fischer 344	Female	3,6,12,24,28	Aminopyrine *N*-demethylase	←→	89
Wistar	Male	3 vs 24	Aminopyrine *N*-demethylase	↓	91
Wistar	Female	3 vs 24	Aminopyrine *N*-demethylase	↑	91
Wistar	Male	1,3,10,20	Aniline hydroxylase	↓	2,79
Fischer 344	Male	4 vs 24	Aniline hydroxylase	↓	88
Fischer 344	Male	3,6,12,24,28	Aniline hydroxylase	↓	89
Fischer 344	Female	3,6,12,24,28	Aniline hydroxylase	←→	89
Wistar	Male	1,3,10,20	Strychnine oxidase	↓	2,79
Wistar	Male	1,3,10,20	*p*-Nitrobenzoic acid reductase	↓	2,79
Wistar	Male	1,3,10,20	*p*-Dimethylaminobenzene reductase	↓	2,79
CFN	Male	1,2,3,12,20,30	Zoxazolamine hydroxylase	↓	83
CFN	Male	3,12,27	Ethylmorphine *N*-demethylase	↓	64
Fischer 344	Male	16 vs 27	Ethylmorphine *N*-demethylase	↓	86
CFN	Male	3,12,27	Benzo(a)pyrene hydroxolase	↓	64
CFN	Male	3,12,27	Benzphetamine-*N*-demethylase	↓	64
Fischer 344	Male	3 vs 23	Benzphetamine-*N*-demethylase	↓	87
Fischer 344	Male	4 vs 24	Benzphetamine-*N*-demethylase	↓	88
CFN	Male	3,12,27	Epoxide hydrase	↑	95
Fischer 344	Male	3 vs 23	7-Ethoxycoumarin *O*-deethylase	↓	87
Fischer 344	Male	3,6,12,24,28	7-Ethoxycoumarin *O*-deethylase	↓	89
Fischer 344	Female	3,6,12,24,28	7-Ethoxycoumarin *O*-deethylase	↓	89
Fischer 344	Male	3,6,12,24,28	Nitroanisole *O*-demethylase	↓	89
Fischer 344	Female	3,6,12,24,28	Nitroanisole *O*-demethylase	←→	89
Fischer 344	Male	4 vs 24	Nitroanisole *O*-demethylase	↑	88

[a] ↓ = decrease with age, ←→ = no change with age, ↑ = increase with age.

The changes in enzyme activities in the mouse are more complex. In males, no general change can be detected from the few data available. An increase (epoxide hydrase, benzpyrene hydroxylase), a decrease (benzphetamine *N*-demethylase, ethylmorphine *N*-demethylase and zoxazolamine hydroxylase) and no change (pyroxidal kinase and pyridoxamine peroxidase) have been found. Studies of Stohs *et al.* [92] with female Swiss Wistar mice reveal decreases in 7-ethoxycoumarin *O*-deethylase, aryl hydrocarbon hydroxylase and aniline hydroxylase.

A few studies have been done on the effect of age on the metabolism of drugs in intact animals. The results of these studies are collected in Table IX. The metabolizing

TABLE VIII

CHANGES IN DRUG-METABOLIZING ENZYME ACTIVITIES WITH AGE IN MICE

Strain	*Sex*	*Age (months)*	*Enzyme*	*Change[a] with age*	*Reference*
Swiss-Webster	Female	6,9,12,15,18	7-Ethoxycoumarin *O*-deethylase	↓	92
Swiss-Webster	Female	6,9,12,15,18	Aryl hydrocarbon hydroxylase	↓	92
Swiss-Webster	Female	3,6,9,12,15,18	Aniline hydroxylase	↓	92
C57Bl/6J	Male	12,25,30	Pyroxidal kinase	←→	96
C57Bl/6J	Male	12,25,30	Pyridoxamine peroxidase	←→	96
C57Bl/6J	Male	3,12,26	Epoxide hydrase	↑	95
C57Bl/6J	Male	3,12,26	Benxphetamine *N*-demethylase	↓	66
C57Bl/6J	Male	3,12,26	Ethylmorphine *N*-demethylase	↓	66
C57Bl/6J	Male	3,12,26	Zoxazolamine hydroxylase	↓	66
C57Bl/6J	Male	3,12,26	Benzpyrene hydroxylase	↑	66

[a] ↓ = decrease with age, ↑ = increase with age, ←→ = no change with age.

capacity seems to be decreased with age in rats, whereas no change in the metabolizing capacity was found in mice.

