Liver and Drugs

edited by F. Orlandi
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Centre for the Study of the Action of Drugs on the Liver, University of Camerino and Division of Gastroenterology, General Hospital, Ancona, Italy

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Contributors

J.-P. BENHAMOU, *Unité de Recherches de Physio-Pathologie Hépatique, Hôpital Beaujon, 92 Clichy, France.*

L. CHIANDUSSI, *Institute of Medical Pathology, University of Turin, Italy.*

L. FEINMAN, *Section of Liver Disease and Nutrition, Veterans Administration Hospital, 139 West Kingsbridge Road, Bronx, New York 10468, USA.*

A. M. JEZEQUEL, *Centre for the Study of the Action of Drugs on the Liver, University of Camerino and Division of Gastroenterology, General Hospital, 60100 Ancona, Italy.*

C. S. LIEBER, *Section of Liver Disease and Nutrition, Veterans Administration Hospital, 139 West Kingsbridge Road, Bronx, New York 10468, USA.*

E. MARUBINI, *Institute of Biometrics and Medical Statistics, Via Venezian 1, Milano, Italy.*

F. ORLANDI, *Centre for the Study of the Action of Drugs on the Liver, University of Camerino and Division of Gastroenterology, General Hospital, 60100 Ancona, Italy.*

R. PREISIG, *Department of Clinical Pharmacology, University of Berne, Berne, Switzerland.*

E. RUBIN, *Section of Liver Disease and Nutrition, Veterans Administration Hospital, 139 West Kingsbridge Road, Bronx, New York 10468, USA.*

B. RUEFF, *Unité de Recherches de Physio-Pathologie Hépatique, Hôpital Beaujon, 92 Clichy, France.*

G. SEGRE, *Institute of Pharmacology, University of Siena, Via Banchi di Sotto, Siena, Italy.*

S. SHERLOCK, *Department of Medicine, Royal Free Hospital, London WC1, England.*

C. SICOT, *Unité de Recherches de Physio-Pathologie Hépatique, Hôpital Beaujon, 92 Clichy, France.*

E. S. VESELL, *Department of Pharmacology, Milton S. Hershey Medical Centre, Pennsylvania State University, Hershey, Pennsylvania 17033, USA.*

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Chapter 1

Individual Variations in Drug Response

ELLIOT S. VESELL

Department of Pharmacology
The Milton S. Hershey Medical Center
The Pennsylvania State University
College of Medicine
Hershey, Pennsylvania

I. INTRODUCTION

For many years variability resulting from marked species and individual differences in response to drugs has plagued pharmacologists. This variability involves either the effects exerted on experimental animals by certain drugs or more commonly the manner in which animals handle some drugs, thereby modifying their duration and intensity of action. Variability in the handling of drugs generally arises because of differences in rates of drug biotransformation in hepatic

microsomes, whereas variability in type or degree of response may be attributed to differences in the receptor sites on which the drugs act. Although these two facets provide a convenient approach to the subject of individual variations in response to drugs, they are clearly interrelated from a therapeutic point of view. Until recently, individual variability in response to drugs was largely ignored as a guide to the administration of therapeutic agents. Now, however, individual variations in drug response are receiving much attention, are being increasingly considered in drug administration and are being investigated intensively at the clinical level.

Recent work has established, among normal individuals, the genetic control of large individual differences in rates of elimination of several drugs (Vesell and Page, 1968a, b, c). The range of these reproducible differences in man is 3- to 4-fold for phenylbutazone and antipyrine but 10-fold for dicoumarol. Larger individual differences in the pharmacokinetics of chlorpromazine have been described and associated with variability in the clinical responsiveness to this drug (Curry and Marshall, 1968). To some extent variable blood levels of chlorpromazine result from non-uniform absorption of the oral form (Hollister *et al.*, 1970). Differences among individuals in rates of drug biotransformation can explain why certain individuals who are slow drug metabolizers develop toxicity on chronic drug administration. On the other hand, fast drug metabolizers may degrade and eliminate certain drugs too rapidly for them to attain sufficient, or sufficiently sustained, concentrations at their sites of action to exert their pharmacological effects. These examples emphasize the dangers of administering the same dose to all patients and consequently the need for ascertaining individual rates of drug metabolism to guide therapy effectively.

