Age and cytochrome P450–linked drug metabolism in humans: An analysis of 226 subjects with equal histopathologic conditions

Objectives: The effect of aging on drug metabolism in humans has not yet been completely described.
Methods: Two hundred twenty-six patients with equal histopathologic conditions were investigated. The cytochrome P450 contents in the liver biopsy samples, the plasma antipyrine clearance rates after oral administration and, as an independent control of vitality, serum testosterone levels were determined.
Results: Cytochrome P450 content in subjects from 20 to 29 years of age was 7.2 ± 2.6 nmol · gm^{-1}, increased during the fourth decade (+7.2%, p = NS), declined after 40 years (−16%, $p < 0.01$) to a level that remained unaltered up to 69 years, and declined further after 70 years (−32%, $p < 0.001$). The antipyrine (phenazone) clearance rate in young subjects was 46.4 ± 18.5 ml · min^{-1}, remained unaltered during the fourth decade, and declined after 40 years by a rate of 0.34 ml · min^{-1} per year toward old age (−29%, $p < 0.001$). The half-life in young subjects was 9.5 ± 2.0 hours and increased after 30 years toward old age (+26%, $p < 0.001$). The volume of antipyrine distribution, 0.46 ± 0.12 L · kg^{-1} in young subjects, decreased after 30 years (−11%). In line with the testosterone content, the decrease in drug metabolism was equal in both sexes.
Conclusion: This study shows a reduction of in vitro and in vivo drug metabolism with age in humans. The data suggest that at least three age groups—young, middle-aged, and elderly—should be included in the evaluation of the pharmacokinetics of a new drug. The reduction of drug metabolism (−30%) after 70 years of age indicates that care is needed in the prescription of drugs for elderly subjects. (Clin Pharmacol Ther 1997;61:331-9.)

Eero A. Sotaniemi, MD, PhD, Arno J. Arranto, MD, PhD, Olavi Pelkonen, MD, PhD, and Markku Pasanen, MD, PhD *Oulu, Finland*

Liver drug metabolism decreases with age in animals.[1-4] The effect of aging on microsomal cytochrome P450 (P450)–dependent drug metabolism in humans has not yet been verified. The rate of drug elimination in vivo, including antipyrine (phenazone), lidocaine, diazepam, and theophylline, declines with age,[3-7] whereas the P450 content or the components of the microsomal electron transport system in vitro remain unaltered.[8-14]

From the Departments of Internal Medicine, Pathology, Pharmacology and Toxicology, and the Central Laboratory, University Hospital of Oulu, and the Deaconess Institute of Oulu.
Supported by the Academy of Finland, Helsinki (research contract 1051029 to Dr. Pelkonen).
Received for publication June 14, 1996; accepted Oct. 6, 1996.
Reprint requests: E. A. Sotaniemi, MD, PhD, Department of Internal Medicine, University of Oulu, Kajaanintie 50 FIN-90220 Oulu, Finland.

0009-9236/97/$5.00 + 0 **13/1/78491**

The reasons for the discrepancies between the experimental and human in vitro data are probably attributable to (1) the range of interindividual variation in a restricted human series and (2) the difficulties of getting representative liver samples from healthy subjects. The wedge biopsy specimens from surgical patients with "normal histopathologic conditions" reflect not only the age of the patients but also the agents used in premedication or anesthesia, as well as the liver process (e.g., gallstones) that caused the operation. A change in the amount and activity of the monooxygenase enzymes located in the smooth endoplasmic reticulum, in spite of a normal histopathologic condition, is possible.[15-18] Therefore, reduced lidocaine and coumarin clearance in the elderly, reflecting the CYP3A4 and CYP2A6 activities, suggests a diminished activity of the microsomal monooxygenase system with age.[19]

The increase of the human life span increases the

number of elderly persons who require drug therapy. To guarantee efficacy and safety of the treatment, the age-associated alterations in the liver-drug metabolism of elderly people should be clarified. We investigated the effect of age on drug metabolism in biopsy specimens from 226 patients with "equal" histopathologic conditions. The study group was selected from among 500 consecutive patients with diagnostic liver biopsies by exclusion of the subjects who had received drugs that influence the liver monooxygenase activity and the patients with "apparently normal" or "severe" histopathologic conditions. The subjects were classified by age in decades. The P450 content of the biopsy samples, the rate of antipyrine elimination from plasma, and the liver function tests were assessed simultaneously. To compare further the age-associated changes in humans, serum testosterone, another indicator of life span,[20,21] was determined independently in 104 healthy subjects.

