The Role of the Liver in Drug Metabolism

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Enzymes located in the endoplasmic reticulum of liver cells protect the organism against an accumulation of lipid-soluble exogenous and endogenous compounds by converting them to water-soluble metabolites which can be easily excreted by the kidney. But only few drugs possess suitable groups which are conjugated with glucuronic or sulfuric acid. Most compounds have to be hydroxylated first. For this purpose the endoplasmic reticulum has at its disposal an enzymic system, completely unspecific, which activates molecular oxygen for the oxidation of lipid-soluble compounds. This takes place at a cytochrome, P_{450}, which is available in the endoplasmic membranes abundantly. The oxidation rate, however, is extremly slow and dependent on the chemical configuration of the compound and on genetically determined differences of the protein moiety of the enzyme.

Since more specific enzymes located in liver cells metabolize most of the endogenous compounds, such as steroids, at a much higher rate, the slow hydroxylation by the unspecific endoplasmic enzyme does not play an important role in their conversion to inactive compounds.

Drugs, however, are mainly converted to less active or inactive compounds. Their effectiveness and their duration of action depend on the rate of metabolism in the endoplasmic reticulum. An overload of the liver cell with numerous lipid-soluble drugs increases drug metabolizing enzymes in the endoplasmic reticulum and augments the smooth membranes in the hepatocytes with the result that all lipid-soluble compounds reacting with cytochrome-P_{450} are oxidized more rapidly.

Because of the lack of specificity of this enzyme, drugs compete for the binding sites if high concentrations of several drugs are present in the liver cells. A slower metabolism of these drugs with less affinity is the result.

Metabolism of drugs by this enzyme system leads sometimes to more active and toxic compounds which produce liver injury, e.g., in the case of carbon tetrachloride. Drug metabolism is inhibited only in severe hepatitis, and exceptionally in liver cirrhosis.

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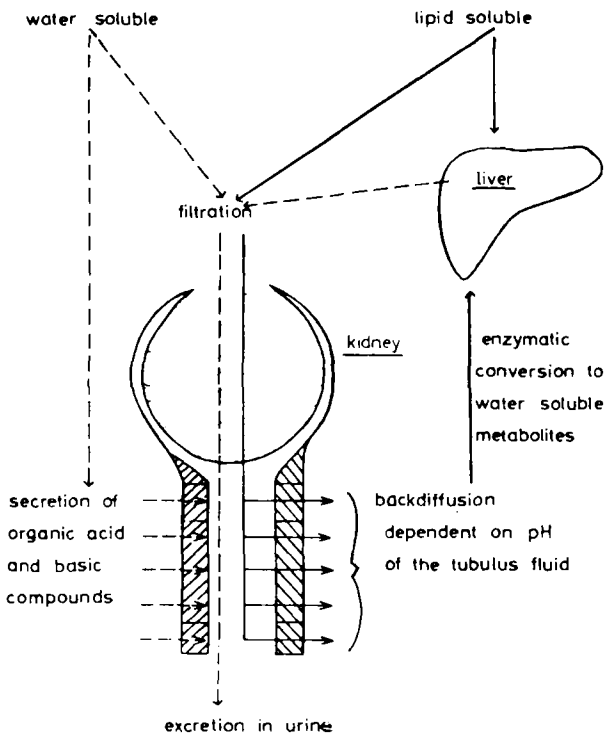

Figure 1. Elimination of drugs.

TABLE I Metabolism of Drugs by Enzymes Located in the Endoplasmic reticulum

Metabolism	Enzyme
Oxidations of aliphatic and aromatic groups: barbiturates, diazepoxides, phenothiazines, meprobamate, phenytoine, etc. antihistamics, phenacetin, aminopyrine, antipyrine and congeners synthetic steroids, contraceptives, anabolic androgens (digitoxin)	Cytochrome P_{450}
Reductions of azo- and nitro-groups: azo-dyes, (chloramphenicol), etc.	Flavin enzymes
Hydrolyses of esters and acidamides: procaine, lidocaine, meperidine, atropine	Esterases
Conjugations of glucuronic acid with following groups: (a) alcoholic and phenolic (b) (carboxyl) (c) (amine)	Transferases

NOTE: Parentheses indicate that the metabolic pathway mentioned plays a minor role in conversion.

To protect the organism against intoxications is one of the main functions of the liver. Significant amounts of foreign compounds altering, if not to say, polluting man's normal environment invade our body as smoke particles and toxic gases, as dyes, preservatives and disinfectants, and last but not least as drugs, strictly defined. The character particularly of sedatives and stimulants has changed completely; formerly used as drugs for therapeutic purposes, now they have to be regarded as mass-products consumed in a similar manner as food.

All these and many other compounds foreign to the organism should be regarded as drugs which, broadly defined, are chemical agents interfering with biochemical reactions in living cells. They are excreted by the kidney in unchanged form only very slowly, if they are lipid soluble. If taken daily they accumulate in the organism and would achieve a dangerous level if the liver had no capacity to convert lipid-soluble drugs into more water-soluble compounds (Figure 1).

Described here is the role played by the liver in drug metabolism, the properties of the enzyme systems involved, and the dependence of the duration and extent of drug actions on the function of an unspecific hydroxylase located in the endoplasmic reticulum. With few exceptions, the well-known drugs are lipid-soluble and are able to penetrate cell membranes. This is why the drugs, after glomerular filtration of the portion not bound to plasma proteins, return to the blood from the ultrafiltrate, passing into the tubule cells by diffusion. The driving force is the concentration gradient produced during reabsorption of water and solutes. The quantity of a substance which is not excreted depends mainly on its ratio of lipid-to-water solubility. In general, the more lipid-soluble a drug is, the greater the proportion retained in the organism.