Responsiveness to many drugs is directly related to their blood levels. For procainamide (Koch-Weser *et al.*, 1969) and digoxin (Smith *et al.*, 1969) drug toxicity is associated with high blood levels, therapeutic efficacy with intermediate, but narrow ranges and therapeutic failures with still lower plasma levels of each drug. Convenient, rapid and inexpensive methods have been developed for the determination of the blood levels of many commonly employed drugs. Such assays are becoming increasingly used in medical centers to tailor the administration of drugs to widely differing individual requirements.

Species, strain and intrastrain differences in drug response are due primarily to variations in rates of drug elimination (Quinn *et al.*, 1958; Vesell and Page, 1968a, b, c, 1969). These variations arise predominantly from differences in rates of drug metabolism within the smooth endoplasmic reticulum (microsomes) of liver cells (Fouts, 1961). For

drugs such as barbital and other therapeutic agents, for instance certain general anesthetics, that are not metabolically transformed to a great extent but eliminated by urinary or pulmonary excretion, much smaller differences in response occur. The relative roles of metabolism as compared to renal elimination depend on the lipid solubility of the drug and also the degree of binding by and storage within tissues. Polar drugs and drug metabolites are rapidly cleared by the kidney, whereas lipid soluble drugs could theoretically remain indefinitely (Butler, 1958). One of the major functions of the hepatic microsomal drug-metabolizing enzymes is to make lipid soluble compounds more polar and thus water soluble, thereby rendering them more susceptible to urinary excretion.

Although drug-metabolizing activities have been identified in the adrenal gland, the intestinal mucosa, the lungs and the skin, it is the liver that performs by far the largest share of drug metabolism. Therefore, this chapter on individual variations in drug action focuses on environmental and genetic factors that affect hepatic microsomal drug-metabolizing activity.

II. The Hepatic Microsomal Drug Metabolizing System

Endogenously generated metabolic intermediates and exogenously administered substances chemically related to them are biotransformed by enzymes that are much more specific than those that handle foreign compounds without endogenous counterpart. The enzymes that degrade exogenous compounds without endogenous counterpart are highly nonspecific, are located in hepatic microsomes and are termed drug-metabolizing enzymes (Brodie *et al.*, 1958). They have not been successfully purified as yet, because their activity declines when they are removed from their lipid envelopes in hepatic smooth endoplasmic reticulum which, as shall be developed subsequently, apparently contain substances necessary for their activity.

The step by which nonpolar exogenous materials are converted to metabolites of greater polarity, rendering them thereby more easily excretable, is the introduction of a polar group through an enzymatically catalyzed reaction classifiable as an oxidation, reduction or hydrolysis. In certain cases a final conjugation reaction occurs in which a glucuronate, sulphate or glycine group is added to the polar group.

Although a few reactions can be performed by enzymes in the mitochondria and cell sap, most are catalyzed by enzymes located in the hepatic endoplasmic reticulum. Gillette (1966) has described the following series of 3 reactions by which these microsomal mixed function oxygenases employ NADPH to reduce A (a microsomal component

which reacts with molecular O_2 to form an "active O_2" capable of oxidizing a drug):

(1) NADPH + A + H^+ → AH_2 + $NADP^+$
(2) AH_2 + O_2 → "active O_2"
(3) "active O_2" + drug → oxidized drug + A + H_2O.

A summation of these 3 reactions may be written as follows:

NADPH + O_2 + drug → $NADP^+$ + H_2O + oxidized drug

It was discovered that the microsomal component A, with which NADPH reacted by way of an electron transport chain and which activated oxygen and transferred it to drugs, was a carbon monoxide sensitive cytochrome called P-450 (Gillette, 1966). With special spectrophotometers capable of measuring small absorbance changes in turbid solutions, the binding of various organic compounds to sites on P-450 was demonstrated (Remmer *et al.*, 1966). Many drugs oxidized by the hepatic microsomal system complexed with the oxidized form of P-450. The concentration of the drug determined the magnitude of the spectral changes. Finally the apparent Km of the reaction for oxidation of certain drugs was similar to the concentration of drug causing half maximal spectral changes (Ksp). Clearly then the initial step in drug oxidation appeared to be a complex between drug and the oxidized form of P-450.

