DRUGS and ALTERED HEPATIC BIOTRANSFORMATION:

CLINICAL and EXPERIMENTAL STUDIES

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by

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CONTENTS

* denotes original clinical/laboratory study

TABLES

FIGURES

SUMMARY

In recent years basic research has contributed greatly to defining the role of the liver and its subcellular components in drug metabolism, and has drawn attention to various factors which may alter the activity of hepatic drug metabolising enzymes (such as enzyme induction). However, the clinical relevance of much of this information remains to be established. In this thesis I have attempted to explore certain drug-liver interactions, with particular reference to the clinical significance of drug stimulation of hepatic biotransformation, and the effect of liver disease on drug metabolism.

Chapter I outlines the evolutionary development of hepatic biotransformation [a more appropriate term than detoxication or drug metabolism since many naturally occurring endogenous and exogenous substrates are metabolised (not necessarily to inactive products) by a microsomal enzyme system of broad specificity]. This process can usefully be considered to occur in two phases, and details of the subcellular localisation and properties of the enzymes involved are reviewed. Rate limiting steps both <u>in vivo</u> and <u>in vitro</u>, and various pharmacological consequences of hepatic biotransformation are also discussed.

Chapter II summarises important genetic and environmental factors affecting hepatic biotransformation. Particular attention is paid to the phenomenon of hepatic microsomal enzyme induction. Various direct and indirect methods (morphological, pharmacological and biochemical) used to measure the activity of hepatic microsomal enzymes in man (and thus to determine the presence of the "induced state)

are reviewed. These include the merits and disadvantages of the measurement of urinary D-glucaric acid excretion, a technique applied in subsequent clinical studies (Chapters IV and V) to assess enzyme induction.

As the liver plays such a central role in the metabolism of both endogenous and foreign compounds, liver disease might be expected to have important consequences for drug metabolism. The conflicting evidence is outlined in Chapter III, together with data obtained from a collaborative study in patients with hepatic cirrhosis, using chlorpromazine as the test drug, and measuring the plasma disappearance and cerebral effects of this phenothiazine. Although no significant difference in plasma clearance of chlorpromazine (compared to normal controls) was found, the cirrhotic group was undoubtedly more sensitive to the sedative effects of this drug. Various mechanisms which might disturb hepatic biotransformation in patients with liver disease are discussed. Pharmacokinetic concepts are used in an attempt to explain the altered sensitivity to sedative drugs which is characteristic of cirrhotics, and a critical appraisal made of previous studies investigating drug metabolism in liver disease.

An excellent example of the clinical application of advances in the basic sciences has been provided by attempts to stimulate hepatic biotransformation in man using enzyme inducing drugs, such as barbiturates. This technique has been used most widely in the treatment of various types of jaundice, and is reviewed in Chapter IV. While undoubtedly

effective in lowering bilirubin levels, the mechanism of action remains obscure as hepatic enzyme induction is accompanied by several other drug mediated effects on the hepatocyte and whole liver. The results of clinical and animal studies undertaken in an effort to determine the relative importance of enhanced bilirubin conjugation compared to increased choleresis in the reduction of plasma bilirubin levels seen after phenobarbitone administration are presented. While not providing a conclusive answer, they emphasise the importance of species variation in response to hepatic enzyme inducing drugs, and the need for caution before extrapolating results from experiments in animals to man.

Additional possible therapeutic applications resulting from enhancement of hepatic biotransformation are considered in Chapter V. Although of theoretical interest in a number of clinical situations (Cushing's syndrome; dissolution of gallstones; treatment of inherited enzyme deficiency states; etc.) the practical value of enzyme induction seems limited. However, adverse effects are certainly of clinical importance, and altered drug pharmacokinetics; enhanced drug toxicity, and teratogenesis are discussed. Much interest has also centred on the possible role of drug mediated hepatic enzyme induction in the production of vitamin deficiency. Clinical studies on anticonvulsant osteomalacia, and folate deficiency, are presented. Our data showed a correlation between the extent of enzyme induction (as measured by the urinary excretion of D-glucaric acid) and both serum calcium and serum/red cell folate levels. These observations provided support for the suggestion that disorders of calcium metabolism after long-term anti-

convulsant therapy may be a consequence of altered hepatic biotransformation (enzyme induction). Moreover they suggested that the folate deficiency commonly seen in treated epileptics (and occasionally in other subjects on long-term therapy with other enzyme inducing drugs) may also be a consequence of hepatic enzyme induction. Although unproven, the hypothesis elaborated fits many of the observed facts. Data from further studies presented in this chapter indicate that hepatic enzyme induction may occur during human pregnancy.

The work described so far was all undertaken at the Liver Unit, King's College Hospital, and provided the basis for further studies on a unique form of drug-liver interaction. These were carried out at the University of California Medical Center, San Francisco, and are described in the final chaper (V1), which examines the curious sensitivity shown by patients with the hereditary hepatic porphyrias, to many drugs (particularly barbiturates).

This group of pharmacogenetic disorders of porphyrin and haem synthesis is characterised by marked idiosyncrasy towards lipid soluble drugs. Interestingly, most of these drugs, which can precipitate acute attacks of porphyria, are recognised as being inducers of the hepatic haemoprotein cytochrome P450, the terminal oxidase in drug metabolism.

The primary genetic defect in the hereditary hepatic porphyrias is partial deficiency of an enzyme in haem synthesis (which may result in secondary de-repression of ALA-synthetase, the initial and rate limiting enzyme in the pathway). We considered whether the unique sensitivity

to drugs in this group of disorders might be related to the genetically determined partial block in haem synthesis. Partial inhibition of haem synthesis was produced experimentally in rats (by administration of lead, which inhibits several enzymes in the pathway). This had only minor effects on ALA-synthetase activity, but greatly enhanced the sensitivity of this enzyme to induction by various drugs and steroids which alone had little or no inducing effect.

These studies provided a rational explanation for many of the previously obscure features of the hereditary hepatic porphyrias. In particular, the "idosyncratic" reaction to many drugs which is such a characteristic feature of this group of disorders, can now be seen to be a direct consequence of the primary genetic defect in haem synthesis. In this context, acute attacks of porphyria precipitated by drugs, can be regarded as another adverse effect of hepatic enzyme induction.

CHAPTER I

HEPATIC BIOTRANSFORMATION

(1) HISTORICAL AND EVOLUTIONARY ASPECTS

The therapeutic effect of decoctions of plants and fruits on ailments of the liver was mentioned in the Ebers papyrus in BC 1550 (Clarke, 1967), but recognition of the central role of the liver in the metabolism of most drugs, and of the influence of many drugs on hepatic structure and function was delayed for almost 3500 years. Claude Bernard's classical studies in the 19th century initiated modern scientific investigation of liver function, and contemporary interest has centred on this organ's role in detoxification processes. Whipple (1913) pioneered the study of the role of the liver in drug metabolism, and more recently over the past 25 years, R.T. Williams in England and Bernard Brodie in the U.S.A have established the study of hepatic biotransformation as an important branch of biochemical pharmacology. The increasing number of scientific publications and new journals devoted to this subject attest to the growing interest in this field.

Although basic laboratory studies have contributed greatly to defining the role of the liver and its sub-cellular components in drug metabolism, the clinical relevance of much of this information remains to be established. This thesis attempts to explore the clinical significance of certain drug-liver interactions with particular reference to stimulation of hepatic biotransformation by drugs, and the effect of liver disease on drug metabolism.

In an evolutionary sense the development of hepatic drug metabolism can be regarded as a relatively recent phenomenon. Aquatic mammals have a poor capacity to oxidise or conjugate drugs, but the disposal of lipid soluble compounds presented no problem before the evolution of terrestrial life since these substances readily diffuse through lipoidal gills to be diluted in the surrounding aqueous environment. Before animals could live permanently on land, another means of disposing of lipid soluble impurities had to be developed, as the kidney is unsatisfactory for this purpose. Examination of renal structure reveals why this is so. Foreign organic compounds flow down kidney tubes lined by a collodial membrane, so that highly lipid soluble compounds will be almost completely reabsorbed, while lipid insoluble substances will pass through into the urine.

Indeed the kidney is so poorly equiped to excrete lipid soluble substances that it has been estimated that highly lipid soluble drugs such as thiopental or quinine would have had a half-life of about 100 years if the body lacked the means of making these substances less lipid soluble (Brodie, 1964). The problem was solved by the evolution of a completely non-specific enzyme system capable of metabolising foreign lipid soluble compounds to polar derivatives which could be excreted by the kidney. These include not only the lipid soluble dietary compounds first faced by land dwelling animals several million years ago (such as hydrocarbons, alkaloids, terpenes and sterols ingested in food, which would accumulate to enormous levels if they were not converted to water soluble excretable derivatives), but also the great variety of lipid soluble drugs, pesticides, food additives and other xenobiotics which man has to contend with today. Thus the evolutionary development of hepatic drug metabolising enzymes may be considered as an adaptive response to the movement of life from an aquatic to a terrestical environment.

