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# FACTORS THAT INFLUENCE DRUG BIOTRANSFORMATION

## INTRODUCTION

An account of the influence of species, sex, age, hormonal status and disease state on drug biotransformation forms the major part of this paper, these parameters collectively being referred to as ‘intrinsic factors’. Reported results of several recent studies of these factors are reviewed, with the aim of indicating their variable nature as well as their interdependence. Thus, for example, we will encounter cases where for certain drugs, there are only subtle differences in the biotransformation routes in different species, while for others, dramatically different pathways are adopted, leading to the formation of vastly different metabolic products. Interdependence of most of these factors is a natural expectation, given that the status of an individual’s metabolizing activity and pathological status vary over a lifetime. Thus, for example, the effects of natural attrition of the metabolizing activity in an aged patient and a specific disease state can interact in a way that results in a unique mode of metabolic clearance of a drug. One significant point emerges from the discussion, namely the variability in the outcomes of drug metabolism observed for different species. This unpredictable element reminds us of the caution that should be exercised in extrapolating from animal studies to humans and the implications that this has in the evaluation of new drugs in the pharmaceutical industry. The final section of this paper summarizes the effects of external (environmental) factors on drug biotransformation, examples of which were encountered in earlier subtopics. Continual introduction of new chemical substances into the environment through waste production and industrial activity remains a major international issue, necessitating inter alia on-going studies of their effects on human drug metabolism.

## INTRINSIC FACTORS

Drugs, as well as other xenobiotics are metabolized via various pathways, including phase I and phase II reactions, which involve participation of numerous enzyme systems. Therefore, it is reasonable to assume that there are many factors that can determine or influence along which pathway a particular drug will undergo biotransformation and the extent to which this will proceed. These factors are usually arbitrarily divided into internal and external factors, with nevertheless considerable interaction between them.

### Species

Examples of species differences in drug biotransformation are numerous, continuously investigated, and encountered in both phases of biotransformation. An interesting observation is that they may involve the same route, but differ in the rate along that particular pathway (i.e. quantitatively different) or they may adopt different pathways (i.e. differing qualitatively). It should be noted as well that there is not always a direct relationship between metabolism, half-life and action of a drug. Selected examples an interesting quantitative species difference in phase I metabolism is known for caffeine, both in terms of total metabolism and metabolite production. Thus, the total metabolism is highest in humans, decreasing in the order - monkey, rat and rabbit. While there are no significant differences in the formation of theobromine, marked differences have been recorded for the other two metabolites, paraxanthine and theophylline, with paraxanthine formation highest in humans and lowest in monkey, whereas the reverse obtains for theophylline. An interesting aspect is the way caffeine biotransformation reactions proceed in higher plants, the variability of caffeine catabolism again being dependent on species and to a greater extent, on the age of different tissues investigated. As an example, it was reported that in young tea leaves, theophylline is re-utilized for caffeine biosynthesis, while in aged leaves of Coffea Arabica, it undergoes further metabolism resulting in 7-methylxanthine accumulation. Other species of Coffea have been proven to convert caffeine to methyluric acids. Obviously, these cases exemplify qualitative differences, as well as species- and age-dependence. A well-known quantitative example is that of species variation in hexobarbitone metabolism, affecting half-life and sleeping time. Investigations have been made on man, dog, mice and the rat. The

Longest half-time was registered for man (~360 min). The sleeping time increased in the following order: mice, rats, dogs and man. The main conclusion of the experiment, apart from demonstrating that the oxidative metabolism of hexobarbitone is markedly influenced by species, was that the biotransformation is inversely related to the half-time and duration of action of the investigated drug, the highest metabolism being registered for mice and decreasing in the opposite order as for the sleeping time for example. A recent example refers to the variation in the metabolism of selegiline ((-)-form of deprenyl) in seven different species. From literature data, it is known that selegiline undergoes N-dealkylation, yielding several metabolites, namely N-desmethylselegiline, methamphetamine and amphetamine.