However necessary P-450 now appears to be for drug oxidation, the amount of P-450 hardly differs between species. Thus these small differences fail to account for species, strain and individual differences in drug metabolism (Davies *et al.*, 1969; Page and Vesell, 1969). Strain differences in drug metabolism appear to be more closely related to P-450 reductase activities than to P-450 content and currently it is believed that drugs may form a complex with only part of the oxidized P-450. This complex may be reduced more rapidly than the non-complexed portion of oxidized P-450 (Gigon *et al.*, 1969). These workers suggest that the rate limiting step is the rate of reduction of the complex between the drug and P-450.

Another difficulty is introduced by the diversity of reactions between drugs and P-450. At present, according solely to spectral criteria, there appear to be at least two types of reactions between drugs and P-450. Certain drugs such as ethylmorphine, amidopyrine, hexobarbital and the majority of other drugs produce a spectral change with P-450 characterized by a trough at 420 mμ and a peak at 385 mμ. This is designated a type I spectral change, and the drugs producing it are referred to as type I drugs. A type II spectral change consists of a

trough at 390 mμ and a peak at 430 mμ. Type II drugs include aniline, antipyrine and nicotinamide. In type I reactions, the rate-limiting step is apparently reduction of the P-450–drug complex; whereas in type II reactions between drugs and hepatic microsomes there occurs decreased reduction of the complex between P-450 and the drug (Gigon *et al.*, 1969).

III. Factors Affecting Drug Metabolism

Even before the details of drug metabolism (developed in the preceding section) were elaborated, pharmacologists were aware that a wide variety of diverse factors could produce alterations in response to drugs. The problem of explaining these factors in terms of the complex mechanism for drug metabolism described in the preceding section has been attempted in some instances, but in many cases the precise locus of action in terms of this model is unknown.

For the present purposes, however, no such attempt will be made. A partial enumeration of these factors is desirable, however. In most instances the factors have been identified in rats and mice. Whether they also are operative in man remains unknown at present for the simple reason that in man they have not been adequately investigated.

To illustrate the numerous genetic and environmental factors capable of altering response to drugs the following experiments performed in mice are presented. In all cases, except in the study on changes in enzyme activity during development and in the study on sex differences, adult 20 to 25 gm male mice were used. Each received a single, hypnotic, intraperitoneal dose of sodium hexobarbital (125 mg/kg). The sleeping time (from the time of injection to the time of restoration of the righting response) was measured by stopwatch. At the time of restoration of the righting reflex the brain was removed and its hexobarbital content determined (Cooper and Brodie, 1955). The hexobarbital oxidase activity of the 9000xg supernatant of liver homogenate was assayed (as described in Vesell, 1968).

Strain differences, presumably on a genetic basis, were observed in the hepatic hexobarbital oxidase activity of 11 strains of mice. These differences apparently accounted for the prolonged sleeping times of AL/N, DBA/2N and C57BI/6N strains (Table I). Hybrids between AL/N and DBA/2N males and BALB/C females exhibited sleeping times and hepatic hexobarbital oxidase activities intermediate between those of their parents. These results suggest that sleeping time, like many other metrical or quantitative characters, may be transmitted polygenically.

At the time of awakening all strains had similar levels of brain hexobarbital (Table I) so that differences in the sensitivity of CNS receptor sites to the drug appear not to account for differences in sleeping time.

TABLE I

Sleeping time, hexobarbital oxidase activity and brain hexobarbital level on awakening in various strains of male mice.
(The numbers in brackets indicate the number of animals used)