It is of interest that in drug metabolism ontogenesis appears to parallel phylogenesis. The development of the endoplasmic reticulum in rat liver is histologically complete within 5 days of birth, but several weeks elapse before the specific activity of the processing (drug metabolising) enzymes reaches adult levels (Dallner et al, 1966). Even at this stage however, the total detoxicating capacity of the liver is several hundred times lower than that of adult liver (Soyka, 1969). This immaturity of the liver microsomal enzyme system responsible for the metabolism of many drugs is seen not only in laboratory animals, but also in the human infant (Pelkonen et al, 1973). Indeed impaired drug metabolism in infants has resulted in sensitivity to many drugs, and occasionally in serious and even lethal toxic reactions (Remmer,1970).

(2) GENERAL FUNCTIONS OF HEPATIC BIOTRANSFORMATION

The biological effect and fate of a drug is determined by various factors (which may be subjected to genetic or environmental control as is discussed in a later section of the thesis) including its absorption, distribution in the tissues, metabolism and excretion (into air, bile and urine).

Table 1

Relative ability to metabolize (unit wt./unit time)

	PHENOBARBITONE (Rat)	THIOPENTONE (Rat)	PHENOL (Guinea pig)	N-ACETYLSEROTONIN (Rabbit)
LIVER	100	100	100	100
KIDNEY	0	52	48	0
BRAIN	0	24	3	-
MUSCLE	0	0	3	-
HEART	0	19	0	0
LUNG	-	-	110	0
SPLEEN	-	-	22	0

From R.T. Williams (1972)

Relative drug metabolising ability of various organs from the rat, guinea pig and rabbit. Adapted from Williams (1972).

The role of drug metabolism or biotransformation will be considered in some detail.

After drugs are absorbed into the body they may undergo three possible fates.

1) They may be excreted unchanged.

2) They may change spontaneously into other compounds, without intervention of enzymes, because they meet the right conditions of temperature and pH for spontaneous breakdown, or because they can react chemically with certain compounds or groups which are present normally in the body. Thalidomide is one example of such a drug (Williams,1972).

3) However, the majority of drugs are metabolised by enzyme systems with the production of more polar, water-soluble, substances which can be easily excreted by the kidney. Although many tissues can metabolise drugs, by far the most active tissue per unit weight is the liver (Table 1). Other tissues including the adrenal, brain, liver, heart, muscle, spleen, intestinal mucosa, testis and skin show minor activity towards certain drugs, and the gut flora can also metabolise drugs in certain circumstances (Scheline, 1973). However, when the size of the liver and its blood flow are considered, its predominance in drug metabolism is readily appreciated (Williams, 1972).

Within the liver cell drug metabolising enzymes are present in the sap (soluble fraction) and mitochondria, but the majority of reactions of drug metabolism are carried out in the liver by enzymes located in the smooth endoplasmic reticulum (Gillette, 1966). This cellular component can be separated after homogenisation by high speed centrifugation to give the fraction called microsomes (operationally defined as the portion of liver homogenate that sediments at 100,000g) which with appropriate cofactors, can be used to study drug metabolism in vitro.

Table 2

Fraction of Liver	Relative rate of metabolism	
	PHENACETIN*	CODEINE+
Whole homogenate	100	100
Nuclei (crude)	8	13
Mitochondria	0	0
Microsomes	0	0
Cytosol (soluble fraction)	0	0
Nuclei + cytosol	15	26
Mitochondria + cytosol	8	0
Microsomes + cytosol	85	75

* 0 - deethylation by rabbit liver

\+ 0 - demethylation by rat liver

From R.T. Williams (1972)

Relative drug metabolising ability of various fractions and recombinations of liver homogenate. Adapted from Williams (1972).

Table 2 shows the relative abilities of the various fractions of liver homogenate to carry out two reactions, namely oxidative de - ethylation of phenacetin to paracetamol (p ethoxy acetanilide→p hydroxy acetanilide), and oxidative demethylation of codeine to morphine (3 methyl morphine → morphine). Apart from whole homogenate, the most active fraction is that containing the microsomes (which contain the drug metabolising enzymes) plus the cell sap/soluble fraction, which contains NADPH (reduced nicotinamide - adenine dinucleotide phosphate) a cofactor necessary for enzyme activity. The soluble fraction can be replaced in an in vitro system by the addition of NADPH, or an NADPH generating system.

(3) PHASES OF DRUG METABOLISM

An important concept introduced by Williams is that drug metabolism takes place in two phases. In the initial phase drugs undergo reactions classified as oxidations, reductions, and hydrolyses in which polar groups (OH, COOH, NH_2, and SH) are introduced into the non-polar drug molecule, or alkyl groups are removed to uncover potential polar groups. This allows the drug metabolite to undergo the second phase which consists of conjugation of the polar derivatives with the formation of glucuronides, sulphates, and amino acid conjugates containing glycine (e.g. hippuric acid) glutamine, or cysteine. Methylation and acetylation of drugs also occur as Phase II reactions. The net effect of these reactions of hepatic drug metabolism is that lipid soluble drugs which can be reabsorbed by the kidney are converted into water soluble polar metabolites which are then readily excreted by the kidney.

The metabolism of benzene is a good example of how the two phases of drug metabolism progressively convert a lipid soluble parent compound to a highly ionised water soluble product. Benzene first undergoes a phase I reaction of oxidation to phenol, a weak acid, which is then glucuronidated to the strong acid phenyl glucuronide which is virtually completely ionised at physiological pH.

This useful concept is necessarily an over simplification as drugs may be subjected to several competing pathways simultaneously. Furthermore not all drugs undergo two phase metabolism, since those which already contain a suitable polar group may undergo only a Phase II reaction

(conjugation), while alcohol for example, only undergoes a Phase I reaction since it is readily oxidised to CO_2.

(4) HEPATIC MICROSOMAL DRUG METABOLISING SYSTEM

(a) Mixed Function Oxidation (Phase I Drug metabolism)

Reactions catalysed by non-microsomal (cytosol) hepatic drug metabolising enzymes are of minor importance. Only two play a clinically significant role in drug metabolism (acetylation of drugs such as isoniazid, certain sulphonamides and phenelzine; and oxidation of alcohol by alcohol dehydrogenase - the microsomal contribution to alcohol metabolism is controversial) and they will not be considered further.

The first description of the metabolism of a foreign compound by hepatic microsomes was given in 1949 by Mueller and Miller who showed that the microsomal fraction of a liver homogenate was capable of metabolising amino-azo dyes. Later Brodie et al (1955) demonstrated that a similar enzyme system located in hepatic microsomes was responsible for the metabolism of many drugs. Recombination of the various cell fractions of liver homogenate revealed a requirement for both soluble and microsomal fractions. However full activity resulted from the microsomal fraction alone when the soluble fraction was replaced by NADPH or by a NADPH generating system consisting of NADP, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase.

Mason (1957,1965) suggested the term " mixed function oxidases " for enzymes mediating this type of reaction, while Hayaishi in 1964 introduced the designation " mono-oxygenases ", considering the NADPH dependent reaction merely a class of the general category of oxygenase reactions (Mannering, 1971). This latter terminology implied that these enzymes catalysed the consumption of one molecule of oxygen per molecule of substrate, with one atom of oxygen appearing in the product and the other undergoing two equivalent reduction. Direct support for this view was obtained in studies employing labelled oxygen and water, which showed that the oxygen utilised in the hydroxylation of acetanilide is derived from molecular oxygen rather than from water (Posner et al, 1961). The microsomal mixed function oxidase system catalyses a wide variety of oxidations including hydroxylation, side

Table 3

1. AROMATIC HYDROXYLATION

Acetanilide
Anabolic/contraceptive steroids
Aniline
Benzene
Naphthalene
Phenformin
Phenobarbitone
Phenylhydrazine

2. ALIPHATIC HYDROXYLATION

Amitryptiline
Antipyrine
Imipramine
Meprobamate
Metronidazole
Pentobarbitone
Tolbutamide

3. N-DEALKYLATION

Aminopyrine
Chlorpromazine
Codeine
Diazepam
Imipramine
Pethidine
Morphine

4. O-DEALKYLATION

Codeine
Griseofulvin
Phenacetin

5. N-OXIDATION

Chlorpromazine
Guanethidine
Imipramine
Nicotinamide

6. SULFOXIDATION

Phenothiazine
Chlorpromazine

7. DEHALOGENATION

CC14
DDT
Halothane
Trichlorethylene
T3, T4

8. EPOXIDATION

Squalene insecticides
(Aldrin, Heptachlor)

9. DEAMINATION

Amphetamine
Ephedrine

Mixed function oxidase (Phase I) reactions, with examples of drugs metabolised by each.

chain oxidation, N-oxidation, sulfoxidation, N, O, and S-dealkylation, deamination and desulfuration (Table 3). However this great versatility of the microsomal drug metabolising system seems less remarkable when the reactions are visualised simply as different kinds of hydroxylation requiring a reducing agent (NADPH) and molecular oxygen (Brodie et al, 1958). These workers suggested that NADPH participated in the reaction by reducing a liver microsomal component X, that then reacted with O_2 to form an " active oxygen complex ", which in turn hydroxylated various substrates by a group of non specific enzymes.