The investigations made during the study referred to, and performed on liver microsomes of different species, in addition to characterizing the potential metabolic variations, also proved the existence of another metabolite, the N-oxide. The rate and extent of formation of this metabolite was found to be markedly influenced by species, the highest rate of production occurring in dog and hamster, being much lower in humans, and zero in the rat. Another example of quantitative variation was revealed from experimental studies investigating the metabolic profile of a relatively novel diuretic. A comparative approach was adopted, aimed at demonstrating its metabolism in experimental animals and human liver microsomes. Increased rates of metabolism were observed in rats and monkeys, and six metabolites, designated RU1, RU2, RU3 and MU1, MU2, MU3 for the respective species, were identified in their urine. Quantitatively, only three of these were considered to be major metabolites in rat and monkey urine, namely RU3, RU1 and MU3 respectively, whereas in the dog, the unchanged drug was observed as the major urinary component. This indicated a net difference between the rat and the monkey, both displaying extensive biotransformation, and the dog, in which only little metabolism occurred. In contrast with dogs, humans showed similarities with rats, suggesting a common metabolic pathway.

Six species have been investigated in connection with the psychoactive drug of abuse 4-bromo-2,5-dimethoxyphenethylamine (2C-B) (street names ‘Venus’, ‘Bromo’, ‘Erox’, ‘XTC’ or ‘Nexus’)

Hepatocytes from human, monkey, dog, rabbit, rat and mouse were incubated with 2C-B in an attempt to identify the resulting metabolites and to monitor possible toxic effects. Investigations established that the drug under study undergoes oxidative deamination with successive formation of two metabolites, which may or not undergo further metabolism by demethylation. Marked differences were noticed with two other, less common metabolites identified, one of these occurring only in mouse hepatocytes, the other in human, monkey and rabbit, but not in dog, rat and mouse, supporting the idea of qualitative interspecies variations. Another aim of the study, as mentioned above, was to compare the toxic effects exerted by 2C-B on hepatocytes of the six investigated species: the differences observed were only minor. However, another important aspect was revealed, namely that large differences in susceptibility of hepatocytes may occur between different individuals. The biotransformation pathways of a relatively novel drug used as an acute oral treatment for migraine, namely zolmitriptan, were comparatively investigated in human and rat liver microsomes.

Although the reports indicated that the drug was metabolized by the same CYP isoform in both types of microsome, the numbers of metabolites nevertheless differed. This suggests that the report presents a reasonable and economical in vitro model for comprehensive studies of zolmitriptan metabolism, including biotransformation pathways, enzyme kinetics, induction and inhibition phenomena, interspecies differences and the possible occurrence of drug interactions. An interesting study, involving both phase I and phase II biotransformations, has been performed in an approach using comparative interspecies data for both prospective design and extrapolations from animal findings to humans. The aim was to reduce the potential for human risk and increase therapeutic benefit. For paclitaxel for instance, markedly different metabolites were observed to occur in rats and humans, which renders metabolic drug-drug interaction investigations in rats practically irrelevant for humans (thus, qualitative differences). In contrast, for zidovudine (AZT), the variations were quantitative, with a high rate of glucuronidation in humans, resulting in a much shorter half-life than that observed in animals, which display negligible glucuronidation. This study revealed more significant features: qualitative differences in phase I biotransformation and quantitative variations in phase II, with no relevant similarities to allow extrapolations and drug-drug interaction predictions from animals to humans.

Advanced analytical procedures (e.g. LC/MS, high field NMR spectroscopy) have been used to examine the potential differences in the biotransformation of efavirenz, a potent and specific inhibitor of reverse transcriptase commonly recommended in the treatment of HIV infections. Metabolites produced by humans, rats, guinea pigs, hamsters and monkeys were investigated. Observations confirmed that efavirenz extensively metabolized by all species, with marked species differences in the metabolites isolated and structurally determined. Although the major metabolite, namely the O-glucuronide conjugate, proved to be common to all five species studied, other metabolites displayed species specificities as follows: the sulphate conjugate was found in rats’ and monkeys’ urine, but not in that of humans, while GSH-related metabolites were identified only in the urine of rats and guinea pigs.