Strain		Sleeping Time min ± S.D.	Hexobarbital Oxidase μmol/gram liver/15 min ± S.D.	Brain Hexobarbital Level (γ/gram) ± S.D.
AI/N	(24)	86 ± 16	0.20 ± 0.01	45 ± 6
BALB/C	(24)	46 ± 6	0.34 ± 0.02	46 ± 4
C3H/HeN	(24)	41 ± 7	0.34 ± 0.02	53 ± 5
C57BL/6N	(24)	73 ± 13	0.23 ± 0.01	52 ± 5
DBA/2N	(24)	85 ± 17	0.21 ± 0.01	48 ± 4
STR/N	(24)	47 ± 6	0.36 ± 0.01	49 ± 4
CAF_1	(24)	72 ± 10	0.24 ± 0.02	46 ± 4
CDF_1	(24)	58 ± 9	0.28 ± 0.02	54 ± 5
NIH	(24)	37 ± 5	0.32 ± 0.03	48 ± 4
CFW/N	(24)	40 ± 7	0.37 ± 0.02	46 ± 3
GP	(24)	42 ± 8	0.37 ± 0.03	52 ± 5

Differences in sleeping time and liver hexobarbital oxidase activity between AL/N, DBA/2N, C57BL/6N, CAF_1 and CDF_1 mice and other strains are significant ($P < 0.05$). From Vesell, 1968.

Table I reveals that the standard deviation (S.D.) of the sleeping time is greater in the longer sleeping strains than in the shorter sleeping strains. The magnitude of the S.D. of the sleeping time had been interpreted as a reliable indication of whether inbred or outbred animals were more suitable for experimental use. Jay (1955) reported that the S.D. of the hexobarbital sleeping time was smaller in inbred than in outbred mouse strains and therefore recommended the use of the former in drug studies. However, McLaren and Michie (1956) reported the opposite results for pentobarbital and claimed that outbred strains displayed a more uniform response to the environmental stress of unconsciousness induced by hexobarbital. The S.D. of the sleeping time is an unfortunate measurement to employ in settling this question of great interest to pharmacology and genetics because, as suggested in Table I and dramatically indicated in Fig. 1, the S.D. of the sleeping time varies directly with the magnitude of the sleeping time. Therefore, in order to use the S.D. of the sleeping time to compare

the uniformity of response in inbred and outbred animals, the magnitude of the sleeping times must be very similar in both groups.

It was shown that littermates had a more uniform response to hexobarbital as judged by the S.D. of the sleeping time than did non-littermates (Vesell, 1968). This occurred both for inbred and outbred mice. The smaller S.D. of littermates may be environmental, in that littermates share a more similar intrauterine environment than do non-littermates, or it may be genetic, in that littermates are probably more similar genetically than non-littermates even of inbred strains.

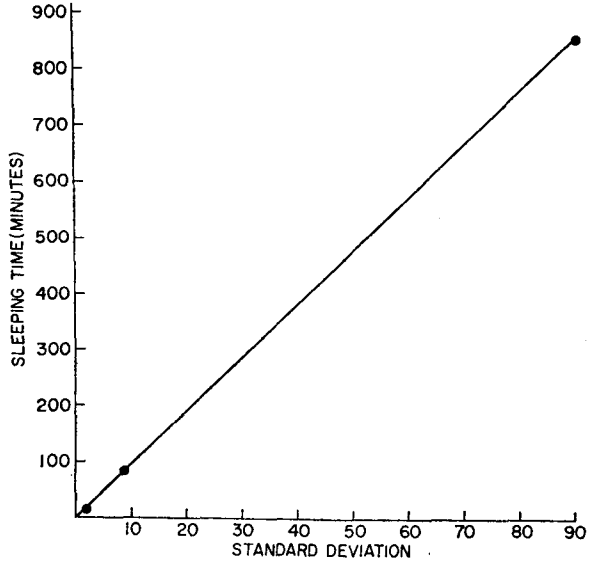

FIG. 1. Relationship between sleeping time and standard deviation of sleeping time. From Vesell, 1968.

A factor influencing responsiveness to hexobarbital that is clearly environmental in nature is the imposition of painful stimuli (Vesell, 1968). Mice are not permitted undisturbed sleep but traditionally have their tails pinched with medium intensity at 1 minute intervals during the measurement of hexobarbital sleeping times. In an investigation of the effect of tail pinching on sleeping time, hepatic hexobarbital oxidase activity and brain hexobarbital level at the time of awakening, Vesell (1968) showed that tail pinching shortened the hexobarbital sleeping time by approximately 40 to 50% in comparison with unpinched mice. The hepatic hexobarbital oxidase activity was similar in both groups, but the brain level of hexobarbital at the time of awaken-

ing was higher in the pinched mice. Therefore, painful stimulation shortens hexobarbital sleeping time by raising the threshold of the CNS receptor sites to the drug so that the end point of awakening occurs earlier and at higher brain hexobarbital levels. For the widely different sleeping times shown in Table I and Fig. 1, the brain hexobarbital levels were similar at the time of awakening in all mice and different sleeping times could be attributed solely to differences in hexobarbital oxidase activity.