Gillette (1963), formulated the overall reaction for oxidation of a drug by the hepatic microsomal system as follows:

1) $NADPH + X + H^+ \longrightarrow XH2 + NADP^+$

2) $XH2 + O_2 \longrightarrow$ " active oxygen "

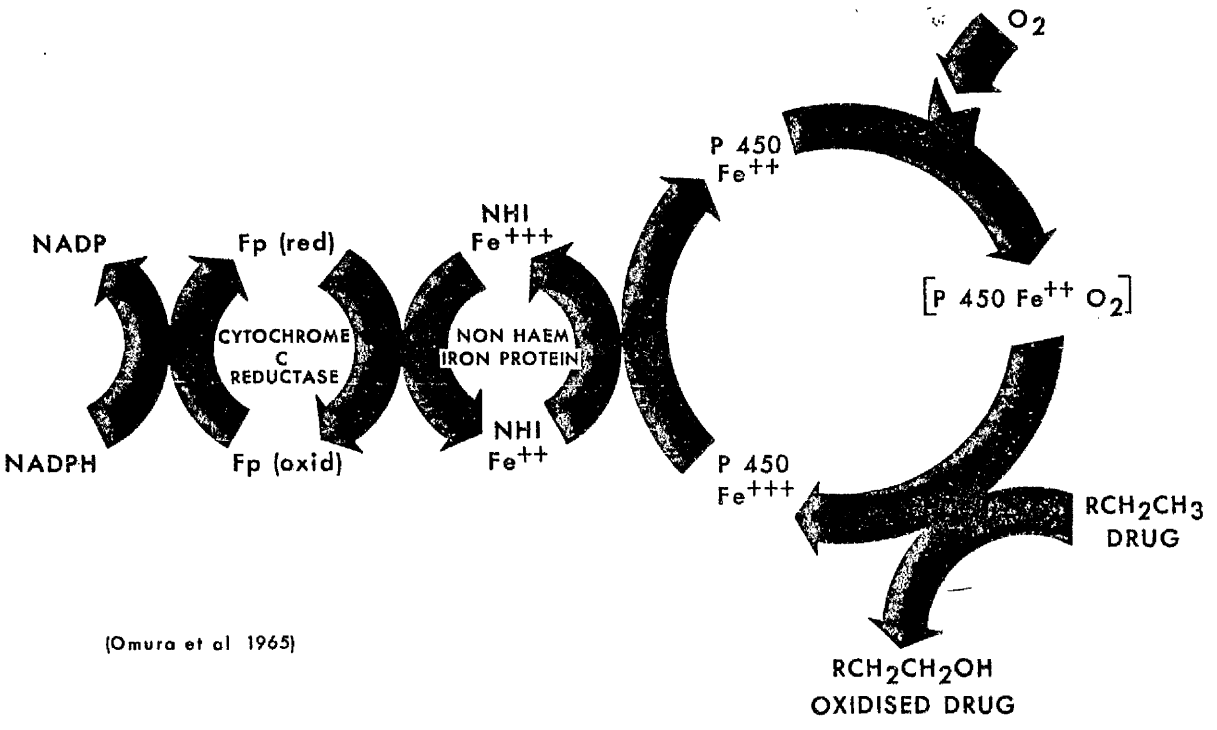
3) " active oxygen" + drug $\longrightarrow$ oxidised drug + $X + H_2O$

$NADPH_2 + O_2$ + drug $\longrightarrow$ $NADP + H_2O$ + oxidised drug

The scheme requires that equivalent amounts of NADPH, oxygen and substrate (drug) be utilised in the reaction. Subsequently it was established that the liver microsome component (X) reduced by NADPH was in fact the CO sensitive haemoprotein cytochrome P450 (Cooper et al, 1965).

There has been much speculation regarding the interaction of the components of the microsomal mixed function oxidase system responsible for drug metabolism, and about the nature of the associated electron transport system. Various elaborate schemes have been proposed (Mannering, 1971) and a relatively simple one is shown in Figure 1 (Omura, 1965) which illustrates our present understanding of the processes involved in the microsomal oxidation of drugs. The flavoprotein enzyme NADPH cytochrome reductase (Fp) is thought to transfer electrons (reducing equivalents) from NADPH to cytochrome P450, either directly or via an unidentified carrier X. This accomplishes the reduction of oxidised cytochrome P450 (Fe^{+++}) to reduced cytochrome P450 which is essential to the overall reaction.

Fig. 1



A simplified scheme showing interaction of components of the microsomal mixed function oxidase system. Electrons are transported from NADPH via flavoproteins for reduction of oxidised cytochrome P 450 (Fe +++) prior to substrate oxidation. Modified from Omura et al (1965).

Reduced cytochrome P450 (Fe^{++}) then combines with molecular oxygen (O_2) to form an " active oxygen complex" (red P450 - O_2). Binding of drug substrate to this complex now takes place which allows activated oxygen to be transferred to the drug molecule to give oxidised drug. The process involves the splitting of a molecule of " activated" oxygen, one atom being transferred to the drug, the other being incorporated into a molecule of water.

(b) Microsomal oxidation and electron transfer

The microsomal mixed function oxidase system has (in common with the mitrochondrial enzyme system) the capacity to transport electrons to a final cytochromal acceptor.

$$D + 2e + 2H^{+} + O_2 \longrightarrow D{:}\ O + H_2O$$

As shown above two electrons are used in the reduction of one molecule of oxygen; one for the reduction of one atom of oxygen to water, the second oxygen atom being incorporated in the substrate (D).

However in the endoplasmic reticulum (unlike mitochondria) there is no evidence that oxidation and electron transport is coupled with the formation of high energy bonds. Reduced NADP (probably derived from glycolysis) functions exclusively as an electron donor. The electrons are picked up by an NADPH dependent flavoprotein, cytochrome-C-reductase, and flow from the reduced flavin enzyme to cytochrome P450. The reduced heme of cytochrome P450 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.

(c) NADPH cytochrome c reductase

This microsomal flavin enzyme, which reduces cytochrome P450 (directly or possibly indirectly via an intermediate carrier) is believed to be the rate limiting step in the overall process, as the rate of drug metabolism (for certain substrates at least) has been shown to be more closely related to the rate of cytochrome P450 reduction, than to the total amount of cytochrome P450 (Davies et al, 1969).

(d) Cytochrome P450

A pigment in liver microsomes was first described by Klingenberg in 1958. A large number of mixed function oxidase reactions had previously been described, and it was recognised that the microsomal fraction of the liver and adrenal cortex was the site of such enzyme systems. However nothing was known about the nature of this pigment, or the mechanism of oxygen activation by these enzyme systems until Estabrook, Cooper, and Rosenthal in 1963 detected a CO-binding pigment in adrenal cortex. This had an absorption maximum at 450nm, comparable to the liver microsomal pigment earlier described by Klingenberg, and was able to hydroxylate progesterone.

For the first time a function could now be assigned to this CO-binding microsomal pigment, which on account of its spectral characteristics was called cytochrome P450 (Omura and Sato,1964) or reticulochrome because of its association with the endoplasmic reticulum. Subsequent studies established that the same cytochrome was responsible for drug hydroxylations (Remmer and Merker, 1965) and that the hypothetical liver microsomal component (X) which Brodie and his colleagues had previously postulated might react with oxygen to form an "active oxygen complex" was in fact this CO sensitive haemoprotein, cytochrome P450 (Omura, Sato, Cooper, Rosenthal and Estabrook, 1965).

(e) P450 binding to drugs

Studies by Remmer et al (1966) and Imai and Sato (1966) showed that drugs and other foreign compounds combined non-specifically with the oxidised form of cytochrome P450 to cause small but significant changes in its absorption spectrum. This reaction preceded the hydroxylation process, and two general types of reversible binding could be distinguished spectrophometrically when lipid soluble drugs were added to microsomal syspensions. The difference in the absorption of microsomal suspensions without and then with added substrates, called the binding spectrum, has been widely used as a tool for studying drug metabolism. Two general types of spectral change have been recognised, and compounds giving these spectra have come to be known as TYPE I or TYPE II drugs.

Type I drugs include hexobarbital, aminopyrine, and ethylmorphine, and cause diminished absorbance at 420nm and a peak at 385nm. Type II compounds include nicotinamide and aniline, and cause maximal and minimum absorbance at 430nm and 390nm respectively. Thus, with opposing Δmax and Δmin, type I and type II spectra are approximately mirror images of each other. However this distinction is not absolute and some substances appear to cause an intermediate type of spectral change.