DRUG-METABOLIZING ENZYMES IN THE ENDOPLASMIC RETICULUM

Fortunately, the endoplasmic reticulum in liver cells prevents accumulation of drugs. Enzymes are attached to the lipid layers of the membranes catalyzing the metabolism of drugs to more water soluble compounds (Table I) [1,2]. It is possible that metabolites formed by the enzymes in the interior of the endoplasmic reticulum do not gain direct access to the cytosol and are discharged either into the bile canaliculi or into the extra-

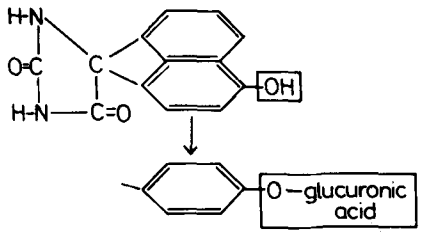

Figure 2. Metabolism of phenytoin (Dilantin) by two consecutive steps shown in the frames.

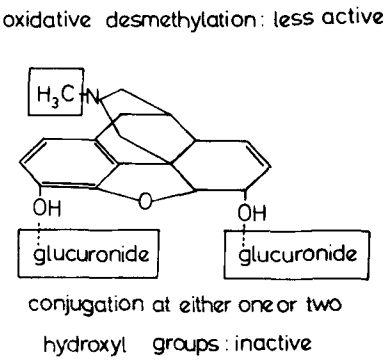

Figure 3. Metabolism of morphine.

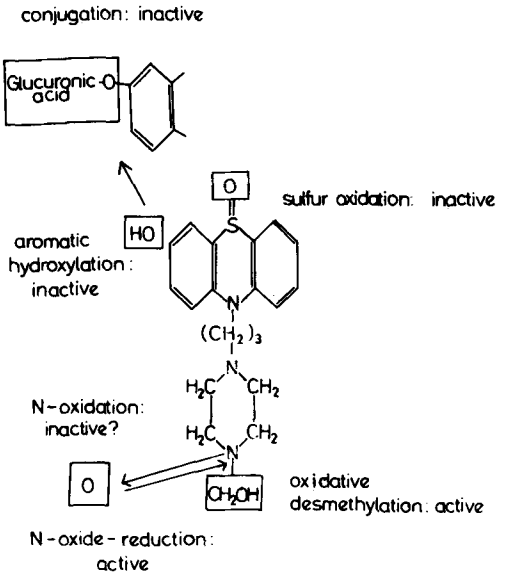

Figure 4. Metabolism of phenothiazines (Perazin): different types of oxidations shown in the frames.

cellular fluid where the endoplasmic membranes are in contact with the cell membrane. But this is still a moot question.

The most important step is an oxidation of nearly all lipid-soluble compounds, whether formed in the body or consumed as drugs, to more polar and water-soluble substances. This may be followed by a conjugation which converts the compound to a highly polar acid that is inactive and readily excreted in the bile or urine. In this article several enzymatically catalyzed drug conversions which occur in the cytosol of the liver cells will not be considered. These reactions are of minor importance, only two playing a significant role in drug metabolism and being deserving of mention, i.e., the acetylation of drugs, such as sulfonamides and isoniazid, and the oxidation of alcohol by alcohol dehydrogenase [3].

MAJOR TYPES OF DRUG METABOLITES FORMED

Only a few examples will be presented to illustrate the type of conversion of lipid-soluble molecules which occur in the endoplasmic reticulum.

(1) Phenytoin is an example of many drugs which are hydroxylated at only one site of the molecule (Figure 2). This step converts the drug into a more water-soluble and inactive compound. When the hydroxyl group is attached, the substance can then easily be conjugated either with glucuronic or sulfuric acid [4]. The hydroxylation is the rate-limiting step in the over-all reaction and determines the duration of phenytoin action. Similar reactions are involved in the metabolism of barbiturates and other hypnotic agents [5].

(2) Many drugs possessing a hydroxyl group can be conjugated directly to inactive, highly water-soluble metabolites, e.g., morphine (Figure 3). The oxidative demethylation that occurs can be regarded as a metabolic side-reaction. This converts morphine to the less active normorphine [3].

(3) The oxidative enzyme system in the endoplasmic reticulum membranes is peculiar insofar as it can convert a drug to various metabolites oxidized at different sites of the compound. Two or even three oxidation steps may occur in the same molecule. As a typical example, phenothiazine metabolism may be cited [6] (Figure 4). One of the main conversion steps involves hydroxylation of the aliphatic methyl group attached to the nitrogen. This group can be readily split off as formaldehyde. The desmethyl-compound remains active. This and the parent drug undergo further oxidations to hydroxylated aromatic metabolites. Hydroxylation can occur as indicated in Figure 4 or at another site of the aromatic ring. However, not all sites are reactive. Other important metabolites are the sulfoxides and the N-oxides.

The sulfoxides and the metabolites hydroxylated

on the aromatic ring are inactive, but it is not yet known whether the N-oxide is an active or inactive compound [7]. It will be appreciated from Figure 4 that numerous metabolites may originate if one or two different oxidations involving the phenothiazine molecule or its desmethyl compound occur.

