British Medical Bulletin (1990) Vol 46, No 1, pp 22–35

Hepatic drug metabolism and ageing

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The hepatic clearance of many drugs is reduced in the elderly Contrary to previously held views we suggest the following:

1. Specific activities of several cytochrome P-450 Phase I enzymes are not reduced with age *per se* in primate and man, nor is enzyme affinity for substrate changed.

2. An important contribution to the reduced hepatic elimination is made by reduction in liver size and blood flow with advancing age.

3 Major changes in liver drug metabolism may occur in frail old people. Future studies of hepatic drug metabolism and ageing must closely define the subject groups under study.

The increased risk of adverse drug reactions in the elderly population has been extensively investigated.[1–5] Whilst it appears that at least part of this apparent excess may be due to increased drug usage[6] epidemiological studies suggest that geriatric patients are up to three times more likely to suffer from a drug side effect; and Type A, dose dependent reactions appear to be especially frequent.

Part of the explanation for this phenomenon may be increased pharmacodynamic sensitivity to drugs, but it has also been shown that the systemic elimination of many agents is decreased in the elderly, leading to higher blood concentrations and hence a higher possibility of Type A adverse effects.[1,3] Since Kato and colleagues[7] had shown in 1964 that the specific activities of the most important hepatic drug metabolising enzymes, the monooxygenases, declined with age in male rats, it has seemed that a simple explanation for impaired drug metabolism in ageing humans exists.

0007–1420/90/0046–0022/$10 00

Recent work from San Francisco[8–10] and Newcastle Upon Tyne[11–13] has, however, cast doubt on this assumption, and this review will discuss the effect of ageing upon the hepatic elimination of drugs and other foreign compounds.

BASIC PRINCIPLES OF HEPATIC DRUG ELIMINATION

The Hepatic Clearance (CL_H) of a drug can simply be described by the formula:

$$CL_H = Q.\frac{Ca - Cv}{Ca} = Q.E$$

where Q is hepatic blood flow, Ca and Cv are the arterial and venous concentrations of the compound respectively, and E is the steady state organ extraction ratio. From this equation it can be seen when E approaches unity, CL_H will approach and be limited by hepatic blood flow. Such drugs are termed flow limited.[14] By contrast, if E is small, CL_H will vary according to hepatic extraction and will be relatively independent of flow. Such agents are termed capacity limited.[14] Hepatic extraction is a variable dependent upon liver size, uptake into hepatocyte, and affinity and activity of drug metabolising enzymes. When considering hepatic drug elimination in age it is therefore important to address the following variables:

1. Activities of drug metabolising enzymes and their induction and inhibition
2. Liver size
3. Liver blood flow and hepatic uptake

METABOLIC PATHWAYS OF DRUG METABOLISM

The metabolic pathways involved in the biotransformation of foreign molecules may be divided into two phases.[15] Phase 1 reactions comprise oxidative, reductive or hydrolytic processes. In general they render the compound less lipophilic, but additionally serve to expose, or to add, functionally reactive sites to which polar groups may be conjugated. Such conjugations, which include glucuronidation, methylation, sulphation, acylation and mercaptan formation are called Phase 2 reactions. Compounds which already possess suitable reactive sites may undergo Phase 2 conjugation without prior Phase 1 conversion. Thus paracetamol, which has an

accessible hydroxyl group, is glucuronidated and sulphated; and the sulphonamides, which have amine groups, are acetylated.[16]

Much attention has been focused on Phase 1 oxidations and ageing, especially those mediated by the hepatic microsomal monooxygenase enzyme system (MMO). Other Phase 1 reactions, and Phase 2 reactions, have been relatively neglected.

PHASE 1 REACTIONS AND AGEING

In vivo studies of hepatic drug clearance

The most widely used marker of hepatic drug oxidation has been antipyrine.[17,18] This drug is rapidly and almost completely absorbed from the gut and has no significant pre-systemic metabolism. It is distributed to body water, and has protein binding of under 10%. 95% is metabolised by hepatic microsomal monooxygenase enzymes, the three major products being norphenazone, 3-hydroxymethylantipyrine and 4 hydroxyantipyrine. In one of the first studies of pharmacokinetics in aged man O'Malley and colleagues[19] showed in 1971 that the half life of antipyrine was lengthened by 45% in the elderly and that this was principally due to a decreased total plasma clearance. Several other studies have since produced comparable results[20–22] and have also shown that the changes may be less marked in women. There is no doubt, that factors other than age are important in determining hepatic drug elimination; and at least some of the apparent age related decline can be accounted for by factors other than chronology. For example Vestal and colleagues[25] have shown, in a study of 307 healthy males, that whilst there is a significant negative correlation between antipyrine clearance and ageing, only 3% of the total variance depended on age itself, 12% being related to different smoking habits in young and old individuals.