Differences in the production of reactive metabolites may sometimes result in species-selective nephrotoxicity. For example, efavirenz was reported to produce renal injury (necrosis of the renal tubular epithelial cells) in rats, but not in monkeys or humans. Here, a species-specific glutathione adduct, produced only by rats, was deemed responsible for this nephrotoxic effect. Species differences involve, as mentioned above, both phases of biotransformation. An interesting study was performed to investigate the maintenance of drug-metabolizing capacities in collagen gel sandwich and immobilization cultures of human and rat hepatocytes. L-proline was added to the medium to improve albumin secretion. As far as most important phase I enzyme systems are concerned, namely the cytochrome P450 dependent monooxygenase (CYP) and microsomal epoxide hydrase (mEH) systems, comparative measurements of enzyme activities in the absence and presence of L-proline, revealed that their biotransformation enzyme activities were not affected by the addition of L-proline. Instead, the activity of an important phase II enzyme, GST, was decreased in rat hepatocytes, whereas in humans it remained almost unchanged. As human hepatocytes showed a better maintenance of GST activities than the rats in the presence of L-proline, species differences were again demonstrated. Another study investigated whether there are also species variations in maintaining certain phase I and phase II enzyme activities after cryopreservation of liver slices prepared from five different species, namely mouse, rat, dog, monkey and human. The conclusion of the study was that although the metabolic patterns and rates of biotransformation varied among these species, the phase I and phase II metabolic capacities of the liver slices were well maintained after cryopreservation. For certain drugs, and depending upon the species investigated, variations have not proven to be very significant. For example, an experiment concerning orbifloxacin metabolism in two species, pigs and calves, aimed at establishing possible species differences, proved that in both species the metabolic pathway of the drug was the same, differing only in the amount of the excreted metabolite. Indeed the final, common metabolite was the glucuronide, excreted in average amounts of 3% and 1% in pigs and calves respectively. In addition, the remainder of the drug was excreted unchanged in both species. However, a qualitative difference was noted, namely that calf urine contained also a product of oxidative metabolism. Quantitative species differences were established for the immunosuppressive drug cyclosporine A (CSA) Investigations were performed on liver and small intestinal microsomes from rat, hamster, rabbit, dog, baboon and man.

The metabolic pathways of CSA are known to result in two principal metabolites, the hydroxylated and N-demethylated CSA, which accounted for most of the CSA metabolized in all tested species. However, marked variations occurred in the biotransformation rate, measuring only 2-8% over 30 min in rats, in contrast to dogs, whose liver microsomes proved to be very efficient, yielding a 70-100% change in the same period. Investigations having been performed on both liver and small intestinal microsomes, another objective of the study was to determine possible differences determined by different tissues of the same organism. Measurements of the formation of the principal metabolites in the two investigated organs indicated a similar metabolic profile, but with differences in the rate of metabolism, that in the small intestine being slightly slower. Differences in the metabolic profiles were the subject of investigation for panomifene, an analogue of tamoxifen, an anti-estrogen for hormone-dependent tumors. Liver microsomes from mouse, rat, dog and human were used. The observed routes of biotransformation were hydroxylation and side chain modifications. Although seven metabolites were detected in the incubated mixtures, there was only one produced by all species that had lost the side chain. Interspecies differences concerned the metabolites with the truncated side chain, as follows: in the case of rodents, the microsomal system led to loss of the hydroxyethylamino group, while for incubated mixtures containing microsomes of all three other investigated species, only the loss of the hydroxyethyl group was detected. Other important observations made during the experiment were (a) that of the seven metabolites detected, three were produced exclusively by the dog and (b) that human liver microsomes produced an oxidized form of the metabolite containing a double bond in the side chain, this compound not being detectable in the other species investigated.

Different profiles, as well as quantitative species differences, were observed in the metabolism of L-775,606, a selective 5-HT1D receptor agonist, developed for the acute treatment of migraine. Species investigated included human, dog, monkey and rat. For three of these (human, monkey and rat), the main metabolites were the hydroxylated M1 and the N-dealkylated M2. In contrast, in the dog the N-oxide metabolite (M3) was prevalent, representing an average of about 40%, whereas in the other investigated species, its formation represented a minor pathway, with the excreted metabolite corresponding to less than 5%. In an interesting experiment accomplished both in vitro and in vivo, the metabolic fate and the toxicity of dapsone were comparatively investigated in rat, mouse and man. The metabolites were determined by HPLC/MS and metHb formation was used as toxic endpoint. The investigations focused especially on the toxic aspects and possible consequences during dapsone administration. As for the in vitro investigations, the results revealed that the greatest toxicity occurred in rats, with a significant difference between sexes: ∼36% metHb formed in males and only 8.2% in females. In humans, the metHb toxic metabolite was found in an amount of ~11%, while in the mouse, only 4% under the same conditions. The rank order of toxicity was in direct relation to the formation of the hydroxylamine metabolite in vitro. However, experiments proved that the microsomes from all tested species were able to reverse the reaction, reducing the hydroxylamine back to dapsone. In contrast, under in vivo conditions the species most susceptible to dapsone toxicity proved to be the human, the sensitivity to toxic effects decreasing in the order: human, mouse, rat. Interspecies and sex differences also occurred in the biotransformation of the drug, in that the hydroxylamine and its glucuronide were detected only in male rats and humans, but not in female rats or mice.