All these conversion products can be found in the urine of man and all are produced by the same enzyme system in the endoplasmic reticulum of the liver cells. The spectrum of the metabolites in the urine varies from species to species. Even individual differences are known, as has been shown in the urine of patients treated with phenothiazines. The composition of the metabolites in human urine also depends upon the phenothiazine compound prescribed. As an example, perazine has been selected [8] (Figure 4), since it is widely used as an antipsychotic drug in Europe.

PROPERTIES OF THE MICROSOMAL HYDROXYLASE (CYTOCHROME$_{450}$)

Discovery of the Drug-Hydroxylase. Brodie, Axelrod and co-workers [1,2] discovered liver microsomes able to hydroxylate numerous drugs. The nature of this enzyme system remained obscure until in 1963 Estabrook, Cooper and Rosenthal et al. [9,10] detected in the microsomes of the adrenal cortex cells a peculiar cytochrome, called cytochrome P_{450}, which is able to hydroxylate progesteron at position C^{21}. Subsequent studies revealed that the same type of cytochrome found in liver cells several years previously [11] is also responsible for drug hydroxylations [12,13]. It is not present in all tissues. The highest content has been found in the liver, much smaller amounts in kidney > lung > intestinal mucosa > skin [14]. The kidney contains, per gram tissue, only one seventh of the amount found in liver. Taking into consideration the 6 times greater weight of the liver, the kidney contributes only 2.5 per cent to the total hydroxylation of a drug in the body. The other tissues mentioned contribute even less.

Comparison Between Oxidations in the Mitochondria and in the Endoplasmic Reticulum. This enzyme system has in common with the mitochondrial enzyme system the capacity to transport electrons to a final cytochromal acceptor. Both consume oxygen, but differ significantly in the type of oxygen activation [10,15] (Table II). The mitochondrial cytochrome oxidase needs four electrons for reduction of one molecule of oxygen. The microsomal hydroxylase located in the endoplasmic reticulum of liver cells belongs to the group of mixed-function oxidases which use two electrons, one for the reduction of one atom of oxygen to water; the second oxygen atom is incorporated into the substrate.

Electron transport in the mitochondria is coupled with the formation of high energy bonds (ATP) which furnish much of the driving force for biochemical reactions. There is no indication that a similar system operates in the endoplasmic membranes, implying that the energy produced by the oxidation process is wasted. The normal oxygen consumption of an isolated and perfused rat liver increases, if a drug is metabolized, up to 40 per cent. This is the amount that is used for maximal drug hydroxylation [16,17].

Electron-Transfer Chains in the Endoplasmic Reticulum. Reduced NADP functions exclusively as an electron donor. The electrons are picked up by an FAD-containing flavoprotein known as NADPH-dependent cytochrome-c-reductase [18]. The electron flows from the reduced flavin enzyme

TABLE II Electron Transfer to O_2 in Mitochondria by Cytochrome Oxidase and in Endoplasmic Reticulum by Cytochrome P450

$$4\,e^- + 4\,H^+ + O_2 \xrightarrow{\text{Cytochrome oxidase}} 2\,H_2O$$

$$X + 2\,e^- + 2\,H^+ + O_2 \xrightarrow{\text{Mixed function oxidase (Cytochrome } P_{450})} X:O + H_2O$$

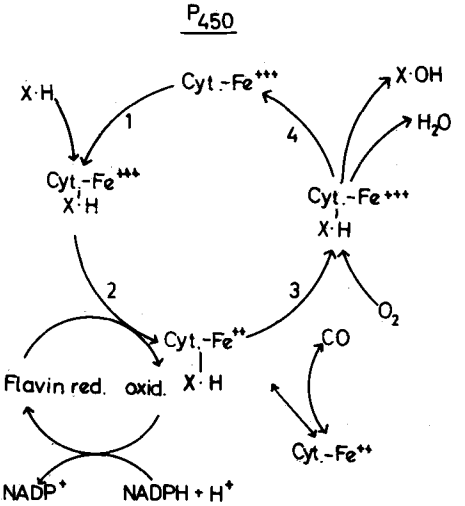

Figure 5. Scheme of electron transport from NADPH to cytochrome P_{450}.

to the cytochrome P_{450} (Figure 5). The reduced heme of the cytochrome is now able to bind one molecule of oxygen, which is activated presumably by picking up one electron from the heme-iron. How the second electron which is necessary for mixed function oxidation comes into play and how one atom of the oxygen reacts with the available substrate is not yet known [15,19]. However, the stoichiometry for this reaction has been elucidated and can be expressed in the following manner, where X stands for any drug as substrate [9,10]

$$X \cdot H + NADPH + H^+ + O_2 \rightarrow X \cdot OH + NADP^+ + H_2O$$

A second electron-transfer system is present in the endoplasmic reticulum. The cytochromal electron acceptor is cytochrome b_5 [15,20] (Figure 6). However, the significance of this system is still obscure.

Determination of Cytochrome P_{450}. It has been possible to measure cytochrome P_{450} in microsomes of liver and adrenal cortical cells by exploiting its peculiar characteristics [9,10]. In the reduced form it reacts with carbon monoxide which has about the same affinity as oxygen, using the sixth ligand as a binding site. By competing with oxygen, carbon monoxide inhibits drug hydroxylations. The spectrum of the carbon monoxide-cytochrome has a striking absorption peak at 450 nm which can easily be measured by differential spectrophotometry in microsomal particles (Figure 6). This permits determination of the amount of cytochrome P_{450} in the liver cells quantitatively. Besides cytochrome B_5 can be measured by utilizing the difference in absorption of the reduced and oxidized forms (Figure 6).