Many other careful pharmacokinetic studies of drugs eliminated by hepatic Phase I metabolism have been carried out (see Greenblatt et al.[25a] for review). These have shown highly variable decreases in the systemic clearance of drugs with advancing age between 0–50%. Lauterberg and Schnegg[25b] compared the clearance of aminopyrine and caffeine—two established markers of hepatic microsomal function with galactose elimination, said to reflect functioning liver cell mass, in healthy subjects over age 70. They found a parallel decline in clearance of aminopyrine and caffeine on the one hand and galactose on the other; they speculated that this might be due to reduced liver cell mass.

There has been a suggestion that some isoenzymic forms of the microsomal monooxygenases may be selectively impaired with ageing, and Posner et al.[26] have described a specific reduction in N-demethylation capacity with age. However, using theophyline as substrate no similar reduction in N-demethylation has been shown by others[27,28] and it is as yet premature to suggest that specific cytochrome P450 forms are preferentially lost with advancing age in humans.

Other Phase 1 enzyme systems

Less attention has been paid to the effect of age on other Phase 1 enzyme systems, although they are of considerable importance.

Ethanol for example undergoes oxidation by both cytosolic alcohol dehydrogenase (75–80%) and by the microsomal ethanol oxidising system MEOS (20–25%). Although age may influence the volume of distribution of ethanol resulting in rather higher blood levels after acute dosing, the metabolism of the drug is little affected by ageing.[29,30]

Esterases are another important group of drug metabolising enzymes,[31] which until recently had been neglected by gerontologists. We have however recently investigated the effect of age upon plasma aspirin esterase, an enzyme which is responsible for the hydrolysis of aspirin to acetate and salicylate, and whose activity correlates well with the pharmacodynamics of aspirin. In addition, activity of the plasma enzyme correlates well with that in liver.[31] Ageing had no effect on the activity of the enzyme in healthy elderly individuals. However, physical frailty, as defined by Woodhouse et al.[32] was associated with a significant decline in plasma esterase activities.[33] Furthermore it appears that, as with MMO enzymes, the affinity of esterases is unaffected by ageing, or indeed by frailty.[34]

In vitro studies of liver enzyme activities

Microsomal monooxygenases (MMO)

Rodents. An enormous body of work exists concerning age related declines in activities of MMO enzymes in animal strains, and these data have been fully reviewed by Schmucker.[35] As early as 1964 Kato and co-workers[7] demonstrated a negative correlation between chronological age and in vitro hepatic MMO activities, using inbred male rats. Similar findings have been reported from

other laboratories.[11,36] Several mechanisms behind this decline have been postulated, and include:

a) A reduction in smooth endoplasmic reticulum in aged rat liver.[37]

b) Alterations in the lipid profile of membranes. This comprises a decline in total phospholipid content and an increase in the cholesterol/phospholipid ratio.[38–41] The relative proportions of specific phospholipid classes appear to change little during ageing,[38,39,42] although the fatty acid composition of the microsomal membrane may alter.[43] The significance of these possible changes in membrane composition remains unclear and reports of ageing effects on membrane fluidity are contradictory.[43,44]

c) Alterations in cytochrome P450 isozymes. There is now good evidence that most of the changes in MMO activities which are seen in elderly animals are sex related. Age associated declines in MMO activities in female rats are much less marked,[68] and it appears that MMO activities are increased by testosterone in young males. Ageing results in an impaired hypotholamic pituitary testicular axis, manifesting in reduced MMO activities and loss of male specific cytochrome P450 forms.[45]

Man and primates. Although well described in male rodents, and widely extrapolated to man, there is no good evidence of an age related decline in MMO activities in humans and non human primates. Our own studies, using aldrin, 7-ethoxycoumarin, and 7-ethoxyresorufin as probe substrates have shown no age related alterations in MMO specific activities in histologically normal human liver.[12,13] Similarly others have found that both microsomal protein and cytochrome P450 content is unaffected by age or sex in rhesus monkeys,[9] and similarly studies of P450 content, NADPH-cytochrome C reductase and benzo(a)pyrine metabolism in microsomes from livers of female pigtailed macaque monkeys (*Macca nemestrina*) have shown no change from age 2 to 21 years.