Sex hormones are known to alter drug metabolism (as reviewed in Gram and Gillette, 1969). However, the effect of sex hormones on hexobarbital sleeping time varies with species and strain (Vesell, 1968). Table II reveals that in certain mouse strains males sleep longer than females, whereas in other mouse strains such sex differences did not occur. In contrast, female rats sleep longer than males (Nichols and

TABLE II

Sex differences in duration of action of hexobarbital in various strains in mice.

		Sleeping time (min) ± S.D.	
		Male	Female
General Purpose	(12)	34 ± 5	31 ± 5
CFW	(12)	29 ± 5	31 ± 5
AL/N	(12)	99 ± 6	58 ± 4
NBL/N	(12)	73 ± 5	35 ± 2

The numbers in brackets are the numbers of animals used. Sex differences between GP's and between CFW's are not significant. Sex differences between AL/N's and between NBL/N's are significant ($P < 0.01$). From Vesell, 1968.

Barron, 1932). Westfall *et al.* (1964) were able to modify sex differences in Swiss-Webster mice either by administering stilbestrol to males, thereby almost halving their sleeping times, or by administering testosterone to females, thereby doubling their sleeping times. In incubation mixtures containing 9000xg supernatant fractions of rat liver homogenates, either norethynodrel or progesterone at 10^{-5} M and 10^{-4} M concentrations markedly inhibited the side chain oxidation of hexobarbital, the ring hydroxylation of zoxazolamine and the *p*-hydroxylation of aniline (Juchau and Fouts, 1966). Pretreatment with these steroids 1 or 2 hours prior to sacrifice inhibited only zoxazolamine and hexobarbital metabolism, but a single dose of norethynodrel administered 10 or 48 hours prior to sacrifice stimulated hexobarbital metabolism, as did subacute (daily for 3 days) or chronic (daily for 3 weeks) pretreatment (Juchau and Fouts, 1966). Pretreatment with

progesterone produced no stimulation of hexobarbital or zoxazolamine metabolism. Significant shortening of hexobarbital sleeping times in mice by "Enovid" suggests that norethynodrel enhanced drug metabolism in this species (Roberts and Plaa, 1966). During pregnancy, drug metabolism is not uniform. Inhibition of aniline hydroxylation and of ethylmorphine N-demethylation occurs during the late stages of gestation in the rat (Guarino *et al.*, 1969).

Drug metabolism changes with age. It declines in the aged rat and for a short period following birth it is negligible in the mouse, guinea pig, rat and human (Jondorf *et al.*, 1958; Fouts and Adamson, 1959; Catz and Yaffee, 1967; Vesell, 1968). Figure 2 reveals rapid development