MATERIAL AND METHODS

Subjects. Two hundred twenty-six patients with equal histopathologic conditions (102 women and 124 men) were investigated (Table I). They were selected from a total group of 500 consecutive patients for whom diagnostic liver biopsies had been performed during a 5-year period. The subjects (n = 180) who were receiving drugs that influence liver microsomal enzyme activity inducers (e.g., phenytoin and phenobarbital) or inhibitors (e.g., cimetidine and isoniazid) were excluded. To investigate the effect of age on subjects with comparable livers, the patients with "apparently normal" and "severe" histopathologic conditions were excluded from the final series (Table I).

The specimens were taken with a Tru-Cut needle[16] from the right lobe of the liver. The subjects met the following criteria: (1) All were investigated as inpatients; (2) the biopsies were performed for diagnostic purposes: unexplained physical findings or abnormal liver tests, follow-up of a liver disease process, or the effect of therapy on it; (3) patients with hepatitis A, B, and C antigens were excluded from the study; (4) at the time of the study, no patients had any significant cardiac decompensation, and kidney function as judged by serum creatinine was normal. Alcohol was classified as an inducing agent only in the subjects with an apparently normal liver histopathologic condition, whereas the others were classified on the basis of liver histologic examination.[16] However, alcoholic patients with apparently normal liver histopathologic conditions were excluded.

A second study group consisted of seven women who had had gallstone surgery. This group was included to determine P450 levels in wedge biopsy samples taken during the operation. They were 33 to 37 years of age and had normal histopathologic conditions. None of these women were receiving continuous drug therapy, including contraceptives, and they took spasmolytic agents only occasionally.

An independent age-related variable, serum total testosterone, was analyzed in 104 healthy subjects, including 80 blood donors (40 women and men) and 24 elderly subjects (12 women and 12 men). They had normal values in liver and kidney function tests before the study.

The control subjects in the drug metabolism studies consisted of 20 subjects from 25 to 55 years of age (10 men and women) who had undergone diagnostic liver biopsies. They had normal liver histopathologic examinations and were not undergoing therapy with drugs that influence the hepatic monooxygenase system.

Protocol. The study plan was congruent with the Helsinki declaration and accepted by the local Ethics Committee of Oulu University. Informed written consent was obtained from each subject before the study. Blood samples for liver function tests were drawn after an overnight fast. The biopsy was performed 3 to 4 hours later. The biopsy material was divided into two parts: one was fixed in formaldehyde for histologic studies and the other was frozen in liquid nitrogen and stored at −80° C until analyzed. Antipyrine (20 mg/kg body weight, dissolved in orange juice) was given to each subject after an overnight fast. No food was permitted for 3 hours to ensure complete absorption of the drug. Blood samples were drawn by venipuncture before and 1, 3, 6, 9, 12, 24, 48 and, in some cases, 72 hours after drug administration.

Drug metabolism studies. P450 content ($nmol \cdot gm^{-1}$) was determined from the whole homogenate of the biopsy material with a method described earlier.[16,18,22] Plasma antipyrine concentrations were determined by a gas-liquid chromatography method, with phenacetin as an internal standard.[16,18]

Liver function tests. Serum bilirubin and albumin contents, activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and prothrombin time were measured with standard Eu-

Table I. Classification of the livers of 320 patients according to their histopathologic diagnoses and age in decades

Liver histologic condition	*Age in decades (yr)*					
	20-29	*30-39*	*40-49*	*50-59*	*60-69*	*>70*
Normal (n = 29)	12	12	2	2	1	0
Slight to moderate (n = 226)	22	40	48	66	40	10
Severe (n = 65)	3	5	13	31	10	3

Table II. Age, sex, and drug metabolism of control subjects and of patients classified by liver histopathologic condition

Liver histologic condition	*n*	*Female/ male*	*Age (yr)*		*Drug metabolism*			
			Mean	*Range*	*P450 (nmol · gm^{-1})*	*Antipyrine $t_{1/2}$ (hr)*	*CL (ml · min^{-1})*	*V_{area} (L · kg^{-1})*
Apparently normal	29	16/13	35 ± 13	21-65	10.2 ± 1.8	8.4 ± 2.1	48.0 ± 18.6	0.50 ± 0.14
Slight to moderate	226	102/124	47 ± 14	21-76	6.5 ± 2.9*	10.1 ± 2.9*	43.4 ± 16.3†	0.43 ± 0.09*
Severe	65	32/33	50 ± 12	22-75	4.7 ± 1.9*	22.2 ± 5.7*	22.9 ± 8.4*	0.46 ± 0.13
Control subjects with normal liver	20	10/10	48 ± 11	25-55	11.1 ± 1.5	7.5 ± 1.8	52.1 ± 15.1	0.51 ± 0.10

Data are mean values ± SD.
$t_{1/2}$, Half-life; CL, clearance; V_{area}, volume of distribution.
*$p < 0.001$ (compared with controls).
†$p < 0.05$ (compared with controls).