The most persuasive evidence that these spectra do in fact result from interaction of drug with cytochrome P450 was the finding that solubilised and partially purified P450 combined with Type I and Type II compounds to give characteristic binding spectra (Lu et al, 1969). It is of interest that compounds which induce microsomal drug metabolism tend to be Type I compounds, and that the diminished ability of microsomes to metabolise certain drugs when stored in the cold, is probably due to loss of Type I binding sites. Storage has little or no effect on Type II binding (Shoeman et al, 1969). Nicotinamide (a Type II compound) was frequently added in the past to incubation mixtures when drug metabolism was studied in vitro, to prevent loss of NADP by inhibiting nucleotidase. However in concentrations commonly used it was found that drug metabolism was inhibited, probably because it binds to P450, and it is now excluded from incubation mixtures (Schenkman et al, 1967).

(f) Different Species of P450

The presence of at least two types of cytochrome P450 was suggested by studies demonstrating a preferential effect of certain inducing agents in causing increased metabolism of some drugs but not others. The mechanism by which phenobarbitone and many other drugs stimulate the synthesis of the microsomal drug metabolising enzymes has long been considered to be different from that by which polycyclic hydrocarbons such as 3 methyl cholanthrene and 3,4 benzpyrene produce their inductive effect (Mannering, 1971). This assumption was based on the evidence that drugs such as phenobarbitone induce the increased metabolism of a much larger number of drugs and other foreign substances than do the polycyclic hydrocarbons, and it was concluded that the polycyclic hydrocarbons caused the synthesis of a modified form of cytochrome P450.

Alvares et al (1967) provided further evidence for this suggestion when they showed that the Δmax of reduced microsomal protein bound to CO obtained after administration of polycyclic hydrocarbons differed slightly from that in untreated animals, being at 448nm instead of the usual 450nm, and named this modified cytochrome, cytochrome P448. Although it is established that administration of polycyclic hydrocarbons causes a change in microsomal haemoprotein, there is still controversy as to whether this change reflects formation of a new molecular species of haemoprotein or whether the change simply represents an alteration in the relative amounts of interconvertible forms of a single haemoprotein. Most of the evidence leads to the conclusion that administration of polycyclic hydrocarbons causes biosynthesis of a molecular species of cytochrome P450 (P448) not normally detectable in appreciable amounts in microsomes from untreated or phenobarbitone treated animals. The possibility that small amounts of cytochrome P448 may be found in untreated animals cannot be excluded. Indeed this might be expected as polycyclic hydrocarbons or other substances capable of inducing the synthesis of cytochrome P448 are present in the atmosphere, and may be ingested in the diet, or produced by intestinal flora. Questions regarding the number of haem containing enzymes in the endoplasmic reticulum cannot be answered definitely at present, but the available facts suggest there are two (Lu et al, 1973). The evidence strongly suggests that this is a relatively non-specific enzyme system. It is difficult to conceive of a large number of enzymes using haem as a cofactor, each reacting specifically with a particular type of chemical configuration (Remmer, 1970). Further evidence for the existance of only a limited number of forms of this haemoprotein is suggested by the finding that certain drugs such as SKF 525A which have a high affinity for cytochrome P450, can inhibit the metabolism of nearly all compounds reacting with this pigment (Anders & Mannering, 1966).

(g) Conjugation (Phase II Drug Metabolism)

The second phase of drug metabolism consists of the conjugation of the polar derivatives of drugs with a number of substances. However, glucuronide formation is the most common route of Phase II metabolism for many drugs (possibly because of the general availability of glucose in biological systems) and quantitively accounts for a major share of drug metabolites (Dutton, 1966).

The reaction involves the condensation of the drug or its biotransformation product with D-glucuronic acid, and does not take place directly, but requires the activation of glucuronic acid by the synthesis of UDPGA from UDP glucose. This is mediated by a dehydrogenase present in the supernatant fraction of liver, while the interaction of UDPGA with the acceptor drug is catalysed by glucuronyl transferase, a microsomal enzyme. In addition to a large number of drugs, many normally occuring substrates such as steroids, thyroxine and bilirubin are also conjugated with glucuronic acid. There is evidence that several glucuronyl transferases exist, but although competition can be shown between substrates for the same transferase reaction, their exact substrate specificity is unclear. In general glucuronide formation abolishes the biological properties of a drug. Glucuronides are more water soluble than their parent drugs because of the large hydrophilic carbohydrate moiety. At the same time they are usually stronger acids, and so more ionised at physiological pH. Such compounds are less likely to penetrate membranes than the parent drugs, are poorly reabsorbed by kidney tubules, and more readily excreted (Smith & Williams, 1966). Drug glucuronide conjugates may be hydrolysed by β glucuronidase, a lysosomal enzyme. Hydrolysis of a drug conjugate by β glucuronidase may restore the free aglycone in sufficiently high concentration to produce toxicity, but the exact function of this enzyme is not clear. It is of interest that a natural inhibitor of β glucuronidase (D-glucaric acid) is produced as an end product of the glucuronic acid pathway in the liver, and that drugs which induce the synthesis of hepatic enzymes, stimulate the production of this metabolite. This observation has been used as the basis for a clinical test of hepatic enzyme induction, and will be discussed in more detail in Chapter II.

Glucuronides are usually excreted by the kidney either by tubular secretion, or for conjugates of higher molecular weight, by glomerular filtration alone. In some species however biliary excretion of glucuronides is predominant. In the rat for example conjugates with a molecular weight in excess of 400 are usually actively secreted in the bile, while lower molecular weight derivatives are less likely to be transferred by this route.

Various factors including species variation, age, and drugs, have been recognised to affect glucuronide conjugation (Dutton, 1966). It is deficient in the cat and the Gunn rat. The former can synthesise UDPGA and is able to conjugate various endogenous compounds as glucuronides, but not so drugs - apparently because cats lack the glucuronyl transferases used in drug conjugation. The Gunn strain of rat is characterised by unconjugated hyperbilirubinaemia and diminished ability to conjugate certain compounds with glucuronic acid. Although UDPGA levels are normal, and aniline and nitrophenol are excreted as glucuronides, o-glucuronyl transferases are deficient, and bilirubin, o-aminophenol and o-aminobenzoic acid do not form glucuronide derivatives (Arias, 1961). A similar situation may explain the congenital unconjugated hyperbilirubaemia in children with the Crigler-Najjar Syndrome (Arias et al, 1969). Drugs which are normally conjugated with glucuromic acid may aggravate kernicterus in these individuals by further impeding the formation of bilirubin. The activity of glucuronyl transferase is influenced by microsomal enzyme inducers and thyroxine. In the newborn of most species, glucuronyl transferase activity is remarkably low (Dutton, 1966). Neurological damage due to unconjugated bilirubin in infants (Kernicterus) can be aggravated by novobiocin, an antibiotic which inhibits glucuronyl transferase activity (Hargreaves and Holton, 1962). Recent attempts to prevent or treat kernicterus by stimulating the activity of conjugating enzymes in the newborn by drug treatment will be discussed in a subsequent section of the thesis on therapeutic implications of hepatic enzyme induction (Chapter IV).

(5) RATE LIMITING STEPS IN DRUG METABOLISM IN VITRO

The rate of drug hydroxylation is extremely low compared with other known enzyme reactions, and differs between substrates, species and individuals. As discussed previously, the rate limiting step in drug hydroxylation is thought to be the reduction of the haem iron of cytochrome P450, catalysed by cytochrome-C-reductase. This varies with the type of substrate and its binding to cytochrome P450 (Schenkman et al, 1967A). It is increased if a type I substrate is bound to the hydrophobic region of cytochrome P450, while basic (Type II) compounds inhibit reduction (Remmer, 1970).

In intermediary metabolism the turnover number (number of substrate molecules converted/mole of enzyme/minute) is many orders of magnitude (10^3 - 10^6) greater than the number of molecules of drug converted by cytochrome P450 (Alvares et al, 1969). This low turnover may be largely explained by the non-specific nature of the microsomal mixed function oxidase system. Despite this most drugs seem to be metabolised at a reasonable speed, because of the surprisingly large capacity of the hydroxylating mixed function oxidase system. It has been estimated that 1g of rat or rabbit liver contains between 1.8 and 2.4mg of cytochrome P450, and that this haemoprotein accounts for greater than 1% of the total liver protein (Remmer, 1970). Human liver contributes only 2% of total body weight (compared to 4% in the rat) and contains about half to one third the amount of cytochrome P450 found per gram of rat liver. This species difference in cytochrome P450 content corresponds to the observation that man metabolises drugs in vivo at a rate 2 to 10 times slower than the rat (Quinn,Axelrod & Brodie, 1958).