For drugs metabolised by 1st order kinetics, specific activities (Vmax) of the MMO enzymes are only one determinant of reaction rate. Equally important is the affinity of enzyme for substrate, of which apparent K_m is good index. Using 7-ethoxyresorufin and aldrin as probe substrates, we have demonstrated similar K_m values and hence enzyme affinities in microsomes from young and old rat livers,[11] and have confirmed the lack of ageing effect in human livers of varying ages using 7-ethoxycoumarin as a

substrate.[12] Similarly, age has no effect on the affinity of rat liver microsomal NADPH cytochrome C reductase for substrates.[46]

Overall therefore it is safe to say that age related declines in MMO specific activities do occur in rodent liver, but that these changes are sex and species specific. Similar reductions in ageing human or primate enzyme activities have not been shown. Furthermore age has no apparent effect on the affinities of MMO enzyme systems for substrates in rat or human liver.

PHASE 2 REACTIONS AND AGEING

In contrast to studies of oxidative drug metabolism in the elderly, conjugation reactions have been relatively neglected. It is widely stated in the literature that conjugation rates are unaffected by ageing, and indeed some work is available to support this,[47–50] however other authors have reported a small decline in drug conjugation with age,[51–53] and clearly further work is required to clarify this issue.

The effect of ageing upon Phase 2 conjugation reactions in vitro has also been less extensively studied, and such data as are available are confusing, because of marked species and sex differences in drug conjugation.[54] Several authors have examined the effect of age upon UDP-glucuronyl transferase activities (responsible for glucuronidation) and have reported no change in Fisher 344 rats or minimal changes only (10–20%) in Wistar rats.[55–58] Similarly sulphation of paracetamol in isolated hepatocytes was reported to be slightly decreased in old rats but not in old mice,[59] when compared to young adults. No data are available on the effects of age on these enzymes in human liver.

Glutathione conjugation is an important detoxification mechanism. The concentrations of glutathione in human liver are unaffected by ageing[13] but the activities of the glutathione transferases have not been studied in man. In animal liver however, the activities of these enzymes show either no change, or minor changes only with ageing, depending upon the substrates chosen.[60–63]

INDUCTION AND INHIBITION OF DRUG METABOLISING ENZYMES

The inhibition of drug metabolising enzymes appears to be similar in young and elderly subjects. Two separate studies have investi-

gated the effect on cimetidine of drug oxidation, and found that the magnitude of inhibition was not related to the age of the patient.[64,65] Similarly studies from this laboratory have shown that acute doses of ethanol result in a similar percentage reduction in acetanilide clearance in young and healthy aged volunteers.[30]

The relationship between ageing and induction of drug metabolism is less clear. Whilst some authors have shown some age related declines in MMO inducibility in some animal strains[66,67] others have reported no age related changes.[68,69]

It has also been suggested that induction of drug metabolism by environmental factors and other drugs is decreased in elderly *humans*, although it has to be said that the evidence for this is inconclusive.[25,70]

AGEING AND LIVER SIZE

One of the earliest and largest studies of liver size in relation to age was undertaken in 1933.[71] 1582 subjects aged between 20 and 80, and dying of acute injury or accident were studied at autopsy. Liver size fell by 18% in women and 24% in men between these ages, with the most marked changes occurring after the sixth decade. In another autopsy study[72] a 25% fall in liver weight was observed between the ages of 20 and 70 years, with a more marked reduction in the very elderly—a fall of 46% between the third and tenth decade. However autopsy studies are difficult to interpret, in that the dead are not representative of the general population, even if death was accidental. For example in the elderly, injury is often a manifestation of physical illness.