Table III. Drug metabolism in 226 patients with "equal" (slight to modern to alterations) in liver histopathologic conditions and serum testosterone level in 104 healthy men and women

Parameter	*Age in decades (yr)*					
	20-29 (young)	*30-39 (young)*	*40-49 (middle-aged)*	*50-59 (middle-aged)*	*60-69 (middle-aged)*	*>70 (elderly)*
P450 (nmol^{-1})	7.2 ± 2.6	7.6 ± 2.5	6.1 ± 2.2†	6.4 ± 2.3†	6.3 ± 2.6†	4.8 ± 1.1*
Antipyrine						
Half-life (hr)	9.6 ± 2.0	9.9 ± 3.1	10.3 ± 3.0	10.6 ± 3.5	10.9 ± 3.2	12.1 ± 3.1‡
CL (ml · min^{-1})	46.4 ± 18.5	46.6 ± 15.4	45.2 ± 14.2	42.0 ± 19.2	33.8 ± 10.7*	32.8 ± 12.2*
V_{area} (L · kg^{-1})	0.46 ± 0.12	0.45 ± 0.07	0.43 ± 0.07	0.42 ± 0.10	0.40 ± 0.07	0.41 ± 0.09
Testosterone (nmol · L^{-1})						
Men	17.2 ± 5.5	16.2 ± 5.8	12.2 ± 3.5[c]	12.9 ± 4.8‡	12.2 ± 4.7‡	10.9 ± 3.1‡
Women	1.1 ± 0.6	1.4 ± 0.2	1.4 ± 0.9	1.0 ± 0.4	1.6 ± 1.1	1.2 ± 0.8

*$p < 0.01$.
†$p < 0.01$.
‡$p < 0.05$.

ropean Community Clinical Laboratory (ECCL) automatic techniques.

Serum testosterone. Total serum testosterone was measured with a coated tube radioimmunoassay (Spectria Testosterone Kit, Orion Diagnostica, Oulu, Finland). The blood samples were taken after fasting from 80 healthy blood donors and 24 healthy elderly subjects. After centrifugation, the serum was kept at −70° C until analyzed. The intraassay and interassay coefficients of variation were <5%.

Liver histopathologic examination. The liver biopsy specimens were fixed in 10% buffered neutral formalin, embedded in paraffin wax, sectioned serially, and stained with hematoxylin and eosin, periodic acid-Schiff's reagent after diastase digestion, van Gieson's stain, or Gomori's reticulin stain.[23,24]

The subjects were classified into three groups on the basis of liver histopathologic examinations, applying standard criteria (Table I): (1) apparently normal liver histologic diagnosis, (2) subjects with

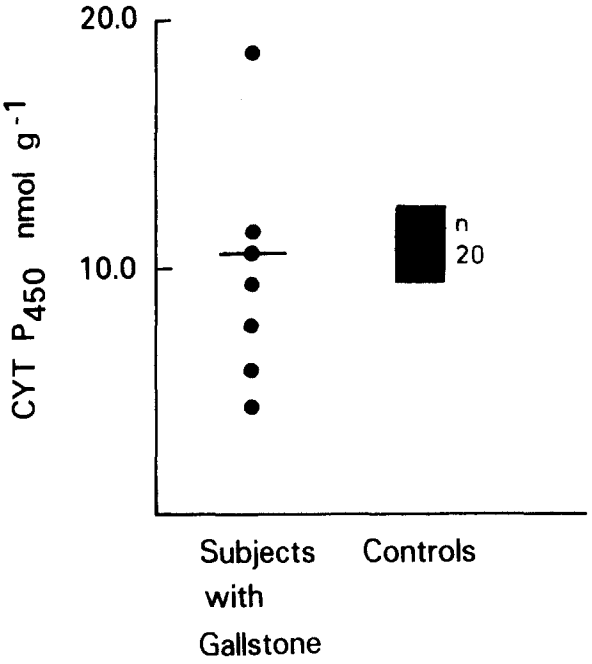


Fig. 1. Cytochrome P450 content in wedge biopsy specimens from seven women undergoing gallstone operation and from matched control subjects. Histopathologic conditions were normal in both groups.