Species differences may also account for stereoselective reactions. Experiments were performed with fifteen O-acyl propranolol (PL) prodrugs, using rat and dog plasma and liver subfractions. The aim of the study was to investigate both species differences and substrate specificities for the stereoselective hydrolysis of the tested prodrugs. As far as species was concerned, significant differences in the hydrolytic activities of prodrugs were established, in rat plasma being in the range of 5-119-fold greater than those in dog plasma. In contrast, dogs displayed a higher hepatic hydrolytic activity, especially in cytosolic fractions. The significant differences in the hydrolytic rates therefore represent quantitative species differences. As for stereoselectivity, the study also revealed important interspecies differences: hydrolysis in dogs generally showed a preference for the (R)-enantiomer, whereas in the rat, for all of the prodrugs containing substituents of low carbon number, the (S)-enantiomer was preferentially hydrolysed. Following a previous report of species differences in the tolerability of rhein (a constituent of rhubarb), with rabbits displaying the highest susceptibility to kidney disturbances, a complex phase I and phase II metabolic investigation was performed in an attempt to elucidate species differences in the biotransformation of this compound. Experiments were performed in vivo, with 14C-labelled rhein; tested species included the rat, rabbit, dog and man. The common major metabolites determined in all tested species were the phenolic monoglucuronide and monosulphate. The urine samples of rabbits showed an additional hydrophilic metabolite fraction. The in vitro experiments performed on subcellular liver fractions of rabbits revealed the presence of several metabolites, including three monohydroxylated metabolites, their corresponding quinoid oxidation products and a bis-hydroxylated derivative. The hydroxylated phase I metabolites were further detected as glucuronides in all tested species, whereas the quinoid product was found only in rabbit urine. It is assumed that this metabolite displays a potential reactivity with endogenous macromolecules and generates that species-dependent susceptibility. Species differences can also impact on inhibition phenomena. Investigations of the inhibition of pentobarbital biotransformation in the presence of empenthrin support this idea.Empenthrin (a synthetic pyrethroid) has been reported to display an inhibitory effect on pentobarbital metabolism, resulting in prolongation of the sleeping time.

This phenomenon was observed for mice (the inhibitory effect being determined as dose-dependent), but not for other species investigated, namely rats, dogs, guinea pigs or hamsters. Further experiments using microsomal fractions expressing human CYPs were performed to determine the possible inhibitory effect of empenthrin on pentobarbital metabolism in humans. The final results revealed that the inhibition of pentobarbital by empenthrin occurred only in mice and not in any other of the other species investigated, including humans. As previously mentioned, species differences may be implicated in several aspects, including qualitative and quantitative differences in drug biotransformation route, influences on stereoselective biotransformations and even on inhibition phenomena. An interesting and relatively recent study revealed the impact of species differences also on the distribution of drug metabolising enzymes. Complex investigations followed the expression of nine CYP450 isoenzymes and three GSTs in the pancreas of several species including humans. The seven species tested in comparison to humans, were mice, hamsters, rabbits, rats, dogs, pigs and monkeys. A first finding was the large variation in the cellular localisation of the enzymes among the eight investigated species, with most of the enzymes expressed only in the pancreas of hamster, mouse, monkey and man. The other tested species were lacking several enzyme isoforms. However, in human tissue, four enzymes were lacking in almost half the cases. All of these observations concerning interspecies differences in the distribution of some of the most important drug-metabolising enzymes support the notion that great caution needs to be exercised when attempting to predict or extrapolate from animal data to humans. This last observation confirms the importance of species differences, especially for drug-design in the pharmaceutical industry, where a suitable model reflecting human patterns of biotransformation and toxicity is desirable.

### Sex

As already indicated in some of the above examples, qualitative and quantitative differences in both phases of drug metabolism are related to sex as well. Initial observations of this feature were made in the early 1930s, when researchers noticed that female rats required only half the dose of a barbiturate compared to male rats to induce sleep. Later investigations indicated that this was due to the reduced capacity of the female to metabolise the barbiturates.