Several non invasive methods of measuring liver volume have therefore been developed, including computerised tomography,[73,74] radionucleotide scanning[75–77] and ultrasonography.[78–80] Of these, sonography is the most acceptable as it involves no exposure to ionizing radiation. Using ultrasound, several studies relating age to liver size have been performed. In 1978, Swift and colleagues[81] examined liver volume using ultrasound in 15 volunteers in the third decade and 11 healthy older subjects aged 75–86 years. Mean liver volume was 990 ml in the aged individuals compared to 1303 ml in the young volunteers—a 24% decline. In our own studies,[80] comparing 65 healthy community based volunteers aged 24–91, we have shown a significant negative correlation between liver volume and age. Linear regression analysis of our results suggests average liver volumes of

1474 ml at age 24 compared to 934 ml at age 91—a fall of 37%. Furthermore the changes were slightly more marked in females than males, the percentage decline in liver volume being 44% in women and 28% in men.

The significance of a reduction in liver volume of between 24% and 44% with ageing is considerable, as it is of similar magnitude to the age related reduction in antipyrine clearance described earlier and to the reported 30–40% decline in the clearance of imipramine[82] and theophylline.[83] We feel therefore that changes in liver size alone are sufficient to explain age related decrements in the clearance of low extraction drugs, without invoking changes in drug metabolising enzymes.

Marchesini and colleagues[84] have also examined liver volume with ageing in man using an ultrasound technique. Estimated liver volume fell by about 33% between ages (approx.) 30 and 85 years. They compared this with galactose elimination in 50 normal subjects and found a close correlation between galactose elimination and estimated liver volume. Using part correlation analysis they suggested that while the decline in liver volume with age would explain most of the decline in galactose elimination there was possibly also an age related, liver volume independent, decline in this marker of 'intrinsic metabolic capacity'.

AGEING AND LIVER BLOOD FLOW

For many years, hepatic blood flow has been said to fall with age. However, until recently, such assertions were based on a study which was not specifically designed to investigate this parameter,[85] and which estimated liver blood flow using a bromsulphthalein (BSP) clearance. Unfortunately, BSP clearance is determined by relative storage capacity (S) and transport rate maximum (T_M), and studies have shown that both may be modified independently by ageing.[86,87] This agent is therefore not suitable as a model for studies of ageing and liver blood flow.

Indocyanine green (ICG) a dye with flow dependent clearance and which has been widely used in studies of blood flow, is probably a better model drug to use, as its hepatic extraction is unchanged between young adulthood and senescence, at least in rodents.[88]

The clearance of ICG, and hence apparent liver blood flow, does fall with ageing in humans.[89] Our own studies of ICG clearance in 65 healthy volunteers aged between 24 and 91 years

(and assuming a constant hepatic extraction ratio of 0.74) showed a decline in apparent liver blood flow of 35% in those over 65 years compared to those under 40 years, even allowing for changes in body weight. In addition, liver perfusion (liver blood flow per unit liver volume) also fell over this age range by a factor of 11%.[80]

As with liver size, these age related changes in liver blood flow are probably of utmost importance. Reductions in systemic clearance of several high extraction drugs (flow limited) have been reported in the elderly, including propranolol (45% fall),[90] chlomethiazole (30% fall),[91] and morphine (35% fall)[92]—all of which are compatible with the decline in apparent liver blood flow. In addition presystemic elimination of such drugs has been reported to be reduced with age.[93,94] This may, however, be less marked in fit elderly than in hospitalized subjects.[95] Factors other than liver size and blood flow are important in determining presystemic elimination including uptake into hepatocytes, functional 'shunting' and saturation of enzymes—all of which could theoretically be affected by ageing, and the relative contributions of these factors to the change in presystemic elimination must be speculative.

CONCLUSIONS

In summary we can draw the following conclusions regarding hepatic drug metabolism and ageing:

1. The total capacity of the liver to metabolise many drugs declines with age in man
2. This decline is very variable, from drug to drug and from person to person
3. Much of the variance may be accounted for by the influence of disease states and 'frailty', environmental factors, co-administration of other drugs, and possibly by genetic factors
4. Liver size falls by about 35% between early adulthood and late old age in humans
5. Liver blood flow also falls in elderly humans, probably to a slightly greater extent than liver size. This reduction in liver size and blood flow with age may well offer a further major explanation for the decrease in hepatic clearance of many drugs in old age
6. Whilst monooxygenase enzyme activities decline in aged rats, this is not the case in humans or non-human primates, although indirect evidence suggests that a few, specific cytochrome P-450 forms may selectively decline

7. Age has no effect on the affinity of monooxygenase enzymes for substrate in rat or human at least for the substrates so far studied, or on plasma aspirin esterase affinities in humans
8. Conjugating enzyme activities are little affected by age in animals, and the same probably applies to humans but data are scarce.