Fasting increases the cytochrome P450 content of hepatic microsomes as does administration of phenobarbitone, hydrocarbons, and many other compounds known to increase in vitro and in vivo drug metabolism. A fuller description of this phenomenon of de novo synthesis of hepatic microsomal enzymes, and of its clinical relevance will be presented in subsequent sections of the thesis. However in addition to changes in the content and kinetic properties of drug metabolising enzymes which alter drug metabolism in vitro, certain other factors may affect the rate of drug metabolism in vivo.

(6) RATE LIMITING STEPS IN DRUG METABOLISM IN VIVO

Although the kinetic properties of hepatic microsomal enzymes are normally of central importance in determining the overall rate of drug metabolism in vitro, non enzymic factors are important in the whole animal, and under certain circumstances marked changes in enzyme activity may have little effect on metabolism of drugs in vivo. The difficulty of extrapolating from in vitro data to the in vivo situation is also emphasised by the recognition that metabolism of drugs in vivo may be affected by factors that are difficult if not impossible to evaluate with tissue homogenates (Gillette, 1971).

These include the following:

(a) Competitive Inhibition

The non-specific nature of the mixed function oxidase system results in the fact that most drugs competitively inhibit each others metabolism *in vitro* (Tephly & Mannering, 1967). However such competition rarely occurs *in vivo* because the free concentration of most drugs in the body is considerably below the concentration required to half-saturate the enzyme (Km). Thus when the free concentrations of two drugs are both below their Km values, competitive inhibition becomes negligible. Even if *in vivo* concentrations of both drugs are as high as their Km values, they will inhibit each others metabolism by only approximately 33% (Gillette, 1971).

(b) Limited Availability of Cofactors

Liver perfusion systems have provided evidence that starvation may decrease the metabolism of drugs by decreasing the level of NADPH in hepatocytes (Gillette, 1971). Fasting and feeding have important regulatory effects on the induction of microsomal enzymes in animals (Conney, 1967; Bonkowsky et al, 1973) but it is not known whether starvation might also impair glucuronic acid synthesis from glucose and hence impair glucuronidation by limiting the availability of UDPGA. Ascorbic acid has been shown to be required as a cofactor in microsomal drug metabolism (Krasner et al, 1974) and in a subsequent section (Chapter V) some evidence is presented to suggest that folate deficiency may also impair drug metabolism both *in vitro* and *in vivo*.

(c) Hepatic Blood Flow

The hepatic extraction ratio (ER) for a given drug is a measure of the capacity of the liver to metabolise that drug. It may be calculated as follows:

$$ER = \frac{Q.(Ca - Cv)}{Q.\ Ca}$$

Where: Q = blood flow to liver

Ca = arterial concentration of drug

Cv = venous concentration of drug

This expression simplifies to $ER = \frac{Ca - Cv}{Ca}$ and with drugs that

are metabolised by very active enzymes in the liver, or rapidly excreted into the bile so that they are cleared in a single passage through the liver, the extraction ratio equals or approaches 1.

Now, clearance (Cl) = Q. ER

Thus with drugs whose extraction ratio approaches one, clearance equals flow. This consideration is important with drugs which have high extraction ratios such as oxyphenbutazone (Whitsett et al, 1971) lignocaine (Thomson, Melmon et al, 1973) and propranolol (Branch et al, 1973). Under these circumstances in vivo drug metabolism is completely limited by the rate of blood flow to the liver, and independent of the activity of drug metabolising enzymes. Thus impaired elimination of such drugs in patients with chronic liver disease may be a consequence of reduced hepatic blood flow, rather than impaired drug metabolising capacity (Chapter III). Most drugs however have relatively low extraction ratios (ER$\approx$0.1) and their in vivo metabolism is not significantly affected by changes in hepatic blood flow (Gillette, 1971).

(d) Drug Distribution and Protein Binding

The half life ($t_{\frac{1}{2}}$) of a drug may be derived from the following formula (Gillette, 1971)

$$t_{\frac{1}{2}} = \frac{0.693\ Vd.\ Ca.\ K}{F.\ Cv.}$$

Where: Vd = volume of distribution of the drug

Ca = arterial concentration of drug

Cv = venous concentration of drug

F = fraction drug unbound to plasma protein

K = kinetic constant

Where the extraction ratio is low, Cv approximates to Ca, and the equation simplifies to:

$$t_{\frac{1}{2}} = \frac{0.693\ .\ Vd\ .\ K}{F}$$

The formula emphasises that drug elimination in vivo is affected both by the volume of distribution of the drug (Vd) and by the fraction of unbound drug in the blood (F). Changes in $t_{\frac{1}{2}}$ are directly related to alterations in Vd, but the effect of changes in the degree of protein binding of a drug on its elimination (metabolism) is more

complex. If the activity of drug metabolising enzymes in the liver is so high that virtually all the drug is cleared from the blood in one pass through the liver (ER = 1) an increase in binding of the drug by plasma proteins could accelerate its metabolism, by increasing the rate at which it is carried to the liver. However for the majority of drugs with low extraction ratios (ER ~ 0.1) the equation holds true and an increase in plasma protein binding of the drug may be expected to prolong its biological half-life. The magnitude of the effect depends on the degree of protein binding. Binding of a drug to albumin prolongs its $t_{\frac{1}{2}}$ by only 11% when 50% of the drug is bound, and only 33% when 75% is bound. This effect becomes therapeutically important when 90% or more of a drug is bound to albumin. At this level small changes in the degree of binding have large effects on biological half-life (Gillette, 1971).

(7) PHARMACOLOGICAL CONSEQUENCES OF DRUG METABOLISM

Hepatic biotransformation does not necessarily imply detoxification, since in addition to conversion of an active drug into a relatively inactive metabolite, the first phase of drug metabolism can change a drug into another compound with similar (or different) pharmacological activity, or it may convert an inactive compound into an active drug. These three possible consequences of Phase I metabolism are illustrated by the following examples.

PHENACETIN - this drug is by itself an active analgesic agent, but its activity is largely due to its oxidation product paracetamol (acetaminophen) formed enzymatically in the liver. This subsequently undergoes a Phase II reaction and is conjugated to form the inactive product paracetamol glucuronide (acetaminophenylglucuronide) which is then excreted.

Alternative pathways of Phase I metabolism of phenacetin also occur as was shown by the investigation of two sisters who readily developed methaemoglobinaemia after exposure to this drug, and who were found to excrete excessive 2-hydroxyderivaties of phenacetin, but relatively little of the normal de-ethylated metabolites (Shahidi, 1968). Recent studies indicate that paracetamol also undergoes a further Phase I reaction, to form a hepatotoxic intermediary (Jollow et al, 1973).

When paracetamol is taken in overdose, sufficient quantities of the toxic metabolite may be produced so that hepatic necrosis, and occasionally death, results (Clark et al, 1973).

PRONTOSIL - this drug has no antibacterial activity in vitro, but is active in vivo. It is converted by reduction in the liver to sulphanilamide, the active chemotherapeutic agent, which is then inactivated by subsequent acetylation (Bernhein, 1941).

PHENOBARBITONE - is partly metabolised and partly excreted unchanged in man. The two Phase process of metabolism involves oxidation to hydroxyphenobarbitone, a relatively inactive metabolite, which is then conjugated to an inactive excretory product (Butler, 1955).

CHAPTER II

GENETIC AND ENVIRONMENTAL INFLUENCES ON HEPATIC BIOTRANSFORMATION

(1) GENETIC FACTORS MODIFYING DRUG METABOLISM

(a) Species Differences

Various studies indicate that drugs which are not subject to metabolic transformation (usually highly lipid insoluble compounds) and which are mainly eliminated from the body through the kidney (a process not particularly different from one species to another) have remarkably similar pharmacological activities in various mammalian species including man. For example the parenteral dose of tubocurarine required to produce a 90-100% neuro-muscular block varies only between 1 and 5mg/kg in 9 species including man (Zaimis, 1953) and the effects of about 300 ganglion blocking agents, all quaternary ammonium compounds, vary even less (100-200%) in 5 species of animals (Nador, 1960).

However, comparative pharmacological studies clearly show that the majority of drugs which are lipid soluble and undergo enzyme catalysed hepatic biotransformation show major differences in both the rate and pathway of drug metobolism between different species (Williams, 1971). The quantitative aspects of species differences in hepatic drug metabolism, which may be related in part to species differences in cytochrome P450 content, were mentioned in Chapter I. Considering the evolution of hepatic drug metabolising enzymes it is perhaps not surprising that the activity of a particular enzyme is vastly different from one species to another, and in some species may be completely lacking. The cat is generally deficient in drug metabolising enzymes, and completely lacks the ability to conjugate drugs as glucuronides (although it is able to conjugate various endogenous compounds with glucuronic acid). In addition the relative importance of multiple pathways of drug metabolism may vary from one species to another. Deamination is the major route of metabolism in the rabbit, but in the dog and rat this reaction is virtually absent, and demethylation (dog) and hydroxylation (rat) are the major pathways. Although the patterns of metabolism of certain drugs in monkeys may be similar to those in man, the relative importance of different pathways vary greatly in new and old world monkeys.