slight to moderate changes in liver parenchyma (fatty liver, fatty liver with fibrosis, degree of fibrosis 0 to 3, and reactive changes such as granulomata or portal infiltrates), and (3) subjects with severe changes (cirrhosis and hepatitis). The patients classified as normal had either completely normal histopathologic examinations or only a marginally altered liver. Those with fatty livers had lipid vacuoles in the hepatocytes that exceeded 10% of the parenchyma. The patients with cirrhosis had a loss of hepatocytes, increased fibrosis, regenerative nodules, distortion of the lobular structure, and an abnormal vascular bed.[18,23]

Classification of patients by age in decades. The effect of age on drug metabolism in humans has been investigated by linear regression analysis.[8,12] To investigate the effect of age in short intervals, the subjects were classified by age decades (Table I). Notable variation in drug metabolism among healthy subjects has been documented.[24] Therefore, only the group of patients with slight to moderate changes in histopathologic condition (at least 10 subjects per decade) was considered to be acceptable for enrollment. Although some young and elderly subjects were categorized with normal and severe histopathologic conditions, their numbers were not great enough to do a complete analysis.

Smoking and drug metabolism. When the case history was taken, each subject was asked about their smoking habits. The smokers (n = 61) had been smoking continuously for at least 1 year before the admission. Exsmokers (n = 40) were excluded. The rest were nonsmokers (n = 125).

Gender and drug metabolism. We did not find any sex-dependent difference in drug metabolism when the subjects were classified by gender. Thus, men and women were considered as one group.

Statistical analysis. Plasma antipyrine concentration (on a log scale) versus time was plotted, and the elimination rate constant (k_e), plasma concentration at zero time (C_0), and antipyrine half-life ($t_{1/2}$) were calculated by the method of least squares. The volume of distribution (V_{area}) and clearance (CL) were determined from the following equations:

$$t_{1/2} = 0.693/k_e$$

$$V_{area} = Dose/C_0$$

$$CL = Dose/AUC$$

AUC was calculated by the trapezoidal rule. ANOVA was used to assess the possible differences in the variables between the groups.

RESULTS

Clinical characteristics

Table I shows the distribution profile of the patients who underwent biopsies, classified by liver histopathologic condition and age in decades. The groups diverged significantly in their liver function tests (data not given). P450 content and antipyrine metabolism were related to liver changes; there were significant differences between the groups of patients with histologic diagnoses ($p < 0.001$; Table II).

Age and hepatic drug metabolism in man

P450 content. P450 content was 7.2 ± 2.6 nmol · gm^{-1} in young subjects (20 to 29 years), increased slightly during the next decade (+7.5%, not significant), declined from 40 to 49 years of age, (−16%, $p < 0.01$), remained unaltered during the next two decades up to 69 years, and declined again in elderly subjects (>70 years, −32%, $p < 0.001$; Table III). The decrease in the P450 content was 0.07 nmol · gm^{-1} per year after 40 years.

Fig. 1 shows a fourfold variation in the P450 content determined from wedge biopsy specimens of seven young women undergoing surgery for gall-

Table IV. Smoking and liver drug metabolism in patients with equal histopathologic conditions

Group	*n*	*Drug metabolism*			
		P450 (nmol · gm^{-1})	*Antipyrine $t_{1/2}$ (hr)*	*CL (ml · min^{-1})*	*V_{area} (L · kg^{-1})*
Smokers	61	6.6 ± 3.6	8.7 ± 2.7*	53.3 ± 23.0*	0.42 ± 0.10
Nonsmokers	125	6.7 ± 2.9	11.1 ± 3.4	35.7 ± 12.4	0.45 ± 0.09

*$p < 0.001$ (compared with nonsmokers).

stones. The subjects were otherwise healthy, with apparently normal liver histopathologic conditions. None of them were using drugs (e.g., oral contraceptives) that influence the monooxygenase activities of the liver. Thyroid function was also within the normal range. The control group consisted of 20 patients with "apparently" normal histopathologic conditions who underwent needle biopsy of the liver.