REFERENCES

1 Editorial Medication for the elderly. J R Coll Physicians Lond 1984; 18 7–17
2 Schmucker DL Age related changes in drug disposition Pharmacol Rev 1979; 30 445–456
3 Greenblatt DJ, Sellers EM, Shader RI Drug disposition in old age N Engl J Med 1982, 306. 1081–1088
4 Schmucker DL. Aging and drug disposition· an update Pharmacol Rev 1985, 37 133–148
5 Vestal RE Aging and determinants of hepatic drug clearance Hepatology 1989, 9: 331–334
6 Woodhouse KW, Mortimer O, Wiholm BE Hepatic adverse drug reactions. the effect of age In: Liver and ageing· liver and brain Amsterdam Elsevier, 1986: pp 75–80
7 Kato R, Vassanelli P, Frontino G. Variation in the activity of liver microsomal drug metabolising enzymes in rats in relation to age. Biochem Pharmacol 1964, 12: 1037–1051
8 Schmucker DL, Wang RK. Effects of aging on the properties of rhesus monkey liver microsomal NADPH-cytochrome (P-450) reductase. Drug Metab Dispos 1987; 15 225–232
9 Sutter MA, Wood WG, Williamson LS et al. Comparison of the hepatic mixed function oxidase system of young adults, and old non-human primates (Macca nemestrina) Biochem Pharmacol 1985; 34 2983–2987
10 Maloney AG, Schmucker DL, Vessey DS et al. The effects of aging on the hepatic microsomal mixed-function oxidase system of male and female monkeys Hepatology 1986, 6. 282–287
11 Wynne H, Mutch E, James OFW, Rawlins M, Woodhouse KW. Effect of age on monoxygenase enzyme kinetics in rat liver microsomes. Age Ageing 1987, 16 153–158
12 Wynne H, Mutch E, James OFW, Wright IP, Rawlins M, Woodhouse KW. The effect of age upon the affinity of microsomal monooxygenase enzymes for substrate in human liver Age Ageing 1988, 17· 401–405
13 Woodhouse KW, Mutch E, Williams FM, James OFW The effect of age on pathways of drug metabolism in human liver Age Ageing 1984, 13· 328–334
14 Roberts RK. Branch RA, Desmond PV, Schenker S The influence of liver disease on drug disposition. Clin Gastroenterol 1979, 8(1)· 105–121
15 Williams RT Comparative patterns of drug metabolism Fed Proc 1967, 26. 1029–1039
16 Smith SE, Rawlins MD. Variability in human drug response. Ch 5, London. Butterworth, 1973
17 Vessell ES The antipyrine test in clinical pharmacology: conceptions of misconceptions Clin Pharmacol Ther 1979, 26: 275–286
18 Stevenson IH Factors influencing antipyrine elimination Br J Clin Pharmacol 1977, 4 261–265
19 O'Malley K, Crooks J, Duke E et al. Effect of age and sex on human drug metabolism Br Med J 1971; 3. 607–609