These qualitative and quantitative differences in drug metabolism limit both the usefulness of laboratory animals for drug screening, and the validity of extrapolating results from animals to man. Some apparent species differences in respect of the effect of inducing drugs on hepatic bile flow and bilirubin metabolism between man and the rat (Maxwell et al, 1973) will be presented in Chapter IV.

(b) Inter-individual variation in drug metabolism

Biochemical differences between individuals are ultimately determined by genetic variability which controls the make up of proteins and enzymes regulating metabolic processes. Because most drugs undergo enzyme catalysed metabolic transformations, it is not surprising that inter-person differences in the rate and manner of drug metabolism (so called "pharmacological individuality") might also occur. Studies in man have confirmed this, and have shown that there are often pronounced individual differences in the disposition and metabolism of drugs, accompanied by differences in response (Alexanderson et al, 1969; Rawlins, 1974). This pharmacological variability may of course be due not only to hereditary differences but also to environmental factors such as drug-drug interactions, and the effects of hepatic enzyme induction, which are often difficult to separate (Conney,1967).

Sir Archibald Garrod, who pioneered the study of inborn errors of metabolism (Garrod, 1902) first suggested that unusual reactions to drugs might be caused by aberrations in metabolic pathways. The term " pharmacogenetics", introduced by Vogel (1959), originally referred only to the study of genetically determined variants detected by their unusual drug effects or responses. This definition, which was rather restrictive, has been extended to include a broad range of topics at the interface of pharmacology and genetics (Motulsky, 1969) and now covers the study of all clinically important hereditary variations in response to drugs (Vesell, 1972; 1975).

The evidence that genetic factors are involved in pharmacological variability in man has come not only from recognition of untoward drug responses in inborn errors of metabolism, but also from population and twin studies utilising accurate pharmacokinetic techniques to estimate the range of variation in drug response or drug metabolism. Two main types of variability may be demonstrated from population

studies. Where the data shows a discontinuous frequency distribution (bimodal or polymodal), and if environmental factors can be largely excluded, genetic differences are likely to account for the subpopulations (Evans, 1971). Classical examples of human genetic polymorphism in drug metabolism revealed by a polymodal distribution include the inactivation of isoniazid (Evans, 1960) and other drugs metabolised by cytoplasmic acetylases such as hydralazine, various sulphonamides and monoamine oxidase inhibitors; and the hydrolysis of succinylcholine by plasma pseudo-cholinesterase. As a generalisation it seems likely that polymorphism in drug metabolism is more likely to be observed with drugs whose metabolism is largely due to a relatively specific enzyme working in functional isolation, as is the case with the plasma pseudocholinesterase or cytoplasmic acetylase (Evans, 1971). However with the majority of drugs which are metabolised by the non-specific mixed function oxidase system of several interdependent microsomal enzymes, it is not surprising that variability has been shown to be unimodal. Here genetic influences are multifactorial, and the contribution to variability from alleles at various loci is of a similar order, and results in a single continuous frequency distribution. Thus for the majority of drugs metabolised by hepatic microsomal enzymes minor variations in the various enzymatic steps encountered in drug metabolism result in a continuous frequency distribution curve. Phenylbutazone is one example of such a drug whose metabolism has been demonstrated to be under polygenic control (Whittaker & Evans, 1970).

An additional approach to the demonstration of genetic factors in drug metabolism has been provided by twin studies. If monozygotic (identical) twins exhibit a virtually identical response when given the same dose of a drug, while dizygotic (fraternal) twins show differences, it is reasonable to infer that genetic factors play a significant role in drug response. Twin studies for a variety of drugs such as isoniazid (Bonicke & Lisboa, 1957), phenylbutazone (Whittaker & Evans, 1970) and antipyrine and dicoumarol (Vesell,1972) have confirmed the importance of genetic factors in drug metabolism.

From such studies it is possible to estimate the " heritability index", a measure of the per cent variation of the parameter studied which is due to genetic factors (Osborne & De George, 1959). For many drugs this has been found to be around 90-95% suggesting that under the conditions of the experimental studies (healthy, adult, non-hospitalised, non-medicated twins) environmental factors played a negligible role in the control of drug metabolism.

There is relatively little information as yet on genetically determined qualitive differences in human drug metabolism. One possible example concerned two sisters who readily developed methaemoglobinaemia after exposure to phenacetin, and who were shown to excrete excessive 2-hydroxy derivatives of phenacetin, but relatively little of the normal de-ethylated metabolites (Chapter I). Pretreatment with enzyme inducing drugs worsened the condition, and the findings were interpreted as suggesting that in the relative absence of the deethylation pathway, an increased amount of alternative metabolites were excreted which were responsible for methaemoglobin formation (Shahidi, 1968).

Many previously obscure adverse effects and idiosyncratic reactions to drugs are now recognised as having a pharmacogenetic basis (Motulsky, 1969; Vesell, 1972). The hereditary hepatic porphyrias are a particularly interesting group of pharmacogenetic disorders, as these patients show unusual sensitivity to enzyme inducing drugs. Some studies which attempt to explain the mechanism of this drug idiosyncrasy will be presented in a subsequent section (Chapter VI).

(2) ENVIRONMENTAL FACTORS AFFECTING DRUG METABOLISM

As a matter of clinical experience physicians have long recognised that when patients are treated for prolonged periods with certain drugs, a progressive diminution in drug response (tolerance) is often observed. In fact the first example of such tolerance to drugs is usually ascribed to King Mithridates who died in 63 BC. He murdered his family and concubines, and trusting no one, is said to have repeatedly taken small doses of poison throughout his life to prevent his enemies from poisoning him.

After his ultimate defeat by the Roman army under Pompey, he was unable to kill himself with poison, and had to order a mercenary to behead him. His actions are commemorated by the term " mithridatism", and by this means the novelist Alexandre Dumas also thwarted attempts to poison his fictional hero, the Count of Monte Cristo.

(a) Hepatic enzyme induction

It is now recognised that a number of environmental factors can regulate the activity of drug metabolising enzymes in liver microsomes, and the subject has recently been reviewed by Remmer (1972). Most attention has been directed to the role of drugs and chemicals, but the activity of these enzymes may also be altered by dietary and nutritional factors, X- irradiation and hormonal changes (Conney,1967). The first evidence that enhanced hepatic biotransformation might provide a partial explanation for drug tolerance came from studies which showed that livers from rats and mice fed commercial diets were considerably more active in metabolising amino-azo dyes in vitro than those fed grain or a purified diet (Brown et al, 1954). Later it was demonstrated that animals slept for progressively shorter periods of time after repeated administration of barbiturates (Conney et al, 1960).

Investigation of the metabolic changes responsible for this adaptive phenomenon showed it to result at least in part from enhanced microsomal drug metabolising activity in the livers of drug treated animals. After administration of certain compounds such as barbiturates and polycylic hydrocarbons the livers of treated animals exhibited an increased capacity in vivo and in vitro to metabolise not only that compound, but also a great many others via the microsomal mixed function oxidase system (Conney & Burns,1959; Conney et al, 1960).

More than 200 drugs including hypnotics, sedatives, CNS stimulants, tranquillisers hypoglycaemic agents and anti-inflammatory (adrenocortical) steroids are now known to stimulate the activity of drug metabolising enzymes in liver microsomes of experimental animals (Conney, 1967; 1969).

In addition many chemicals present in the environment have this property. Examples include the polycyclic aromatic hydrocarbons found in cigarettes smoke, polluted city air and certain cooked foods (Welch et al, 1969), halogenated hydrocarbon (organochlorine) pesticides (Hunter et al, 1972) urea herbicides (Kinoshita et al, 1966) and certain food preservatives (Creaven et al, 1966). Some colouring agents and various anutrients present in food-stuffs such as caffeine (Mitoma et al, 1968), flavones (Wattenberg et al, 1968) terpenes and safrole (Parke & Rahman, 1969) as well as cedar-chip bedding material (Ferguson, 1966) are potent stimulators of drug metabolism in laboratory animals.

(b) Factors controlling drug stimulation of hepatic enzymes

Stimulation of hepatic microsomal drug metabolising enzymes has been observed in all mammalian species including man, and is dose-dependent (Breckenridge et al, 1973) but the response is highly variable, depending on the agent used, species, strain and sex of animal studied, and its hormonal and nutritional status (Conney, 1967). In addition the ambient temperature and circadian rhythm can play a role in defining the response of microsomal enzymes to enzyme inducing agents. (Food, Cosmet, Toxicol, 1970) As a generalisation, the higher the intrinsic rate of drug metabolism in a given species, the greater the dose required to stimulate drug metabolising enzymes (Remmer, 1970).