Antipyrine metabolism. The antipyrine clearance rate from plasma was 46.4 ± 18.5 ml · min^{-1} in young subjects (20 to 29 years), remained unaltered during the next decade, and declined linearly after 40 years by the rate of 0.34 ml · min^{-1} per year toward old age (>70 years, −29%, $p < 0.001$; Table III).

Antipyrine half-life, 9.6 ± 2.0 hours in young subjects (20 to 29 years), increased linearly after 30 years toward old age (>70 years, +26%, $p < 0.001$). The apparent volume of antipyrine distribution, 0.46 ± 0.12 L · kg^{-1} in young subjects (20 to 29 years), declined linearly after 30 years toward old age (>70 years, −11%; Table III).

Smoking and drug metabolism. Smoking activated antipyrine metabolism by +42% (Table IV), whereas the P450 content remained unaltered. The results from smokers and nonsmokers did not diverge by age.

Serum testosterone. Testosterone levels in men declined throughout life (Table III). The values were 17.7 ± 5.2 nmol^{-1} in young subjects (20 to 29 years), slightly lower (−4%, not significant) in the fourth decade, and significantly lower (−31%, $p < 0.01$) at the from 40 to 49 years of age, and then declined slowly up to the elderly category (>70 years). In women, testosterone levels increased slightly from the level of 1.2 ± 0.68 nmol^{-1} observed in the young group (+18%, not significant) to the values recorded at 30 to 49 years of age, and declined (−27%) through 50 to 59 years of age. The changes in women were not significant.

Relationship between in vitro and in vivo drug metabolism. Our data show decreases in the P450 content and antipyrine metabolism with age in humans.

Table V. Correlations between in vitro and in vivo drug metabolism parameters (*r* values)

Liver histologic condition	*Antipyrine*	
	$t_{1/2}$ versus P450	*CL versus P450*
Normal	−0.324	0.480
Slight to moderate	−0.356	0.262
Severe	−0.309	0.341
All	−0.395	0.418

The changes of the in vitro and in vivo parameters occurred in diverging ways (Table III). The P450 content declined from 40 to 49 years of age, whereas the antipyrine clearance rate was altered significantly at 60 years of age. This indicates that drug metabolism in vivo is dependent on a complex set of multiple factors and not only on the amount of metabolizing enzymes (Table V). The clearance rate was positively related and the half-life was reversely related to the P450 content. The *r* values remained below 0.500, indicating that many other factors apart from the P450 content itself influence the rate of antipyrine elimination in vivo.

DISCUSSION

The microsomal drug-metabolizing enzyme system of the liver is under genetic control and can be influenced by various factors, such as diet, alcohol, environmental factors, and diseases.[24-27] Our data showed that age as such has a decreasing effect on drug metabolism in humans. The decrease was equal in both sexes. Experimental studies have shown that a decline in the rate of microsomal monooxygenases in vitro, a loss of hepatic smooth endoplasmic reticulum, and alterations in the physicochemical properties of microsomal membranes have been associated with senescence in animals.[1,2,28-31] Similar alterations probably occur with age in human hepatocytes. This explains why no age-induced decrease was observed in the earlier studies when monooxygenase activity was compared by linear regression analysis.[8,12] Kato et al.[1] characterized neonatal, pu-

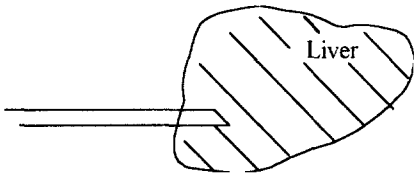
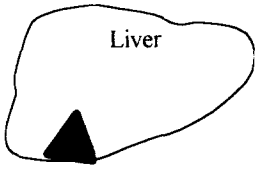
	Present study	Earlier studies
Subjects		
n	**226**	**< 100 in every study**
Age	**In decades**	**Whole group by linear regression analysis**
Histology	**"Equal"**	**Apparently "normal"**
Biopsy	**Tru - Cut**	**Wedge biopsy/ Menghini needle**
		
Material collection	**One hospital**	**Many hospitals**

Fig. 2. Comparison of the Material and Methods sections in this study and in earlier studies.

bertal, mature, and senescent type of P450 activities. In agreement with the classification of Kato et al.,[1] we demonstrated young, middle-aged, and elderly type of P450 activities. According to an animal study,[1] drug metabolism has been shown to be regulated by the sex hormones: testosterone activates the monooxygenase system, whereas estrogen only has a slight effect or no effect. Serum testosterone reflects synthesis in the gonads and metabolism of the hormone in the extrahepatic tissues and in the liver. The levels are related to age[20-21] and considered to be an excellent parameter of androgenicity and vitality of the life span. The serum testosterone in women is about 10% of that in men, and the hormone originates from the ovary, the adrenal cortex, and conversion of the steroids in peripheral tissue. We found that the decrease of serum testosterone started after the 30 years of age in men and 10 years later in women (Table III). A significant reduction in the testosterone content occurred only in men after 40 years of age. This suggests that changes in liver drug metabolism can also be considered to be an indicator of the vital activity of the human life span.