20 Liddell DE, Williams FM, Briant RH. Phenazone (antipyrine) metabolism and distribution in young and elderly adults Clin Exp Pharmacol Physiol 1975; 2· 481–487
21 Wood AJJ, Vestal RE, Wilkinson GR et al. Effect of aging and cigarette smoking on antipyrine and indocyanine green elimination Clin Pharmacol Ther 1979; 26 16–20
22 Swift CG, Homeida M, Halliwell M et al. Antipyrine disposition and liver size in the elderly Eur J Clin Pharmacol 1978, 14: 149–152
23 Greenblatt DJ, Divoll MK, Harmatz JS et al Antipyrine absorption and disposition in the elderly. Pharmacology 1988; 36: 125–133
24 Greenblatt DJ, Divoll M, Abernethy DR et al. Antipyrine kinetics in the elderly. prediction of age-related changes in benzodiazepine oxidizing capacity J Pharmacol Exp Ther 1982, 22-. 120–126
25 Vestal RE, Norris AH, Tobin JD et al Antipyrine metabolism in man. influence of age, alcohol, caffeine, and smoking. Clin Pharmacol Ther 1975; 18 425–432
25a Greenblatt DJ, Sellers EM, Shader RI Drug disposition in old age N Engl J Med 1982; 306. 1081–1088
25b 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 Posner J, Danhof M, Teunissen MWE et al. The disposition of antipyrine and its metabolites in young and elderly healthy volunteers. Br J Clin Pharmacol 1987, 24: 51–55
27 Crowley JJ, Cusack BJ, Jue SG et al. Aging and drug interactions II. Effects of phenytoin and smoking on the oxidation of theophylline and cortisol in healthy men. J Pharmacol Exp Ther 1988, 245 513–523
28 Vestal RE, Cusack BJ, Mercer GD et al. Aging and drug interactions. I Effect of cimetidine and smoking on the odication of theophylline and cortisol in healthy men J Pharamcol Exp Ther 1987, 241 488–500
29 Vestal R, McGuire E, Tobin J, Andres R, Norris A, Mezey E. Aging and ethanol metabolism Clin Pharmacol Ther 1977; 21: 343–354
30 Wynne HA, Mutch E, Williams FM, James OFW, Rawlins MD, Woodhouse KW The relation of age to the acute effects of ethanol on acetanilide disposition Age Ageing 1989, 18 123–126
31 Williams FM Serum enzymes of drug metabolism Pharmac Ther 1987, 34. 99–108
32 Woodhouse KW, Wynne H, Baillie S, James OFW, Rawlins MD. Who are the frail elderly? Q J Med 1988 68(255). 505–506
33 Williams FM, Wynne H, Woodhouse KW, Rawlins MD Plasma aspirin esterase· the influence of old age and frailty. Age Ageing 1989, 18 39–42
34 Summerbell J, Yelland C, Woodhouse K The kinetics of plasma aspirin esterase in relation to old age and frailty Age Ageing 1989 (in press)
35 Schmucker DL. Aging and drug disposition. An update. Pharm Rev 1985; 37. 133–148
36 Schmucker D, Wang R. Age related changes in liver drug metabolizing enzymes Exp Gerontol 1980, 15: 321–329
37 Schmucker D, Mooney J, Jones A. Stereological analysis of hepatic fine structure in the Fischer 344 rat. Influence of sublobular location and animal age. J Cell Biol 1978; 78· 319–337
38 Grinna L Age related changes in the lipids of the microsomal and the mitochondrial membranes of rat liver and kidney. Mech Ageing Dev 1977, 6 197–205
39 Grinna L, Barber A Age related changes in membrane lipid content and enzyme activities Biochem Biophys Acta 1972; 288· 347–353