Sex differences have been intensively studied, not only in relation to sex-dependent metabolism of various xenobiotics, but also with the aim of correlating sex-dependent pharmacokinetics, pharmacodynamics, efficacy, and the possible occurrence of adverse reactions. Sex differences, sometimes related to species or age, are now being observed for a wide range of substrates, including commonly prescribed drugs or even endogenous compounds, including steroid sex compounds. Like other factors that influence drug metabolism, sex differences are considered to determine also biotransformation variations. Therefore, before introducing a new drug into therapy, combined studies investigate both species and sex differences on the metabolic profile of the candidate. As an example, we refer to such a combined study for the in vitro investigation of sex and species differences in the metabolism of BOF-4272, a drug intended for the treatment of hyperuricaemia. Rats, mice and monkeys of both sexes were used in the study. The results of the investigations made on various incubation mixtures revealed that both the pathways involved (i.e. types of metabolites resulting) as well as the rates of biotransformation of the tested drug were significantly influenced by both sex and species differences. On the other hand, results of other investigations examining the influence of sex and age on different enzyme activities showed no significant differences.

### Age

It has long been recognized that the newborn, young and elderly display marked differences in drug biotransformation and are more susceptible to drug action. These differences are chiefly due to the enzymatic systems involved in drug biotransformation and the development of their metabolising capacity. Thus, the increased sensitivity of neonates may be related to their very low, undeveloped metabolising capacity, until adult levels of enzyme activity are achieved. On the other hand, in the elderly, the decrease in drug-metabolising capacity also appears to be dependent on these factors, important changes in the overall metabolism occurring with ageing. An important aspect to be borne in mind is that the factors influencing drug metabolism are split arbitrarily and that they are interrelated. Examples have been given so far regarding species, sex and age. We should also highlight the fact that the status of enzymatic systems and their metabolising capacity may develop in many different ways, the patterns varying and being dependent on the species and sex. Thus, a very recent study in fact reviewed the influence of age and sex on CYP enzymes in relation to drug bioequivalence.

The concern for controlling drug therapy, especially in the elderly to provide desired pharmaceutical effects at lower risks, continues to be a principal aim of research. Specific aims include efforts to try and prevent adverse reactions and to optimise therapy for the individual patient. Unfortunately, as already mentioned, important changes in drug metabolism do indeed occur with ageing. For example, the significant reduction in liver volume accompanying ageing will be reflected in a reduction in the total amount of cytochrome P450 produced, and this could be associated with reduced ability of these enzymes to function. Other problems occurring with ageing, still not very well understood and needing to be revisited in view of recent advances, include the following: the effect of age on extrahepatic enzymes (especially CYPs), the impact of induction and inhibition phenomena on enzymatic systems in the elderly, the effect of the environment on drug metabolism in the aged given the increasing complexity of the CYPs involved in human metabolism, pharmacology and function of transporters, the decline in general metabolic capacity, and general frailty of older people. Taking cognisance of the above, it is understandable and expected that all these conditions will result in altered drug handling and especially, altered pharmacodynamic responses. Recognizing the central role of the liver in the general metabolism of both drugs and other xenobiotics, we should also mention, besides the reduction of hepatocyte mass (with corresponding effects on the hepatic enzyme system activity), the reduction of hepatic blood flow and changes in sinusoidal endothelium. These changes will affect drug transfer and oxygen delivery, resulting in reduced hepatic drug clearance. Another current problem in the elderly is related to renal clearance reduction, which is generally disease-related. Altered pharmacokinetics and pharmacodynamics are expected also in patients with cardiovascular diseases. Also worth remembering is the effect of age on pancreatic secretion. But perhaps one of the major problems resulting in adverse reactions and drug-drug interactions is the still very common practice of polypharmacy, responsible for increased morbidity and mortality in the elderly. This is another aspect that is peculiar to elderly patients, who consume a disproportionate amount of prescription and non-prescription medications. Such practice can obviously lead to many negative consequences, primarily placing the elderly at risk of developing significant drug-drug interactions, which often go unrecognized clinically and which are responsible for increased morbidity in this sector of the population. Drugs can interact to mutually alter absorption, distribution, metabolism or excretion characteristics, or interact in a synergistic or antagonistic fashion altering their pharmacodynamics. In addition, one must be aware that co-administered drugs, foods and nutritional supplements can also alter the pharmacological actions of a medication. These alterations may cause the action of a drug to be diminished or enhanced. Another major issue is that drugs may also interact with diseases, potentially worsening disease symptoms. Therefore, prudent use of medications and vigilant monitoring are essential for preventing the elderly from the high risk of adverse reactions and drug-drug interactions, whose unfortunate consequences have been noted above. Considering the physiological changes in main organ functions in the elderly as well as the pharmacokinetic parameters of various drugs, accumulations of drug metabolites presents another important problem. In this context, particular attention should be paid to an adequate treatment scheme designed to ensure the optimum therapeutic effect with a minimum risk of toxic effects. In fact, a starting dose which is 30-40% less than the average dose used in adults is generally recommended, not only for renally excreted drugs, but also for compounds metabolised and excreted by the liver. Ageing is directly related to ovarian hormonal activity, and progesterone metabolites, specifically, have been proven to affect the response to various psychotherapeutic agents, resulting in increased risk of adverse effects. Studies on benzodiazepines, for example, demonstrated that their metabolism is altered, either resulting in a decrease in their clearance or an alteration of the effect-concentration relationship. These effects may result in increased risk of adverse reactions, particularly in older patients with anxiety disorders. Therefore, establishing the appropriate low dose for optimal treatment will minimise adverse effects. The intimate mechanisms involved are not completely understood, but it has been suggested that they could be related to modulation of the GABA-antagonist receptor by neurosteroids. Other drugs that were investigated with respect to the role of drugmetabolising enzymes and the effects of age included different alkylphenoxazone derivatives, benzodiazepines and neuroleptics, bisphosphonates (BPs) as therapeutic drugs for osteoporosis, anxiolytics and others. A special category includes ‘ultra-aged’ patients. Aspects concerning decreased drug absorption, metabolism and excretion, decline of protein binding, lower blood flow, disturbance of blood brain barrier, adverse reactions and drug interactions for this category of patients have been reviewed, with the purpose of establishing proper therapeutic management.