Two important phase II reactions have been extensively studied in relation with age (Table X). The first involves the different forms of glutathione and glutathione-related enzymes. With respect to the liver glutathione content with age no change [95,101], a decrease [90,101] and an increase [95] have been reported. These discrepancies might be explained by the fact that some investigators [90,95] determined total glutathione content, while Stohs *et al.* [101] determined the reduced (GSH) and oxidized (GSSG) forms of glutathione. In general, it may be deduced that changes with age in the rodent capacity to conjugate foreign substances with glutathione are small. Therefore, these changes are unlikely to be of great importance. Besides glutathione conjugation, glucuronidation is also an important phase II reaction. Changes with age in UDP-glucuronyltransferase again reveal a decrease [90], an increase [90] and no change [89] with age.

TABLE IX

CHANGED METABOLISM OF DRUGS WITH AGE

Species	*Strain*	*Age (months)*	*Sex*	*Drug*	*Change[a] with age*	*Reference*
Rat	Wistar	1,2,4,6,12	Male	Carisoprodol	↓	97
Rat	Wistar	1,2,4,6,12	Male	Pentobarbital	↓	97
Rat	BN/Bi	3,12,24,30	Female	Digitoxin	↓	9
Rat	WAG/Rij	3 vs 25	Male	Digitoxin	↓	98
Rat	WAG/Rij	3 vs 25	Female	Digitoxin	↓	98
Mouse	$C_3H/T/B$	3,12,18	Female	Nicotine	←→	99
Mouse	$C_3H/T/B$	3,18,14,24	Male	Pentobarbitone	←→	77

[a] ↓ = decrease with age, ←→ = no change with age.

TABLE X

CHANGES[a] WITH AGE IN PHASE II METABOLISM

Species	*Strain*	*Sex*	*Age (months)*	*Characteristic*	*Change*	*Reference*
Rat	Wistar	Male	8 vs 30	4-Nitrophenol glucuronidation	↓	4
Rat	Wistar	Male	8 vs 30	Phenolphthalein glucuronidation	↓	4
Rat	CFN	Male	3,12,27	Glutathione-*S*-transferase	←→	95
Rat	CFN	Male	3,12,27	Glutathione content	←→	95
Rat	Fischer 344	Male	4,12,24	Glutathione content	↓	90
Rat	Fischer 344	Male	4,12,24	UDP-glucuronyl transferase towards *O*-aminophenol	↓	90
Rat	Fischer 344	Male	4,12,24	UDP-glucuronyl transferase towards phenolphthalein	↑	90
Rat	Fischer 344	Male	4,12,24	Glutathione reductase	↓	90
Rat	Fischer 344	Male	4,12,24	Glutathione peroxidase	↑	90
Rat	Fischer 344	Male	4,12,24	Glutathione *S*-transferase towards CDNB[b]	←→	90
Rat	Fischer 344	Male	4,12,24	Glutathione *S*-transferase towards DCNB[c]	↓	90
Rat	Fischer 344	Male	4,12,24	γ-Glutamyltranspeptidase	↑	90
Rat	Fischer 344	Male	3,12,24, 28	UDP glucuronyltransferase	←→	89
Rat	Fischer 344	Female	3,6,12,24, 28	UDP glucuronyltransferase	←→	89
Mouse	C57Bl/6J	Male	3,12,26	Glutathione-*S*-transferase	←→	95
Mouse	C57Bl/6J	Female	2,5,9,12, 18	Glutathione peroxidase	←→	100
Mouse	C57Bl/6J	Male	3,12,26	Glutathione content	↑	95
Mouse	CBF-1	Male	3,9,12,15, 18,21,24	Glutathione (GSH)[d] content	←→	101
Mouse	CBF-1	Male	3,9,12,15, 18,21,24	Glutathione (GSSG)[e] content	↓	101
Mouse	Swiss-Webster	Female	3,9,18	Glutathione (GSH) content	↓	102

[a]↓ = decrease with age, ←→ = no change with age, ↑ = increase with age.
[b]CDNB = 1-chloro-2,4-dinitrobenzene.
[c]DCNB = 1,2-dichloro-4-nitrobenzene.
[d]GSH = reduced form of glutathione.
[e]GSSG = oxidized form of glutathione.

These apparent discrepancies might be results of the different changes with age in the different molecular forms of this enzyme, as was observed, for example, by Kitahara *et al.* [90] with respect to UDP-glucuronyltransferase towards *O*-aminophenol and phenolphthalein. Changes in glucuronidation reactions with age seem to be of minor importance.