40 Hawcroft D, Martin P Studies on age related changes in the lipids of mouse liver microsomes Mech Ageing Dev 1974, 3. 121–130
41 Hegner D, Platt D Effects of essential phospholipids on the properties of ATPases of isolated rat liver plasma membranes of young and old animals. Mech Ageing Dev 1975; 4. 191–200
42 Rubin P. Swislocki M, Sonnenberg M. Changes in rat liver plasma membrane phosphoslipids during aging. PSEBM 1975, 142 1008–1010
43 Schmucker D, Wang R, Vessey D, James J, Maloney A Age dependent alterations in the physiochemical properties of rat liver microsomes. Mech Ageing Dev 1984, 27· 207–217
44 Armbrecht H, Birnbaum L, Zesner T, Davis B. Changes in hepatic microsomal membrane fluidity with age. Exp Gerontol 1982; 17· 41–48
45 Gustafsson J, Mode A, Norstadt G, Skett P. Sex steroid induced changes in hepatic enzymes Annu Rev Physiol 1983, 45. 51–60
46 Schmucker D, Wang R. The effect of aging on the kinetic profile of rat liver microsomal NADPH cytochrome c reductase Exp Gerontol 1983c, 18 313–321
47 Kraus JW, Desmond PV, Marshall JP, Johnson BS, Schenker S, Wilkinson GR. Effects of aging and liver disease on the disposition of lorazepam. Clin Pharmacol Ther 1978; 24. 411–419
48 Kendall MJ, Quarterman CP. The effect of age on the pharmacokinetics of oxpenolol. Int J Clin Pharmacol Ther Toxicol 1982; 20, 3: 101–104
49 Greenblatt DJ, Divoll M, Harmatz JS, Shader RI Oxazepam kinetics. Effects of age and sex. J Pharmacol Exp Ther 1986, 215. 86–91
50 Murray TG, Chiang ST, Koepke HH, Walker BR. Renal disease age, and oxazepam kinetics Clin Pharmacol Ther 1981, 30· 805–808
51 Divoll M, Abernethy DR, Ameer B, Greenblatt DJ. Acetaminophen kinetics in the elderly Clin Pharmacol Ther 1982; 31 151–156
52 Greenblatt DJ, Allen MD, Locniskar A, Harmatz JJ, Shader RI Lorazepam kinetics in the elderly. Clin Pharmacol Ther 1979, 26. 103–113
53 Briant RH, Dorrington RE, Cleal J, Williams FM. The rate of acetaminophen metabolism in the elderly and the young. J Am Geriatr Soc 1976, 24, 8. 359–361
54 Fevery J Class 2 reactions in aging In: Bianchi L, Holt P, James OFW, Butler RN eds , Ageing in liver and gastrointestinal tract. Lancaster MTP Press, 1988, pp. 267–273
55 Ali M, Nicholls PJ, Yoosuf A The influence of old age and of renal failure on hepatic glucuronidation in the rat. Proceedings of the BPS April 4–6 1979, pp. 498–9
56 Jayaraj A, Hardwick JP, Diller TW, Richardson AG Metabolism covalent binding, and mutagenicity of aflatoxin B_1 by liver extracts from rats of various ages J Natl Cancer Inst 1985; 74. 95–103
57 Borghoff SJ, Birnbaum LS. Age related changes in glucuronidation and deglucuronidation in liver, small intestine, lung and kidney of male Fischer rats Drug Metab Dispos 1985; 13· 62–7
58 van Bezooijen CFA. Influence of age related changes in rodent liver morphology and physiology on drug metabolism—A review Mech Ageing Dev 1984; 25: 1–22
59 Sweeny DJ, Weiner M. Metabolism of acetaminophen in hepatocytes isolated from mice and rats of various ages. Drug Metab 1985; 13 377–9
60 Fujita S, Kitagawa H, Ishizawa H, Suzuki T, Kitani K Age associated alterations in hepatic glutathione-S-transferase activities. Biochem Pharmacol 1985; 34: 3891–4
61 Spearman ME, Leibman KC. Effects of aging on hepatic and pulmonary glutathione-S-transferase activities in male and female Fischer 344 rats. Biochem Pharmacol 1984; 33. 1309–13