Clinical studies have been limited by the difficulties in assessing hepatic enzyme activity in man, and the number of drugs presently recognised as being clinically important enzyme inducers is relatively small and almost certainly incomplete (Chapter V). In addition genetically determined individual variations in the magnitude of response to these drugs in man appear to be important, as shown by inconsistent responses to standard doses of inducing agents (Whittaker & Evans, 1970), and also by twin studies which have demonstrated large genetically determined differences in the inductive response to phenobarbitone (Vesell & Page, 1969). Interestingly, the greatest inductive responses occurred in those subjects with the longest drug half-lives before administration of the inducing agent.

The majority of lipid soluble compounds metabolised by the microsomal mixed function oxidase system (and some that are not, such as alcohol and barbital) are potential stimulators of drug metabolising enzymes. However clinically important hepatic enzyme induction appears to be limited to lipid soluble compounds with a long half-life, low hepatic extraction, and which achieve intracellular concentrations capable of initiating enzyme induction when given in usual therapeutic doses (Remmer, 1970).

(c) Morphological and Biochemical aspects

The exact mechanism responsible for the increased synthesis of microsomal enzymes after administration of drugs is still obscure, but the morphological and biochemical changes accompanying this adaptive response have been investigated. Administration of phenobarbitone to an experimental animals results in proliferation of the hepatic endoplasmic reticulum (visible with the electron microscope) and concomitant hypertrophy of the liver (Remmer & Merker, 1963). These morphological changes are accompanied by a co-ordinated sequence of biochemical events associated with an increase in phospholipid and protein moieties of the endoplasmic reticulum. The earliest change observed in rat liver is an increase in total microsomal protein synthesis, maximal at 3-4 hours after phenobarbitone injection (Kuriyama et al, 1969). This suggests the establishment of a primary membrane on which subsequently induced enzymes are deposited. Within 4-6 hours (in the rat) an increase in the activity of δ amino-laevulinic acid synthetase, the rate controlling enzyme in haem synthesis, is detectable and becomes maximal at about 12 hours. This response is followed in turn by increased haem synthesis, and is assumed to provide the additional haem necessary for increased synthesis of haemoproteins, and in particular for cytochrome P450 which accounts for more than 80% of the haem synthesised by induced liver cells (Meyer & Marver, 1971). A maximum increase in cytochrome P450 is seen 24 hours after a single injection of phenobarbitone in the rat, and if drug treatment is continued, a further increase in cytochrome P450 occurs until a new steady state is reached within 4-5 days with levels about three to four times above pretreatment values (Tephly, Hasegawa & Baron, 1971).

Increase in cytochrome P450 concentration is accompanied by stimulation of NADPH cytochrome c reductase (Ernster & Orrenius,1965) and an increased rate of both glucuronide formation (Zeidenberg et al, 1967) and overall drug metabolism in vitro (Burns & Conney,1965). Drugs which stimulate the activity of hepatic microsomal drug metabolising enzymes tend to be type I compounds, and (like phenobarbitone) generally have a non-specific effect in that they increase not only their own metabolism, but also that of many other unrelated compounds. This ability of a large number of single drugs to stimulate the rate of metabolism of a host of other unrelated compounds stems from the apparent lack of substrate specificity of the cytochrome P450 dependent enzyme system. In contrast some of the carcinogenic polycyclic hydrocarbons show considerable specificity (Conney, 1967). A possible explanation for this specificity is that polycyclic hydrocarbons cause a selective increase in the synthesis of a specific species of cytochrome (P448) which participates in only a limited number of detoxification reactions (Chapter I).

(d) Regulation of hepatic drug metabolising enzyme activity

Enzyme levels in mammalian cells are regulated by three mechanisms:- activation which involves conversion of a pre-existing biologically inactive protein (zymogen) to an active form (for example conversion of pepsinogen to pepsin) and does not require synthesis of new protein; stabilisation which affects the level of enzymes by regulating their rate of breakdown - a diminished rate of degradation resulting in an absolute increase in enzyme concentration (Schimke et al, 1965), and induction or de novo protein synthesis which increases the rate of formation of protein, usually in response to a specific stimulus.

According to the theory of Jacob and Monod (derived from study of bacterial protein synthesis) the control of protein synthesis by induction is mediated by inactivation of a normal regulatory genetic repressor of protein synthesis (i.e de-repression). Drugs appear to affect the level of hepatic proteins and enzymes involved in biotransformation by at least two of these mechanisms. Studies of

the kinetics of isotopically labelled precursors and the use of inhibitors that interfere with various steps in protein synthesis have shown that phenobarbitone increases microsomal phospholipid by stabilisation (Holtzman & Gillette, 1968; Stein & Stein, 1969) while microsomal cytochrome P450 is augmented by de novo protein synthesis (Arias et al, 1969; Greim & Remmer, 1970). However NADPH cytochrome c reductase seems to be increased by both stabilisation and induction (Jick & Shuster, 1966; Kuriyama et al, 1969).

(3) EVALUATING THE ACTIVITY OF THE HEPATIC MICROSOMAL ENZYME SYSTEM IN MAN

There is growing awareness that a major contribution to inter-person variability in response to a fixed dose of many drugs is due to differences in individual rates of hepatic biotransformation (Sjoquist & Von Bahr, 1973). Among the major factors known to influence the activity of the hepatic microsomal drug metabolising system in man are genetic make-up, age, sex and various environmental factors, including treatment with certain drugs or other foreign chemicals (Chapter V). Considerable effort has been spent in recent years in investigating the clinical implications of some of these factors. Investigation of the phenomenon of hepatic enzyme induction in particular has greatly furthered understanding of certain drug interactions, drug detoxification, and the mechanism of action of some hepato-toxins. In addition, this important concept has provided new and potentially valuable modes of therapy as well as insight into the pathogenesis of various metabolic disorders. (Chapters V & VI)

Blood or plasma concentrations of a drug correlate more closely with pharmacological response than does administered dose. As tissue drug levels are determined by a balance between the rate of drug administration and the rate of elimination, quantitation of individual differences in drug metabolism assumes clinical importance. Considerable success has been achieved in individualising dosage regimens of drugs excreted primarily by the kidney (Peck et al, 1973). This success is due to the availability of simple tests of renal function (urea, creatinine or insulin clearances) which correlate in a predictable manner with the renal clearance of many drugs.

Table 4

MORPHOLOGICAL TECHNIQUES

Electron microscopy to detect proliferation of hepatic SER.
Qualitative - identify enzyme inducing compounds
Quantitative - morphometry

PHARMACOLOGICAL TECHNIQUES

Qualitative identification of enzyme inducing drugs and the induced state in vivo.

i. Alteration in drug effects
(Animals - hexobarbital sleep time; zoxazolamine paralysis.
Man - effect on dicoumarol anticoagulation).

ii. Alteration in pharmacokinetics
Plasma drug kinetics; urinary drug metabolic excretion,

Quantitation of activity of hepatic microsomal enzymes in vivo.

i. Use of model compounds (e.g. antipyrine)

ii. ^{14}C labelled drugs and $^{14}CO_2$ breath analysis.

BIOCHEMICAL TECHNIQUES

In vitro - measurement of hepatic drug metabolism, or components of hepatic mixed function oxidase system (e.g. p 450).

In vivo - measurement of endogenous products of hepatic metabolism.

i. Plasma gamma glutamyl transpeptidase (GGT) activity.

ii. Urinary 6B hydroxycortisol excretion

iii. Urinary ascorbic acid or D-glucaric acid excretion.

Methods of evaluating the activity of hepatic microsomal enzymes in man and laboratory animals.

However, the lack of suitable techniques for assessing liver microsomal activity in man has hampered quantitative studies of drugs eliminated primarily by hepatic metabolism. Most studies on hepatic enzyme induction have been limited to experiments on animals using techniques inappropriate to clinical investigation, and with findings which may not be applicable to man (Conney, 1967; Remmer, 1972). Indeed it has been stated that one of the urgent needs of medical research is " the development of methodology for measuring changes in drug metabolism in patients that could be used as readily as the common tests that are now used to examine liver function" (Committee on Problems of Drug Safety of the National Academy of Sciences) This section of the thesis attempts a critical review of the currently available methods for assessing qualitatively or quantitatively the activity of the hepatic drug metabolising enzymes, with special reference to the clinical detection of hepatic enzyme induction and the identification of enzyme inducing agents.

The techniques available can be classified, into three categories: Morphological; Pharmacological; and Biochemical (Table 4). Methods available within each category will be outlined, together with some examples of their practical application in clinical studies, and a brief assessment of their assets and limitations.