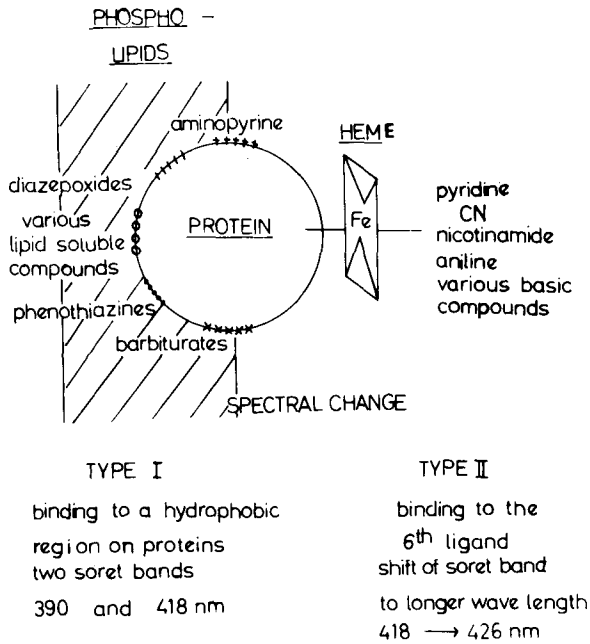

Figure 6. Spectrums of cytochrome P_{450} with a peak at 450 nm and cytochrome b_5 with a maximum at 426 nm in suspensions of microsomes (2 mg protein/ml) isolated from control rats (——), benzpyrene-treated rats (3×, 20 mg/kg) (– – – –), phenobarbital-treated rats (3×, 80 mg/kg) (. . . .); ♀ rats with a body weight of 60 to 70 gm were starved for forty-eight hours previous to decapitation. For method see [22, 24].

Unspecific Reaction of Cytochrome P_{450} with Drugs. The oxidized form of cytochrome P_{450}, the spectrum of which can be identified only with difficult procedures [21,22] reacts unspecifically with nearly all organic lipid-soluble compounds [23,24]. This reaction precedes the hydroxylation process (Figure 5). So far, two types of reversible binding can be distinguished spectrophotometrically (Figure 7) when lipid-soluble substrates are added to microsomal preparations. The difference in the absorption of microsomal suspensions without and then with added substrates, called binding spectrums, can be easily determined. This measurement is widely used as a tool for studying drug metabolism.

One enzyme or many? The question whether there is only one enzyme in the endoplasmic membranes or there are many using heme as a cofactor cannot be answered definitely, but the known facts speak for one or two [25,26]. It is also difficult to conceive that a huge number of different protein moieties are formed, each of which reacts specifically with a particular type of chemical configuration. The idea of many enzymes is not compatible with the fact that drugs which have a high affinity to cytochrome P_{450}, such as SKF-525 A, inhibit the metabolism of nearly all compounds reacting with cytochrome P_{450} [27]. Moreover, the available kinetic data describing the reactions of the hydroxylase with drugs offer no evidence for the presence of many enzymes.

Rate of Hydroxylation. The hydroxylation rate is extremely low compared with well known enzymic reactions occurring in cells, and differs considerably from substrate to substrate [24]. The rate-limiting step of the hydroxylation is the reduction of the heme iron, which increases if a substrate is bound to the hydrophobic region (type I) and decreases if a basic compound occupies the sixth ligand [28]. This indicates that the drug-cyto-

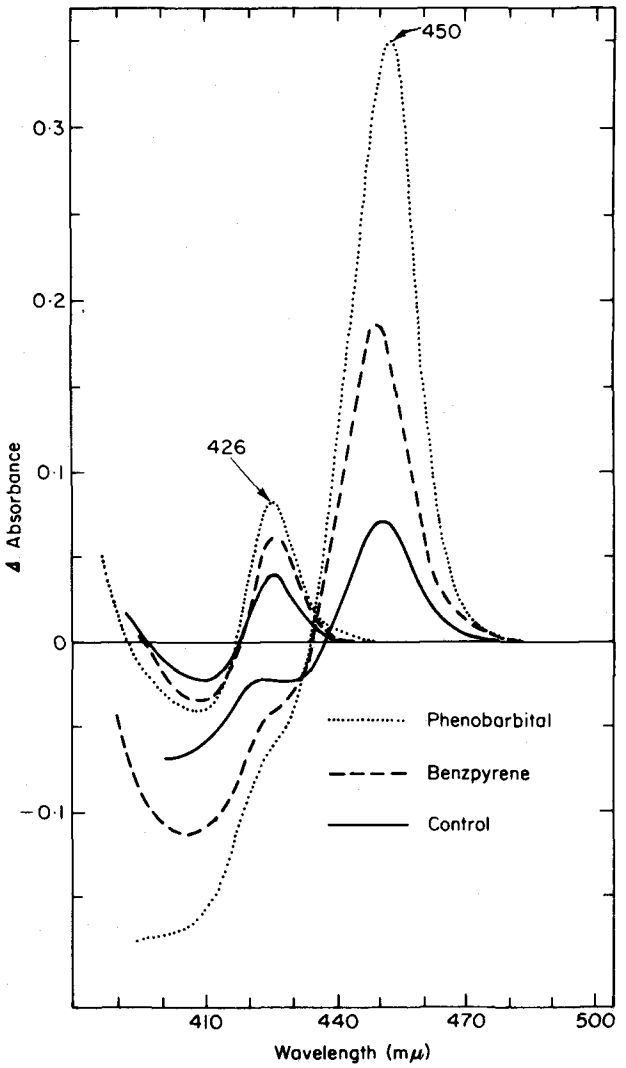

Figure 7. Scheme of drug reactions with cytochrome P_{450} [22].

chrome-P_{450}-complex with a soret band at 390 nm is reduced more easily than the unchanged cytochrome. However, the binding of a drug to the sixth ligand inhibits electron transfer.