4. DISCUSSION

In studying the effect of age on the *in vivo* metabolism of drugs by the liver, it should

be taken into consideration that many factors besides the capacity of the liver to metabolize drugs may be of importance. Even within the liver, many factors not directly related to the hepatic smooth endoplasmic reticulum responsible for drug metabolism play a role in the age-related changes in the capacity of the liver to metabolize drugs. Among these, changes in liver weight/body weight ratio and the spontaneous occurrence of specific liver lesions are of major importance. Studies on the excretory capacity of the liver with age indicate that compounds which are not metabolized by the liver prior to biliary excretion are removed at a slower rate by this means in older animals.

For the phase I reaction of hepatic drug metabolism, the mixed-function oxidase system is of particular importance. It is a complex biological system, as the components cytochrome P-450 and NADPH-cytochrome *c* reductase are multienzymatic in nature and are dependent on a continuous supply of NADPH, which is itself generated by other multienzyme systems. A serious drawback of all of the studies on the mixed-function oxidase and drug metabolism presented in Tables VI, VII and VIII is that they were performed with microsomal preparations. In these preparations, an important part of the regulation, namely the substrate and cofactor supply, is entirely missing. Diffusion of oxygen, transport of a drug to binding sites of cytochrome P-450 and the delivery of NADPH to the flavoproteins may be important factors which determine the effect of age on the metabolizing capacity of the liver. There are indications that the metabolic processes which regulate the supply of NADPH may be unchanged with age (see section 3.2.3).

Thurman and Kauffman [103] supplied a partial list of factors that influence rates of drug metabolism in intact cells. These factors are present in the microsomes, in the cytoplasm or in the mitochondria. When using microsomal preparations, age-related changes in the cytoplasmic and mitochondrial factors will not be detected and will not influence the results. Since all of these factors are present in the *in vivo* situation, the results indicating changed metabolism of drugs with age (Table IX) can be indicative of age-related changes in the overall liver drug metabolism. However, these *in vivo* studies also have many drawbacks. In such studies, complicating extrahepatic factors such as neurological and endocrinological as well as circulatory aspects may influence the metabolic capacity of the liver during aging. In addition, changes in drug absorption, drug distribution and kidney function with age should be taken into account in making conclusions concerning age-related changes in the liver metabolic capacity in rodents.

In view of the various limitations mentioned above for the *in vivo* studies, an experimental system based on the use of isolated intact hepatocytes has the advantage that extrahepatic influences can be excluded and that the cells contain all factors involved in drug metabolism. Many recent articles indicate (for reviews, see Billings *et al.* [104], Andersson *et al.* [105], Thurman and Kauffman [103], and Sirica and Pitot [106]) that the metabolism of compounds by hepatocytes isolated from young rats clearly resembles *in vivo* metabolic rates. In addition, changes in interactions occurring between intermediate metabolism and mixed-function oxidation with age can be investigated by using hepatocytes isolated from rats or mice of different ages. Therefore, data obtained

on the drug-metabolizing capacity of those isolated hepatocytes can be expected to provide useful information on the role of the liver in the age-related changes in the kinetics of drug metabolism of rodents *in vivo*. The metabolism of digitoxin by hepatocytes isolated from 3- and 30-month-old male BN/BiRij rats has been recently investigated [107,108]. The capacity of hepatocytes isolated from rats of these different ages reveal no qualitative changes in the pattern of digitoxin metabolites with age. In addition, the apparent K_m did not change with age, but the V_{max} decreased by about 30%. Richardson *et al.* [109] studied age-related changes in the capacity of hepatocytes to metabolize aflotoxin B1 (AFB1). The rate of AFB1 metabolism by hepatocytes from 30-month-old male Fischer 344 rats was approximately one-half that observed with hepatocytes isolated from 6-month-old rats. The age-related changes in digitoxin and AFB1 metabolism appear to result from the decline in the activity of the monooxygenase system. Digitoxin metabolism is initially mediated through two different pathways: hydrolysis and 12β-hydroxylation. Both reactions are mediated by the hepatic microsomal cytochrome P-450 system [110]. The relevance of the *in vitro* data is strengthened by the findings that the digitoxin *in vitro* data [107] are in agreement with the *in vivo* data that the amount of digitoxin metabolites excreted via the bile is decreased with age [9]. A strong indication for the involvement of the monooxygenase system in the decrease in drug metabolism with age is also the fact that the AFB1 metabolites formed by the monooxygenase system declined to a similar extent with age, as did the monooxygenase activities in the liver microsomes [109]. Furthermore, another AFB1 metabolite the formation of which is catalyzed by a cytoplasmic enzyme did not decline with age [109].

However, it should always be kept in mind that, among others, age-related changes in drug metabolism in rats are strongly sex dependent, which is not the case in man. Therefore, one should always be cautious in applying data obtained in rats to the human situation.

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