In line with the other in vivo studies, the metabolism of antipyrine declined with age.[5,25] Antipyrine is absorbed nearly completely from the gastrointestinal tract, has no significant presystemic metabolism, is slightly protein-bound, and is distributed in the body water. The drug is metabolized in the liver by at least 10 CYP enzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) and is excreted as metabolites in the urine.[26,32,33] The compound is widely used as a marker of hepatic microsomal enzyme activities.[27] Antipyrine metabolism is related to liver parenchymal changes and elimination decreases in an altered liver[16,18] (as was also observed in this study), and the inducers of drug-metabolizing enzymes activate its elimination.[27,32] We demonstrated that the clearance rate and half-life of antipyrine behaved differently with age. The half-life was influenced by the distribution and elimination processes and increased with age, whereas the plasma clearance rate, reflecting directly the microsomal enzyme activity, remained unaltered for the third and fourth decades and declined after the sixth decade. This study agrees with the suggestion of Sharer and Wrighton[33] that antipyrine clearance is a general marker of P450 oxidative capacity in all age groups.

This study raises one of the most important ethical questions: What kind of people can be taken to represent "normal" or "control" subjects in investigations of drug metabolism in humans?[26] We demonstrated earlier that a subject who represents "normal" (i.e., standard) life must have been exposed to various chemical agents in food preservatives, drink, pollution, and drugs.[26] Furthermore, "normal" sub-

jects have had diseases during their childhood, some virus infections such as common cold, and later had various degenerative changes in the body. The response to environmental factors is then modified by each person's own genetic and environmental factors.

In this study, we found a relatively poor correlation between P450 content and antipyrine metabolism, as indicated by the *r* values, in the patients in the study. This is expected because individuals have variable spectra of P450 isoforms in their livers, each contributing to the total P450 content, but only nine of them contribute to antipyrine elimination. In addition, other factors apart from metabolizing enzyme activity, such as liver size, hepatic blood flow, liver uptake, hepatic collagen content, availability of reduced nicotinamide adenine dinucleotide phosphate, and first-pass metabolism and protein binding[18,26,27,34-37] must be taken into consideration when evaluating in vivo clearance of drugs in humans. In alcoholic subjects grouped on the basis of uniform histopathologic conditions (e.g., subjects with fatty livers), the correlation between in vitro and in vivo parameters is relatively good,[26] but in subjects with various degrees of liver change it is poor.[34] Thus the total P450 content, as seen in this study, or the CYP2A6 or CYP3A4 activities[38] need not necessarily predict drug metabolism in vivo. Moreover, a liver specimen obtained by means of a wedge biopsy during surgery (Fig. 2) contains more fibrotic tissue than the central part of parenchyma and therefore is less representative of the liver parenchyma than a specimen taken by a Tru-Cut needle from the right lobe. Therefore the effect of age on monooxygenase activity may have been masked when age was compared with monooxygenases in surgical patients with "normal" livers. Our data also show the necessity of the use of a small age range as a unit when comparing age-induced changes in liver metabolism.

Furthermore, environmental factors may have different effects on in vitro than on in vivo parameters, as shown here by the improved antipyrine metabolism in smokers, in spite of an unaltered P450 content. The reason for this is that smoking does not enhance the cytochrome P450 content of the liver but may alter the amount of some minor P450 fractions, such as CYP1A1 in extrahepatic tissues and CYP1A2 in the liver.[39-42] From an ethical point of view, it is not possible to collect liver samples from healthy persons. We therefore had to use subjects with identical case histories and similar liver histopathologic conditions as control subjects. After this assumption, the effect of aging on the human drug-metabolizing ability seems to be logical and resembles that observed in experimental studies.