In intermediary metabolism the turnover number (number of substrate molecules converted/mole of enzyme/minute) ranges from 1,000 to several millions in the case of catalase whereas the number of molecules converted by cytochrome P_{450} of the rat varies between 0.2 and 15. A similar range seems also to be characteristic for the cytochrome present in the human liver [29,30]. Despite this unusually low turnover number most of the drugs seem to be hydroxylated at a reasonable speed because of the surprisingly large amount of the hydroxylating system available. The liver of rats and rabbits contains about 30 to 40 mμM cytochrome P_{450}/gm [31]. If we assume that the molecular weight of this enzyme (which is not yet known) is in the same range as that of hemoglobin and albumin and comes to about 60,000, 1 gm of liver would have at its disposal 1.8 to 2.4 mg cytochrome P_{450}. More than 1 per cent of the total liver protein should be accounted as cytochrome P_{450}. This is a very high content compared with all the mitochondrial cytochromes, which are present in liver cells in smaller numbers. The high concentrations of the enzymes involved in drug hydroxylations signify the important role that cytochrome P_{450} plays in the liver.

The human liver contains less cytochrome P_{450} [29,30]. From the figures available it can be estimated that about 10 to 20 mμM per 1 gm of liver are present. This is from one third to one half of the amount found in 1 gm of rat liver. If one also takes into consideration the much smaller relative liver weight in man, only 2 per cent of the body weight whereas rats have liver weighing 4 per cent of the body weight, the rat has at its disposal four to six times more cytochrome P_{450}. This corresponds to the observations of many investigators indicating that man metabolizes drugs in vivo at a rate two to ten times slower than the rat [32].

Development of the Hydroxylation Enzyme During Ontogenesis. The enzyme in the endoplasmic reticulum is not fully developed before birth or several days and weeks afterward [33,34]. This results in a low rate of drug metabolism which has caused serious and even lethal toxic reactions in premature babies. The conjugation of drugs in particular is strikingly diminished. This was discovered after chloramphenicol was introduced as an antibiotic in the treatment of premature babies. The increase in the mortality rate

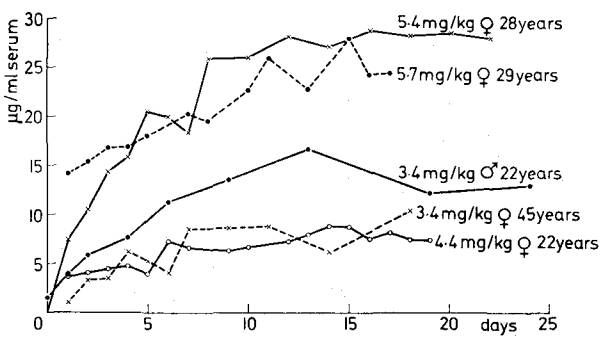

Figure 8. Variation in the plasma steady-state level of phenytoin in five patients who received 0.3 gm daily.

observed could be related to unusually high plasma levels of chloramphenicol. The same glucuronidation system located in the endoplasmic reticulum conjugates bilirubin, and the insufficiency of the system is also responsible for the appearance of neonatal jaundice.

Dependence of Activity on Nutrition. The efficacy of drug metabolism in the liver is lowered if animals receive a calorically sufficient but protein-free diet [35]. This is also true during complete starvation, in which the liver weight decreases considerably [36]. A limited supply of the reducing agent NADPH in vivo significantly enhances the decrease in the hydroxylation rate [16]. This may provide one explanation for the many observations on incompatibilities of drugs in undernourished patients. Because of the diminished metabolism the level of numerous drugs may rise and produce toxic side effects.

VARIATIONS IN DRUG HYDROXYLATIONS

Differences Between Species. In addition to quantitative differences in drug-metabolizing enzymes in different species, qualitative species variation in drug metabolism makes any prediction of the fate of a compound in man difficult even though it may be known how the same drug is handled in several laboratory animals [3,37]. The speed and type of oxidation may differ considerably. It is therefore difficult to devise any rule which may be useful if the fate of a drug is under consideration. Species X, which converts an administered drug A at a higher rate than species Y, may metabolize drug B at a lower speed than Y. Pentobarbital is metabolized in dogs four times more rapidly than in man, but the opposite is true for thiopental, a closely related barbiturate [1]. Also, the metabolites formed may not be the same because species hydroxylate preferentially at different sites of the drug molecule [3,37]. Many drug conversions in the liver are sometimes dependent partly or exclusively on enzymes not located in the endoplasmic reticulum, a circumstance which may modify the type and speed of metabolism even more. As an example tolbutamide may be cited [38].