Two other important aspects of age-related changes are sensitivity to environmental factors and nutritional effects on hepatic drug metabolism in the elderly. The cited works review pharmacodynamic and toxicokinetic changes in absorption, distribution, metabolism, excretion and sensitivity, as well as age-associated differences in hepatic drug metabolism, and the effects of nutrition on drug bioavailability, distribution and hepatic metabolism. An important issue in improving the quality of life of the elderly has recently been reviewed and concerns CoQ10 implications in energetic metabolism, a well-known anti-oxidant effect with relevance to health food and medical drugs. At the other end of the scale, special attention is paid to neonates and children, as regards the development of their enzymatic systems. Unpredictable developmental changes in drug biotransformation have been proven to play a role not only in the pharmacokinetic profile, but also in the pathogenesis of adverse drug reactions in children. Most of these developmental changes have a genetic determinant, which causes variations in different metabolising enzymes, whereby normal, therapeutic drug doses can result in functional overdoses due to drug accumulation. This relative overdosing is determined by inefficient elimination via the affected pathways. Furthermore, idiosyncratic forms of toxicity may occur when a relative increase in reactive metabolite formation is due to imbalances in bioactivation and detoxification processes. Phenotyping and genotyping would be very helpful under such circumstances to prevent these effects. Extra-hepatic metabolism has to be considered as well, the renal clearance and volume of distribution being at least as important as hepatic metabolism. Typically, drug metabolism is significantly reduced in the neonatal period because of lack of enzymatic activity. A recent investigation reviewed the effect of age on the biotransformation of four drugs. The subjects were infants and children, and the tested drugs included caffeine, midazolam, morphine and paracetamol. The first observation was that in the neonatal period, for all four tested drugs, clearance was markedly reduced. Further observations confirmed that (with the exception of paracetamol) this reduced clearance is maintained in infants and children under the age of two years, and that there is considerable inter-individual variation in clearance values for all ages and for all tested drugs, appearing to be the greatest for midazolam. The third important observation suggests that for children older than two years, the mean plasma clearance values for all four drugs are more or less similar to those in adolescents and even adults.