In conclusion, we can point out that age has a reducing effect on liver drug metabolism in humans. Before this conclusion can be reached, specimens must be obtained from persons with uniform histopathologic conditions. P450 content, an in vitro parameter, was altered stepwise, whereas antipyrine metabolism, an in vivo index, declined linearly after 40 years. Our data have importance for the selection of subjects for pharmacokinetic studies of new drugs: at least three age groups—namely, young, middle-aged, and elderly—ought to be included. Our data also suggest that caution is needed when drugs that are eliminated by the liver to are prescribed elderly subjects because the possibility of high blood levels caused by reduced metabolism or drug-drug interaction is increased.

References

1. Kato R, Vassanelli P, Frontino G, Cheara E. Variation in the activity of liver microsomal drug-metabolizing enzymes in rats in relation to age. Biochem Pharmacol 1964;13:1037-51.
2. Kamataki T, Maeda K, Shimada M, Kitani K, Nagai T, Kato R. Age-related alterations in the activities of drug-metabolizing enzymes and contents of sex-specific forms of cytochrome P450 in liver microsomes from male to female rats. J Pharmacol Exp Ther 1985;233:222-8.
3. Loi CM, Vestal RE. Drug metabolism in the elderly. Pharmacol Ther 1988;36:131-49.
4. Durnas C, Loi CM, Cusak BJ. Hepatic drug metabolism and aging. Clin Pharmacokinet 1990;189:359-89.
5. O'Malley K, Crooks J, Duke E, Stevenson IH. Effect of age and sex on human drug metabolism. Br Med J 1971;3:607-9.
6. Abernethy DR, Greenblatt DJ. Impairment of lidocaine clearance in elderly male subjects. J Cardiovasc Pharmacol 1983;5:1093-6.
7. Hust LK, Fleishaker JC, Peters GR, Hardy JD, Wright M, Ward P. Effect of age and gender on tirilazad pharmacokinetics in humans. Clin Pharmacol Ther 1994;55:378-84.
8. James OFW, Rawlins MD, Woodhouse K. Lack of ageing effects on human microsomal monooxygenase enzyme activities and on inactivation pathways for reactive metabolic intermediates. In: Kitani K, editor. Liver and aging, Amsterdam: Elsevier Biomedical Press, 1982:395-406.
9. Woodhouse KW, Mutch E, Williams FW, Rawlins MD, James OFW. The effect of age on pathways of