Individual Variations of Drug Hydroxylation. What makes studies of drug metabolism even more complicated and rational therapy so difficult is the fact that variations in the speed of drug metabolism have also been found when the hydroxylation rates of inbred strains of mice and rats are compared [32]. This is true also for different subjects in a population consisting of different strains, e.g., in mongrel dogs [39]. The magnitude of individual variation and its significance for therapy may be explained by presenting as an example determinations of phenytoin (Dilantin®) in the plasma of epileptic subjects receiving the same dose, 4 to 5 mg/kg (0.3 gm/daily) [40]. This drug was selected for the study because it is absorbed completely, and only 2 to 5 per cent of the dose administered is excreted unchanged by the kidney. It is eliminated as a conjugated oxidation product [4] (Figure 2). The rate-limiting step is the extremely slow hydroxylation in the endoplasmic reticulum, with a turnover number of less than 0.1. The long plasma half-life varies between eight and fifty hours. Consequently phenytoin belongs to that category of drugs which accumulate during therapy until a steady state is reached, reflected by a constant plasma level if the daily dose ingested equals the amount hydroxylated. The large variation in the plasma level (Figure 8) chiefly reflects differences in the hydroxylation rate and is still greater in the fifty patients receiving the same recommended standard dose of 0.3 gm daily studied to date. The range observed varied between 4 and 60 μg/ml. This is why phenytoin is ineffective in some patients if the dose is not increased, and why toxic reactions occur in others if the dose is not lowered. Below a plasma concentration of 10 μg/ml phenytoin exerts no significant antiepileptic action whereas toxic side effects appear if levels of about 20 μg/ml are achieved [41,42].

Differences in the amount of cytochrome P_{450} available in the liver and in the affinity of the enzyme for the substrate may explain the large variation in the hydroxylation rate. A true genetic basis for the striking variation in phenytoin hydroxylation has been found in one family, of which several members have been treated with phenytoin [42].

Determinations of drug levels in the blood of identical and fraternal twins have provided the best evidence that drug metabolism is governed by genetic factors. The individual differences in the handling of drugs which were obvious in fraternal twins were not observed in identical twins [43].

ACTIVATION AND INHIBITION OF DRUG METABOLISM

A great variety of lipid-soluble drugs increase their own metabolism and the metabolism of other

compounds not related pharmacologically or chemically by inducing the drug-hydroxylating enzyme system in the endoplasmic reticulum of the liver [44,45]. This nonspecific phenomenon, which has been observed in all mammalian species so far investigated, can be viewed as an adaptive process which protects the organism against an overload of foreign compounds [46].

Induction of the Hydroxylating System. The first enzyme that increases during induction is δ-aminolevulinic synthetase, which is involved in the synthesis of heme [49]. This is followed by cytochrome P_{450} and the specific flavin enzyme for electron transport. All the other enzymes not involved in drug hydroxylation increase later on to a much smaller extent. This is the case for cytochrome b_5 and the relevant unspecific esterase [12].

After one injection of phenobarbital the maximal induction of cytochrome P_{450} is achieved after twenty-four hours. The newly formed enzyme disappears very quickly, with a half-life of about one day. This seems to be the normal half-life of cytochrome P_{450} [46,47].

If treatment with an inducing agent is not terminated the increase in cytochrome P_{450} continues until a maximal amount is formed after three to five days (Figure 6), and a new steady state is achieved. So long as the inducing agent is present in the liver cells it enhances the rate of synthesis of the heme as well as of the protein moiety by causing an increase in messenger-RNA formation in the nucleus [48]. This occurs presumably by reaction with a repressor compound. The protein breakdown seems passively to follow the increased synthesis rate [47].

Increase in Smooth Endoplasmic Membranes. A significant enhancement of drug-metabolizing enzymes is visible with the electron microscope [12,50]. The smooth (but not the rough) membranes in the liver cells increase considerably. With the growth of the endoplasmic reticulum the liver becomes larger, predominantly by hypertrophy. This does not mean that any enlargement of the endoplasmic reticulum is necessarily associated with an increase in drug-metabolizing enzymes, but this point has to be elucidated further.

Age-Dependence of Induction. The magnitude of induction is also highly dependent on the age of the animals. One tenth of the phenobarbital dose in weanling rats produces nearly the same induction as the full dose in adult animals [51]. This, of course, seems to be the reason also why it is possible to induce the glucuronidation system in babies with small doses of phenobarbital and to prevent icterus of the newborn, a method now widely used in therapy [52–54].

Activation of Drug Metabolism During Therapy. Observations during therapy and clinical studies show that an increase in drug metabolism can occur in man also [45,54,55]. However, the number of drugs known so far to act as inducers of the hydroxylating system during therapy is small; these include barbiturates, glutethimide, meprobamate, phenylbutazone, phenytoin and the antifungal agent griseofulvin [55–57]. This list is far from complete. The inducing action of these drugs in man, however, is exceedingly inconsistent, and many patients treated with the same compound at the same dose levels did not respond with increased drug metabolism [56,58,59]. Species, strain and individual variations in the magnitude of induction make any prediction as to the appearance and extent of this phenomenon in human therapy very difficult. However, several rules can be stated which permit a forecast whether a drug possessing inducing properties in animal experiments will exert a similar action in man.

Conditions Necessary for Induction of the Microsomal Hydroxylase in Man. (1) The majority of lipid-soluble compounds metabolized by the microsomal hydroxylase have some inducing properties. This is true even for ethanol and for those drugs such as barbital which cannot be hydroxylated in measurable quantities during the period of testing. Alkaloids, however, seem to have no inducing capacity.

(2) As can be expected, the inducing action is dose-dependent. However, many drugs are active only at nearly toxic doses; a few, however, such as long-acting barbiturates can induce the hydroxylating enzyme system when therapeutic doses are taken [50].