### Pathological status

The way in which the body clears drugs is affected by many disease states. Among them, those of primary concern are considered to be diseases affecting the liver: cirrhosis, alcoholic liver disease, cholestatic jaundice, and liver carcinoma. Other factors responsible for variation in drug metabolism are the endocrine disorders, such as diabetes mellitus, hypo-and hyperthyroidism, pituitary disorders, and various types of infections (bacterial, viral, malaria). In cirrhosis for example, replacement of parts of the liver by fibrous tissue leads to a reduction in the number of functional hepatocytes. In this situation, it seems absolutely reasonable that drug metabolism should be impaired. It is known for example that human cytochromes P450, particularly the CYP2A6 isoform, catalyse the bioactivation of various drugs and even carcinogens. Recent studies proved that in cases of liver disease, including cirrhosis (but also viral hepatitis or parasitic infestation), this isoform is over-expressed, and as such may therefore be considered a major liver catalyst in pathological conditions. An important consequence of such liver disease (or other organ impairment) arises during transplantation processes; it is well known that prior to transplantation, organ dysfunction may occur because of stress and anxiety, and this may result in altered pharmacokinetic behaviour of some psychotropic agents. In case of cirrhotic patients, an increased drug bioavailability due to portosystemic shunting was noted, which therefore therefore required drug dosage adjustment. Studies on different psychotropic agents suggest that a selection of these, concurrently administered with an appropriate dosage adjustment, could ensure the lowering of risk of drug accumulation. Another recent article reviews the implications of oxidative stress and the role of cytochrome P450s and cytokines in drug-induced liver diseases, which according to some recent studies can be also induced by immunological mechanisms. In this context, we should mention that especially in the last few years, great importance has been attributed to antioxidants in the treatment of druginduced liver oxidative stress, due to the central role of this organ in the general metabolism. Effects of natural antioxidants have been investigated in vitro on liver redox status by biochemical, analytical and histological methods, in order to assess the overall free radical-antioxidant balance.

Studies have also been performed in animal models and in humans with Gilbert’s disease and alcohol liver disease. The results confirmed the role of free radicals in alcoholic patients, stressing the greater vulnerability of women to alcohol toxicity. As regards Gilbert’s disease, investigations found no alterations of free radical-antioxidant balance, but in contrast, an improvement in the non-enzymatic antioxidant defense system. The impact and consequences of drug-induced liver diseases on drug pharmacokinetics and toxicity in the case of pathogenesis are continuously investigated. Recently, the role of polymorphism of drug metabolising enzyme systems has been reviewed. A comparative study was performed on normal mice to investigate the effects of drug-induced liver injury using prednisolone (PSL) versus Angelica sinensis Polysaccharides (ASP), on hepatic metabolising enzyme activities of both phases. ASP was shown to increase content and catalytic activity of several enzymes viz. CYTP450, different demethylases and hydroxylases, and GSH-related enzymes. In contrast, PSL significantly decreased the liver mitochondrial glutathione content, whereas all other enzyme activities were increased. An important observation was that treatment with ASP could restore the GSH content, which is important for detoxication (by glutathione conjugation) of certain xenobiotics, including drugs. An interesting aspect recently investigated concerns the CYTP450 superfamily. The multiple CYP450 isoforms (CYPs) are well known as being involved in the biotransformation of numerous drugs, other chemicals, as well as endogenous substrates. Unfortunately, the hepatic CYPs may also be involved in the pathogenesis of several liver diseases, due to their catalytic activity mediating activation of certain drugs to toxic metabolites. Incidences of drug-induced hepatotoxicity, as well as nephrotoxicity and cardiac failure are well known and unfortunately relatively frequent. The most frequently cited examples of hepatotoxicity refer to halothane and acetaminophen. There are usually several mechanisms involved in drug-induced liver disease. One of them is an immunological one, presumably determined by the covalent binding of the metabolite to CYP, which will result in formation of anti-CYP antibodies, leading to so-called ‘immune-mediated hepatotoxicity’. Another mechanism, related to the CYP2E1 isoform, is associated with lipid peroxidation and production of reactive oxygen species, resulting in damage to hepatocytes and mitochondrial membranes. The explanation for involvement of this particular CYP isoform relies on the observation that in alcoholic patients, its levels are significantly increased. Thus, it was first associated with alcohol-liver disease and non-alcoholic steatohepatitis.