62 Spearman ME Interaction of endogenous and exogenous chemicals with rat glutathione-S-transferases: implications of age related changes In. Kitani K ed Liver and Aging Amsterdam. Elsevier, 1986: p. 45
63 Kanai S, Kitani K, Fujita S, Kitagawa H. The hepatic handling of sulf obromphthalein in aging Fischer 344 rats: in vivo and in vitro studies Arch Gerontol Geriatr 1985; 4. 73–85
64 Feely J, Pereira L, Guy E, Hockings N. Factors affecting the response to inhibition of drug metabolism to cimetidine—dose response and sensitivity of elderly and induced subjects Br J Clin Pharmacol 1984, 17. 77–81
65 Divoll N, Greenblatt D, Abernathy D, Shader R. Cimetidine impairs clearance of antipyrine and desmethyldiazepam in elderly J Am Geriatr Soc 1982, 30· 684–689
66 Schmucker D, Wang R. Effects of aging and phenobarbital on the rat liver microsomal drug metabolising system. Mech Ageing Dev 1981c, 15 189–202
67 McMartin D, O'Connor J, Fasco M, Kaminsky I Influence of aging and induction on rat liver and kidney microsomal mixed function oxidase systems. Toxicol Appl Pharmacol 1980; 54: 411–419
68 Birnbaum L, Baird M Induction of hepatic mixed function oxidases in senescent rodents. Exp Gerontol 1978a, 299–303
69 Birnbaum L, Baird M. Induction of hepatic mixed function oxidases in senescent rodents. *II Effect of polychlorinated biphenyls*. Exp Gerontol 1978b, 13· 469–177
70 Pearson MW, Roberts CJC. Drug induction of hepatic enzymes in the elderly Age Ageing 1984, 13· 313
71 Boyd E Normal variability in weight of the adult human liver and spleen. Arch Pathol 1933, 16. 350–372
72 Calloway NO, Foley CF, Lagerbloom P. Uncertainties in geriatric data II organ size J Am Geriatr Soc 1965; 13· 20–28
73 Fritschy P, Robotti G, Schneckloth G, Vock P. Measurement of liver volume by ultrasound and computed tomography J Clin Ultrasound 1983, 11 299–303
74 Van Thiel DH, Hagler NG, Shade RR, Skolnick ML, Pollitt Heyl A et al. In vivo hepatic volume determination using sonography and computed tomography. Gastroenterology 1985; 88. 1812–1817
75 Stadalnik RC, Vera DR, Woodle ES, Trudeau WL, Porter BA et al Technetium-99m NGA functional hepatic imaging preliminary clinical experience J Nucl Med 1985; 26(11) 1233–1242
76 Roolo FD, DeLand FH The determination of liver mass from radionuclide imagines. Radiology 1968, 91· 1191–1194
77 Kan MK, Hopkins GB Measurement of liver volume by emission computed tomography J Nucl Med 1979; 20: 514–520
78 Carr D, Duncan JG, Railton R, Smith CB Liver volume determination by ultrasound· a feasibility study. Br J Radiol 1976; 49. 776–778
79 Rasmussen Sn Liver volume determination by ultrasonic scanning Br J Radiol 1972, 45: 579–585
80 Wynne HA, Cope E, Mutch E, Rawlins MD, Woodhouse KW, James OFW The effect of age upon liver volume and apparent liver blood flow in healthy man Hepatology 1989; 9 297–301
81 Swift CG, Homeida M, Halliwell M, Roberts CJC Antipyrine disposition and liver size in the elderly. Eur J Clin Pharmacol 1978, 14· 149–152
82 Abernethy DR, Greenblatt DJ, Shader RI Imipramine and desipramine disposition in the elderly J Pharmacol Exp Ther 1985; 232: 183–188
83 Antal EJ, Kramer PA, Mercik SA, Chapron DJ, Lawson IR Theophylline pharmacokinetics in advanced age Br J Clin Pharmacol 1981, 12 637–645
84 Marchesini G, Bina V, Brunori A, Bianchi G, Pisi P, Fabri A, Zoli M, Pisi E.

Galactose elimination capacity and liver volume in ageing man Hepatology 1988, 8 1079–1083
85 Sherlock S, Bearn AG, Billing BH, Paterson JCS. Splanchnic blood flow in man by the bromsulfalein method: the relation of peripheral plasma bromsulfalein level to the calculated flow J Lab Clin Med 1950; 35 923–932
86 Thompson EN, Williams R Effect of age on liver function with particular reference to bromsulphelin excretion. Gut 1965; 6. 266–269
87 Kitani K, Zurcher C, Van Bezooijen K. The effect of ageing on the hepatic metabolism of sulfobromophthalein in BN/Bi female and WAG/Rij male and female rats Mech Ageing Dev 1981, 7: 381–393
88 Kitani K, Setsuko K, Miura R. Hepatic metabolism of sulfobromophthalein in ageing rats. In· Kitani, ed Liver and Ageing Amsterdam· Elsevier, 1978· pp 145–157
89 Wood AJJ, Vestal RE, Wilkinson GR, Branch RA, Shand DG. Effect of ageing and cigarette smoking on antipyrine and indocyanine green elimination. Clin Pharmacol Ther 1979; 26 16–20
90 Castleden CM, George CF. The effect of ageing on the hepatic clearance of propranolol Br J Clin Pharmacol 1979; 7 49–54
91 Naturon RL, Learoyd B, Barber J, Triggs EJ The pharmacokinetics of chlormethiazole following intravenous administration in the aged. Eur J Clin Pharmacol 1976, 10. 407–415
92 Baillie SP, Bateman DN, Coates PE, Woodhouse KW. Age and the pharmacokinetics of morphine Age Ageing 1989; 18 258–262
93 Castleden CM, George C The effect of ageing on hepatic clearance of propranolol British J Clin Pharmacol 1979, 7· 49–54
94 Robertson DRC, Waller DG, Renwick AG, George CF. Age-related changes in the pharmacokinetics and pharmacodynamics of nifedipine Br J Clin Pharmacol 1988; 25. 297–305
95 Regardh CG, Landahl S, Larsson M et al Pharmacokinetics of metoprolol and its metabolite OH-metoprolol in healthy, non-smoking, elderly individuals Eur J Clin Pharmacol 1983, 24. 221–226