(3) Most important is the maintenance of a high concentration in liver cells for a certain time. The dose of hexobarbital has to be increased several times if induction is to be produced in smaller animals in which hexobarbital is metabolized very rapidly (Table III). Besides, a drug with a short action, such as hexobarbital, has to be given repeatedly in order to cause a measurable increase in the enzyme. A similar rule has been found when dogs were treated with long-acting phenobarbital. The more rapidly a dog metabolized barbiturates, the higher the daily

TABLE III Half-life of Hexobarbital in Various Species Compared with the Pretreatment Schedule of Hexobarbital for Increasing Its Own Metabolism

Species	Half-Life (min)	Pretreatment		Increase in Hexobarbital Metabolism (%)
		Days	Daily Dose (mg/kg)	
Mice	55	6	100	+ 40
Rabbits	81	3	50	+ 35
Dogs	180	3	30	+ 60
Men	360	1	10	+ 70

dose which induces drug-metabolizing enzymes had to be [60].

These observations provide a clue to an understanding of so many inconsistent reports about the induction of drug metabolism in man. Only those drugs induce hydroxylating enzymes which have a long half-life and accumulate to a certain extent in the organism.

The maintenance plasma level of phenobarbital which caused doubling of the hydroxylation rate in dogs was 15 to 30 μg/ml plasma [60]. The level producing induction in patients seems to be similar, but it will not be achieved in all patients receiving a daily dose of 0.1 to 0.2 gm phenobarbital. Taking into account the extremely large variation in plasma phenobarbital levels, which should be of the same order as those in patients treated with phenytoin [40–42], we should expect significant induction only in patients in whom barbiturates are metabolized slowly and phenobarbital reaches a plasma steady-state level of 10 to 30 μg/ml.

The large variation in phenytoin metabolism already mentioned may also be partially explained by an inconsistent increase in the amount of drug-metabolizing enzymes in some patients [56,59].

Inhibition of Drug Hydroxylations. Any drug which can be hydroxylated competes with another drug for the enzyme. Depending on the affinity and on the concentration of each drug in the liver, inhibition of hydroxylation can in fact occur [27]. Even ethyl alcohol, with a very small affinity for cytochrome P_{450}, can lower the metabolism of barbiturates in rats by competitive inhibition because of the extremely high concentration of alcohol which is reached in the liver, exceeding the level of barbiturates about 100 times if the alcohol concentration in the blood reaches 100 mg per cent [61,62]. Any drug which is effective only in a high dosage range is a potential inhibitor of drug-metabolizing enzymes and may interfere with the metabolism of a second drug prescribed. This can lead to a prolonged and stronger effect of the second compound administered. Many reports have been published about a decrease in drug action because of enhanced drug metabolism due to enzyme induction, but observations showing the opposite effect, i.e., an increase in drug action due to inhibited metabolism of the drug have been scanty and inconsistent thus far.

IMPLICATIONS OF THE PRESENCE OF THE MICROSOMAL HYDROXYLASE

Hydroxylation of Endogenous Compounds. The unspecific action of the microsomal hydroxylase extends to endogenous lipid-soluble compounds. All steroids are hydroxylated at different sites of the molecule, but to a very small extent [45] because the liver has at its disposal more specific enzymes for the inactivation of steroid hormones. Two types of enzymes should be mentioned, those which reduce steroids to the tetrahydro compounds, which are conjugated easily, and those which form 17-ketosteroids. However, synthetic steroids, e.g., those which possess an alkylgroup at C^{17} and have little or no affinity for the steroid metabolizing enzymes (such as the contraceptives) obviously are hydroxylated by the microsomal hydroxylase to various metabolites which are then excreted as conjugates in the urine [63].

Another endogenous compound metabolized in endoplasmic reticulum is the heme formed in mitochrondria and oxidized to bilirubin by cytochrome-P_{450} (Marver, personal communication). It is not yet clear whether the well known hydroxylations during the formation of bile acids are also catalyzed by the same enzyme [64]. Hydroxylation may be impaired in cholestasis. Probably more endogenous compounds will be discovered which are hydroxylated by the unspecific enzyme system in the endoplasmic reticulum.

Formation of Active Compounds. Not every hydroxylation of a drug in the liver leads to ineffective or less active metabolites. Some well known drugs are oxidized to compounds with nearly identical effects. Thus phenylbutazone is hydroxylated to oxyphenbutazone, which is now also widely employed as an anti-inflammatory agent. This is also true for phenacetin, the ethyl-group of which is oxidatively split off giving rise to N-acetyl-p-aminophenol (acetaminophen), and for aminopyrine which is demethylated to aminoantipyrine [3]. Another example is codeine, which is demethylated to the more active morphine, signifying that its

action depends to a great extent on the formation of morphine [3].

However, all the active metabolites mentioned are oxidized by a second or third step to inactive compounds, or they are conjugated directly, as is the case for morphine, acetaminophen and many other drugs. Sometimes the drug has to be oxidized to become active, but this is exceptional. Only two examples may be cited: Cyclophosphamide itself is inactive, oxidation in the endoplasmic reticulum converts it to an active alkylating drug useful in cancer therapy [65]. The well known pesticide parathion owes its activity to conversion to paraoxon by the hydroxylating enzyme [3].

Toxic metabolites may arise during the conversion of drugs. This is the case if small amounts of hydroxylamine are formed as side-products when aromatic amino-compounds such as sulfonamides are metabolized [66].