drug metabolism in human liver. Age Ageing 1984;13: 328-34.
10. Wynne HA, Murch E, James OFW, Wright P, Rawlins MD, Woodhouse KW. The effect of age upon the affinity of microsomal monooxygenase enzymes for substrate in human liver. Age Ageing 1988;17: 401-5.
11. George G, Wynne HA, Woodhouse KW. The association of age with the induction of drug-metabolizing enzymes in human monocytes. Age Ageing 1990:19: 364-7.
12. Schmucker DL, Woodhouse KW, Wang RK, Wynne H, James OF, McManus M, et al. Effects of age and gender on in vitro properties of human liver microsomal monooxygenases. Clin Pharmacol Ther 1990;48: 365-74.
13. O'Mahony MS, Woodhouse KW. Age, environmental factors and drug metabolism. Pharmacol Ther 1994; 61:279-87.
14. Woodhouse KW. Modification of drug metabolism in relation to age in humans [abstract]. Therapie 1995; 50(suppl):25.
15. Schaffner F, Popper H. Cholestasis is the result of hypoactive hypertrophic smooth endoplasmic reticulum in the hepatocyte. Lancet 1969;2:355-9.
16. Sotaniemi EA, Ahlquist J, Pelkonen RO, Pirttiaho H, Luoma PV. Liver parenchymal changes and indices of drug metabolism in alcoholics. Eur J Clin Pharmacol 1977;11:295-303.
17. Sotaniemi EA, Sutinen S, Sutinen S, Arranto AJ, Pelkonen RO. Liver injury in subjects occupational exposure to chemicals in low doses. Acta Med Scand 1982;212:207-15.
18. Sotaniemi EA, Niemelä O, Risteli L, Stenbäck F, Pelkonen RO, Lahtela JT, et al. Fibrotic process and drug metabolism in alcoholic liver disease. Clin Pharmacol Ther 1986;40:46-55.
19. Sotaniemi EA, Rautio A, Lumme P, Arvela P. Age and CYP3A4 and CYP2A6 activities marked by the metabolism of lignocaine and coumarin in man. Therapie 1996;51:363-6.
20. Vermeulen A, Stoica T, Verdonck L. The apparent free testosterone concentration: an index of androgenecity. J Clin Endocrinol 1971;33:759-67.
21. Vermeulen A, Rubens R, Verdonck L. Testosterone secretion and metabolism in male senescence. J Clin Endocrinol 1972;34:730-5.
22. Greim H, Schenkman M, Klotzbucher M, Remmer H. The influence of phenobarbital on the turnover of hepatic microsomal cytochrome b5 and cytochrome P450 in the rat. Biochim Biophys Acta 1970;201:20-5.
23. Arranto AJ, Sotaniemi EA. Morphologic alterations in patients with alpha-methyldopa–induced liver damage after short and long-term exposure. Scand J Gastroenterol 1981;16:853-63.
24. Arranto AJ. Histological and morphological studies on xenobiotics induced liver injury [thesis]. Acta Universitatis Ouluensis, Oulu, Finland, 1982. 53 p.
25. Vestal RE, Norris AH, Tobin JD, Cohen BH, Shock NW, Andres R. Antipyrine metabolism in man: influence of age, alcohol, caffeine, and smoking. Clin Pharmacol Ther 1975;18:425-32.
26. Sotaniemi EA, Pelkonen RO. Drug metabolism in man: in vivo and in vitro correlations. In: Sotaniemi EA, Pelkonen RO, editors. Enzyme induction in man. London: Taylor & Francis, 1987:79-93.
27. Vesell ES. Genetic and environmental factors affecting drug disposition in man. Clin Pharmacol Ther 1977;17:48-56.
28. Kappas A, Anderson KE, Conney AH, Alvares AP. Influence of dietary protein and carbohydrate on antipyrine and theophylline metabolism in man. Clin Pharmacol Ther 1976;20:643-54.
29. Schmucker DL, Wang RK. Age-related changes in liver drug metabolism: structure vs function. Proc Soc Exp Biol Med 1980;165:178-87.
30. Schmucker DL, Mooney JS, Jones AL. Stereological analysis of hepatic fine structure in the Fischer 344 rat: influence of sublobular location and animal age. J Cell Biol 1978;73:319-37.
31. Schmucker DL, Vessey DA, Wang RK, James J, Maloney A. Age-dependent alterations in the physicochemical properties of rat liver microsomes. Mech Ageing Dev 1984;27:207-17.
32. Engel G, Hoffman U, Heideman H, Cosme J, Eichelbaum M. Antipyrine as a probe for human oxidative drug metabolism; I: identification of the cytochrome P450 enzymes catalyzing 4-hydroxyantipyrine, 3-hydromethylantopyrine, and norantipyrine formation. Clin Pharmacol Ther 1996;59:613-23.
33. Sharer JE, Wrighton SA. Identification of the human hepatic cytochromes P450 involved in the vitro oxidation of antipyrine. Drug Metab Dispos 1996;24:487-94.
34. Brodie MJ, Boobis AR, Bulpitt CJ, Davies DS. Influence of liver disease and environmental factors on hepatic monooxygenase activity in vitro. Eur J Clin Pharmacol 1981;20:30-46.
35. Sotaniemi EA, Pirttiaho HI, Stenbäck FG. Drug interaction involving changes in liver size and fibrogenesis. Int J Clin Pharmacol Ther Toxicol 1992;30:523-35.
36. 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;35:923-32.
37. Adir J, Miller AK, Vestal RE. Effects of total plasma concentration and age on tolbutamide plasma protein binding. Clin Pharmacol Ther 1982;31:488-93.
38. Anttila M, Laakso S, Nyländen P, Sotaniemi EA. Pharmacokinetics of the novel antiestrogenic agent

toremifene in subjects with altered liver and kidney function. Clin Pharmacol Ther 1995;57:628-35.
39. Conney AH. Pharmacological implications of microsomal enzyme induction. Pharmacol Rev 1967;19:317-66.
40. Pasanen M, Haaparanta T, Sundin M, Sivonen P, Vähäkangas P, Raunio H, et al. Immunochemical and molecular biological studies P450-dependent monooxygenase activities. Toxicology 1990;62:175-87.
41. Sesardic D, Pasanen M, Pelkonen O, Boobis AR. Differential expression and regulation of members of the cytochrome P450 1A gene subfamily in human tissues. Carcinogenesis 1990;11:1183-8.
42. Pelkonen O, Pasanen M, Kuha H, Gachalyi B, Kairaluoma M, Sotaniemi EA, et al. The effect of cigarette smoking on 7-ethoxyresorufin O-deethylase and other monooxygenase activities in human liver: analyses with monoclonal antibodies. Br J Clin Pharmacol 1986;22:125-34.