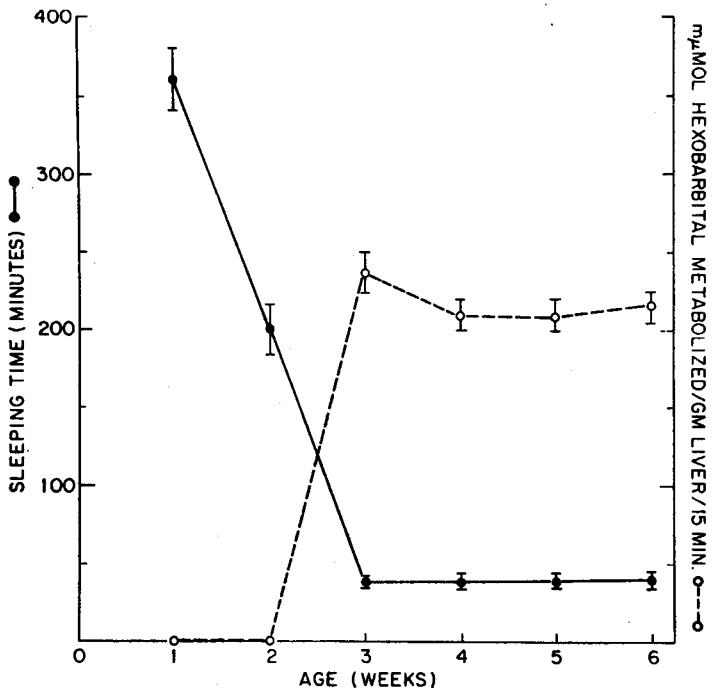

FIG. 2. Development of hexobarbital oxidase activity of 9000xg supernatant of liver homogenate and decrease of sleeping time with age. Each point represents the mean of 12 male NIH mice with standard deviations. From Vesell, 1968.

of adult levels of hepatic hexobarbital oxidase activity between 2 and 3 weeks of age in mice. This is the time that the mice are abruptly weaned and consequently become dependent on their own drug metabolism to detoxify exogenous materials ingested as food.

In addition to materials consumed as food, many mammals are exposed to a variety of environmental agents capable of enhancing their hepatic drug metabolism (Conney, 1967). These inducing agents include certain insecticides, plant alkaloids, polycylic hydrocarbons and, in the case of laboratory animals, soft-wood bedding (Vesell, 1968; Wade *et al.*, 1968; Fujii *et al.*, 1968). Figure 3 reveals the dramatic, but

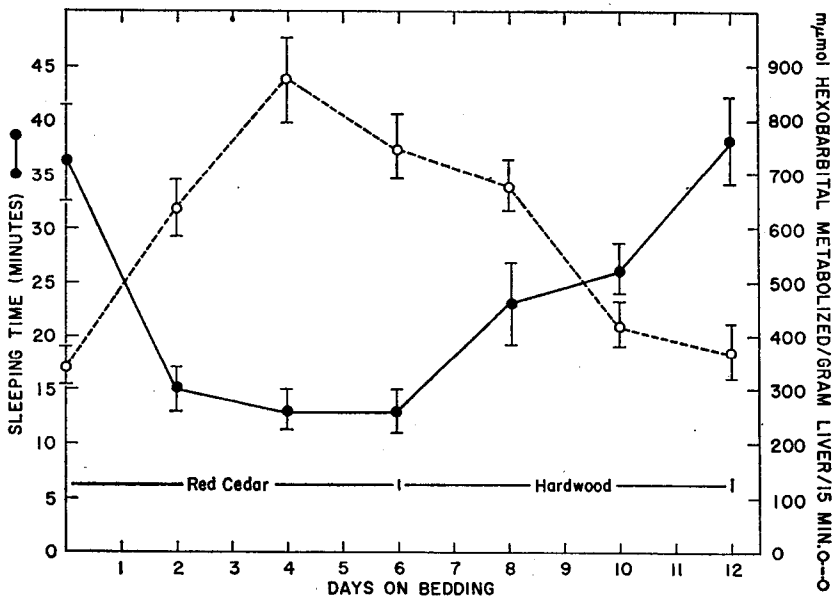

FIG. 3. Each point represents sleeping time (with standard deviations) of 10 NIH male mice and hexobarbital oxidase activity of 9000xg supernatant of liver homogenate. Mice were on red cedar bedding for 6 days and then on a combination of beech, birch and maple bedding for 6 subsequent days. From Vesell, 1968.

reversible, change in hexobarbital sleeping time and hepatic hexobarbital oxidase activity on exposure of mice to red cedar bedding.

We were surprised to observe lower hepatic microsomal drug-metabolizing activities in wild rat strains than in domestic ones (Page and Vesell, 1969). Although such differences between wild and domestic animals in drug metabolism may be due in part to genetic factors, the presence of these differences in another species, the rabbit (Cram *et al.*, 1965), suggests exposure of domesticated animals to potent inducing agents in our laboratories. Apparently animals in nature are largely spared. The extent of individual variation in their drug-metabolizing enzyme activities is much greater than that of laboratory animals (Cram *et al.*, 1965; Page and Vesell, 1969).