However, due to its ability to activate carcinogens, investigations also suggested a possible role of this isoform in hepatocellular carcinoma. Considering the liver as the main location for the most important enzymatic systems, it is expected on the other hand that in patients with liver diseases, drug metabolism should be impaired. Particularly vulnerable isoforms have been proven to be different CYPs such as 1A, 2C19 and 3A, while others (2D6, 2C9, 2E1) appeared to be affected to a lesser extent. An interesting feature is that the pattern of CYP isoenzyme alterations differs with the etiology of the liver disease, with the most severe modifications occurring in cirrhosis. Other liver diseases have also been proven to alter drug metabolism by altering the activities of metabolising enzymes. A prime example is alcohol-induced disease, unfortunately the most common type of chronic liver disease in many countries. An important aspect revealed by one study is that alcohol can interact with other factors of risk for hepatic disease, especially hepatitis C infection and also concurrent consumption of hepatotoxic drugs (acetaminophen, for example), resulting in more severe disease and increased risk of adverse reactions and drug-drug interaction occurrence, than occurs when alcohol alone is the risk factor present. Another interesting aspect to mention, demonstrated in a recent investigation on rats, is that hepatic and extrahepatic (e.g. intestinal) metabolic activities involving especially the cytochrome P450 system are influenced by surgery and/or drug-induced renal dysfunction. The most marked decreases (of about 66%) were observed for the hepatic CYP3A metabolic activities, in the case of nephrectomy. Less marked, but nonetheless significant decreases were observed also in drug-induced renal dysfunction following i.m. injection of glycerol (about 60%), and i.p. injection of cisplatin (about 49%). In contrast, the intestinal metabolic CYP3A activity was weakly increased in rats injected with glycerol, and remained practically unchanged in the case of injected cisplatin or surgery (nephrectomy).

These results suggest a dependence of the extent of lowering of hepatic P450 activities on the etiology of renal failure. In addition, the experimental observations led to the conclusion that alteration of the same enzyme activity in extrahepatic tissues (particularly in intestine, where this tissue was examined experimentally) cannot always be correlated with that in the liver.

### Hormonal control of drug metabolism – selected examples

Hormones, known to play a major role in the general metabolism, have similarly been proven to control the biotransformation of drugs, in direct connection with other factors such as age, sex, or in particular physiological states, such as pregnancy. An example is the apparent connection between certain sex-specific drug- and steroid-metabolising enzyme activities in rats and the sexdependent expression of those specific enzymes, under gonadal steroid and growth hormone control. Another sex and age connection with the control of the growth hormone (GH) was the focus of interesting cDNA cloning investigations. The study examined especially cytochrome P450, it being established that GH is involved in the control of rat hepatic drug- and steroid-metabolism, particularly through the action of this enzymatic system. The results showed low levels of CYTP450 in neonates, and an increase after one month, both in male and female rats. At adult stage, important sex differences were recorded, in female rats the content being about three times higher than in male rats. Thyroid status contributes to differences for several drugs administered in equi-active doses on several forms of UDPGTs. As experimental animals, rats having different thyroid hormonal status were employed, namely normal (control group), hypothyroid and hyperthyroid. The drugs tested were ciprofibrate, bezafibrate, fenofibrate and clofibrate. The responses were markedly modulated by the thyroid status, with an average increase of about 5% in hyperthyroid animals. The results confirmed the role of hormonal control upon the enzyme induction displayed by certain drugs (or other xenobiotics). The hypothalamo-pituitary-liver axis has also been proven to function as a hormonal control system in the metabolism of drugs and endogenous compounds.

## ENVIRONMENTAL FACTORS

These are usually considered to be those influences in our surroundings that can affect (sometimes markedly) drug metabolism. Of course, there are a large number of environmental chemicals that potentially could affect drug biotransformations, usually grouped into heavy metals, industrial pollutants and pesticides. The most important industrial pollutants are typically aromatic or aromatic polycyclic compounds and polychlorinated biphenyls. Many of these have been already discussed under different circumstances (inductive enzyme effects, procarcinogenic effects). Polychlorinated biphenyls (common industrial pollutants).

Pesticides are also of various types (insecticides, herbicides), and are considered environmental contaminants in air, soil, water and food. They will not be discussed further in the present monograph.

## FURTHER OBSERVATIONS

As has been discussed, there are numerous factors (some of them interactive) that can affect drug metabolism, therefore making its control an extremely complex problem. With the exception of genetic factors, all the rest are considered variable during a lifetime, so predictions are made with reservation. Also, since most of the studies are performed either in vitro or on experimental animals, extrapolations from the in vitro to the in vivo situation, or from animals to humans must be approached with extreme caution.