Liver Damage Caused by Carbon Tetrachloride. Nothing is perfect in nature. Thus, enzymic reactions which prevent accumulation of drugs may thereby have deleterious consequences. An example is the metabolism of halogenated hydrocarbons, especially carbon tetrachloride. Paradoxically, newborn rats are much more resistant to the toxic effects of carbon tetrachloride than adults, due to the low hydroxylation rate in newborn animals. In accord with this view is the observation that phenobarbital accelerates the metabolism of CCl_4 and increases its toxicity [67,-68,70]. Electron microscopic examinations of liver cells after exposure to carbon tetrachloride have shown that the lesion starts in the endoplasmic reticulum. However, even several hours after CCl_4 intoxication, when the membranes begin to disintegrate and lose their ribosomes, no significant morphologic changes may be visible in the mitochondria [67]. The loss of enzyme activity in the endoplasmic reticulum as well as the persisting normal function of the mitochondria are in accord with the morphologic observations [69].

With the decrease in cytochrome P_{450} the hydroxylation of drugs decreases [71]. Ribosomal protein synthesis declines similarly [72]. This is also true for glucose-6-phosphatase, an enzyme located in the endoplasmic reticulum. On the other hand the flavin enzyme called cytochrome-c-reductase, which transports electrons from NADPH to cytochrome P_{450}, cytochrome b_5 and the unspecific esterase which hydrolyses drugs, is not impaired three hours after treatment with lethal doses of CCl_4 [73]. This specific impairment of microsomal enzymes confirms the hypothesis that a free radical is formed from CCl_4 which reacts predominantly with the phospholipid moiety of cytochrome P_{450} and leaves the protein portion of the enzyme intact. Only enzymes which can be easily split off from the membranes of the endoplasmic reticulum and solubilized by detergents without any loss of activity resist the action of CCl_4. However, enzymes such as cytochrome P_{450} hydroxylase and glucose-6-phosphatase lose their activity strongly bound to the phospholipids.

Similar effects are caused by lipid peroxidation which also leads to breakdown of the endoplasmic membranes and damages only those enzymes whose activity depends on the integrity of the phospholipids in the membranes [69,74]. Lipid peroxidation and CCl_4 action seem to be related [67]. Free radicals react with phospholipids during the peroxidation process. Thus, it is likely that free radicals are also produced from CCl_4 by an unknown mechanism in which the hydroxylating system in the membranes is involved. This concept is supported by the fact that, thermodynamically, CCl_4 can be converted to a free radical more easily than any other organic solvent [75].

It is likely that related free radicals may be formed occasionally from chloroform and halothane, although this is much more difficult to establish thermodynamically. Although the metabolic steps in the conversion of CCl_4 are not yet elucidated, drug-metabolizing enzymes appear to be involved in the formation of the reactive CCl_4 metabolite.

A similar mechanism may be the reason for liver injury produced by a variety of drugs, e.g., phenothiazines, tetracyclines, sulfonamides and many other compounds. If one considers the enormous variation in drug metabolism it is possible that small amounts of free radicals are formed during the metabolism of these compounds in the endoplasmic reticulum membranes, reacting somewhat like CCl_4. It is even possible that drug metabolites act as haptens with proteins, causing a hypersensitivity reaction locally in the liver.

Drug Metabolism in Liver Diseases. The activity of the hydroxylase probably is decreased in acute hepatitis if the cell injury is comparable with the cell damage after administration of small doses of carbon tetrachloride. Because all sedatives, hypnotics, tranquilizers and similar drugs acting on the central nervous system belong to the category of drugs which are converted to inactive metabolites in the liver, their action would be intensified

and prolonged. This concept would agree with the clinical impression that patients with liver disease tolerate drugs less well than normal persons. The few reports published, however, do not fully demonstrate that drug metabolism, hydroxylations as well as conjugations, is generally impaired by hepatocellular diseases. Even severe hepatitis with high transaminase levels in the plasma does not affect drug metabolism consistently. On the other hand, several patients with mild forms of hepatitis metabolized drugs more slowly than one should expect [76–78].

Surprisingly, drug hydroxylations decrease only occasionally in patients with liver cirrhosis [79,80]. This behavior is very hard to understand in view of the decrease in the number of cells functioning in a cirrhotic liver. Taking into consideration the dependence of the hydroxylation rate on the amount of enzyme available in the liver, one would expect a fair correlation between the extent of the cirrhotic process and the effect on drug metabolism. But this is not the case. The huge variation in drug metabolism even in normal livers makes any comparison of the conversion rate in normal subjects and in patients with liver diseases extremely difficult [78]. The nearly normal rate of drug metabolism in most cirrhotic livers might be explained by an increase in drug-metabolizing enzymes in the parts of the liver still functioning. This might occur also in some types of hepatitis. It is known that the smooth endoplasmic reticulum increases during inflammatory processes in the liver. So long as the membranes in one part of the liver remain hyperactive we would expect that their enhanced function could compensate for the decreased metabolism in other parts of the liver in which the injurious process has progressed and converted the hypertrophic endoplasmic reticulum into a hypoactive form. This hypothesis is based on the course of hepatic damage produced by several drugs administered chronically [81]. An hyperactive hypertrophic endoplasmic reticulum in hepatocytes induced by the action of the drug is progressively converted into an hypoactive form which, however, still appears in the electron microscope as smooth membrane hypertrophy.

CONCLUSION

The liver is the organ which prevents drug and other intoxications. This report attempts to explain how detoxication is accomplished in the endoplasmic reticulum of the liver cells. Calling these processes "detoxication," incidentally, is typical teleologic thinking. How questionable such a concept is when it is realized that the same enzymic reaction which "detoxifies" one drug can convert another to a more toxic compound which produces lesions in the liver or in other tissues! The scientist who considers the reality of nature can only state that lipid-soluble compounds, endogenous as well as exogenous, are converted to more polar metabolites by the hydroxylating enzyme system, and can then be excreted more easily via the urine or bile.

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