(a) Morphological techniques

Examination of the hepatic parenchymal cell under the electron microscope reveals proliferation of the smooth surfaced endoplasmic reticulum (S.E.R) in animals treated with phenobarbitone (Remmer & Merker, 1963). Other non-specific inducers of liver microsomal enzymes such as tolbutamide, nikethamide, chlordane and DDT also cause proliferation of the endoplasmic reticulum (Conney,1967). However the carcinogenic polycyclic hydrocarbons, which show considerable specificity as inducers, have a much smaller effect on this organelle (Fouts & Rogers, 1965).

In clinical studies the value of this morphological approach is restricted by ethical and practical considerations which limit the availability of liver biopsy material.

Where clinical indications have permitted biopsy, electron microscopy of liver tissue has shown typical proliferation of S.E.R. (and thus presumptive evidence for hepatic enzyme induction) in children treated with barbiturates (Thaler et al, 1972) after rifampicin and diazepam therapy (Jezequel et al, 1971; 1974), chronic alcohol ingestion (Rubin & Lieber, 1971) and interestingly, during pregnancy (Perez et al, 1971). Schaffner & Popper (1969) have speculated that hypertrophy of the smooth endoplasmic reticulum might under certain circumstances be associated with hypoactivity of microsomal enzymes, for example during certain stages of viral hepatitis. However, there is little evidence that hypertrophy of the S.E.R. is ever dissociated from increased in vitro activity of the hepatic microsomal drug metabolising enzymes.

While electron microscopic detection of proliferated hepatic S.E.R. can provide a qualitative index of induction of hepatic microsomal enzymes, observer variability in detecting and quantitating these changes is a major problem. Inability to quantitate changes may be partially overcome by the use of morphometric techniques (Weibel et al, 1969; Jezequel et al, 1974). However these are time consuming, technically demanding, and require specialised equipment.

(b) Pharmacological techniques

Various simple pharmacodynamic and pharmacokinetic tests have been utilised experimentally to identify possible enzyme inducing compounds (or to infer the presence of hepatic enzyme induction) in man and to quantitate the in vivo activity of the liver microsomal drug metabolising system.

i) Identification of enzyme inducing drugs and the induced state.

- alteration in drug effect. Hexobarbital (a short acting barbiturate sedative) and zoxazolamine (a muscle relaxant) are useful test drugs for screening potential inducing agents in experimental animals because their duration of action is regulated largely by the activity of hepatic microsomal enzymes.

Most inducing agents decrease the action of one or both drugs. For screening purposes it is necessary to study both, since the polycyclic hydrocarbons selectively accelerate the metabolism of zoxazolamine, while both test drugs are metabolised more rapidly after treatment of an animal with one of the non-specific inducers such as phenobarbitone (Conney, 1967). Measurement of sleeping time (return of righting reflex) after hexobarbital, and duration of paralysis following zoxazolamine correlate well with in vitro drug metabolising activity. Pentobarbital, another short acting sedative, may be used in place of hexobarbital (Borzecella & Manthei, 1957; Breckenridge et al, 1971). In a similar way, reduction in the intensity of coumarin anticoagulation has been used to identify indirectly those compounds which can cause clinically important induction in man. Interactions between enzyme inducing drugs and coumarin anticoagulants (which are also metabolised by microsomal enzymes - Ikeda et al, 1968) are common, therapeutically important, and relatively easy to detect as both the pharmacological action and plasma concentration of the coumarins can be measured (Koch-Weser & Sellers, 1971). When induction of enzymes that metabolise coumarin anticoagulants occurs, the metabolism of these drugs is accelerated, their plasma half-life is shortened and their total and free concentrations in plasma decrease. The resulting change in half-life and decreased anticoagulant effect reflects the degree of enzyme induction caused by the suspected inducing agent. This increase in the rate of coumarin metabolism following administration of an inducing drug has been shown to be reproducible (Breckenridge & Orme, 1971), but there is considerable inter-person variability in the degree of inducibility of in vivo metabolism, which may be largely genetically determined (Vesell & Page, 1969).

The administration of therapeutic doses of many drugs, including barbiturates, diphenylhydantoin, griseofulvin, glutethimide and dichloralphenazone has been shown to increase coumarin metabolism and thus they are considered to be inducers of hepatic drug metabolising enzymes in therapeutic doses (Breckenridge et al,1971; Aggeler & O'Reilly, 1969; Catalano & Cullen, 1966; Cucinell et al, 1965; MacDonald et al, 1969).

This indirect technique for testing whether drugs are clinically important hepatic enzyme inducers has a number of limitations. Because of the well known inter-individual differences in drug metabolism (Vesell & Page, 1969; Sjoquist & Von Bahr, 1973) it is usually necessary to study the same individuals before and after administration of the drug under investigation. Where such a paired comparison is not possible, large numbers of subjects may have to be studied. If the end point to be studied is a change in the steady state level or pharmacological effect of coumarin, observations may have to be extended over many weeks (Breckenridge et al, 1971) with the inconvenience of multiple venepunctures, and uncertainty regarding patient compliance in taking the test medication. Furthermore, use of anticoagulant drugs is not without hazard. Although the concomitant administration of vitamin K will lessen the risk of bleeding without altering coumarin metabolism (O'Reilly et al, 1962), the ability to follow clotting parameters is lost. Recent work has demonstrated the complexity of interactions involving warfarin and has revealed another major difficulty in using changes in coumarin kinetics or pharmacological effects as a clinical test of hepatic enzyme induction. (Lewis et al, 1974) After administration of phenylbutazone, instead of simple displacement of warfarin followed by enzyme induction as expected, the drug was found to have opposite effects on the metabolism of the R and S enantiomers of warfarin. Phenylbutazone caused a more rapid rate of disappearance of R, while slowing the disappearance of S. Since the enantioners of warfarin have different pharmacological potencies, ($S \succ R$), the net result is usually an increased anticoagulant effect. The significant differences in the metabolism of the R and S isoners suggests that warfarin is not an ideal drug for use in studies of enzyme induction.

- alterations in drug kinetics.

(i) Plasma drug studies:

Another indirect measure of changes in hepatic microsomal drug metabolising activity can be provided by demonstrating an alteration in the rate of plasma disappearance (or steady state level) of a suitable test drug.

Such a drug must of course be metabolised by liver microsomal enzymes, and be sensitive to changes in the activity of these enzymes. Thus a suitable test drug should have a low hepatic extraction ratio- that is to say its clearance must be limited by the capacity of hepatic microsomal enzymes, and be unaffected by changes in hepatic blood flow (Gillette, 1971).

Antipyrine, a non-toxic anti-pyretic agent which is well absorbed orally and distributes into total body water, has some of these properties. It is not appreciably bound to plasma proteins nor excreted unchanged by the kidney (Soberman et al, 1949), has no stereo-isomers, and is eliminated by hepatic metabolism. In addition antipyrine is relatively easily measured and so has been widely used to investigate factors regulating drug metabolism. Furthermore it can be measured in saliva, allowing kinetic studies to be performed without the necessity for repeated venepunctures.

Antipyrine has been widely used in clinical studies of hepatic microsomal enzyme activity. These have included studies investigating the effect of physical stress, thyroid function, and chronic renal failure on hepatic drug metabolism, and the role of genetic factors in controlling hepatic enzyme induction (Vesell & Page, 1969). In addition the metabolism of antipyrine and phenylbutazone have been used as indices of microsomal activity in studies investigating the effect of chronic exposure to DDT in workers manufacturing the insecticide (Kolmodin et al, 1969; Poland et al, 1970), and the effect of oral contraceptives (O'Malley et al, 1972; Carter et al, 1974) and of various hypnotics (Stevenson et al, 1972) on hepatic microsomal function. These agents have also been used to study the effect of concomitant therapy with enzyme inducing drugs on microsomal enzyme function in patients with liver disease (Levi et al, 1968) as well as in the investigation of the effect of various potential enzyme inducers on coumarin metabolism (Breckenridge et al, 1971).

The importance of genetic factors in explaining individual differences in the rate of plasma disappearance of drugs metabolised by hepatic microsomal enzymes is now well established (Vesell, 1972; 1975), but it is not clear whether the metabolism of different drugs is under separate regulatory control (Poland et al, 1970).

Although an excellent correlation has been demonstrated between the degree of change in plasma warfarin levels and change in half-life of antipyrine after administration of an enzyme inducing drug (Breckenridge & Orme, 1971), cross over studies of the in vivo metabolism of two or more drugs in the same individual have shown poor correlations between drugs of different classes (Sjoquist & Von Bahr, 1973; Davies et al, 1973). Antipyrine appears to be a more suitable test drug than phenylbutazone, as the latter is less sensitive to alterations in drug metabolising capacity in man (Stevenson et al, 1972; Carter et al, 1974). In addition phenylbutazone carries a small but definite risk of hypersensitivity reactions.

The pharmacokinetic approach to the clinical study of alterations in hepatic biotransformation is limited by the considerable inter-individual genetic differences in drug metabolism and response to enzyme inducing agents (Vesell & Page, 1969; Whittaker & Evans,1970). Consequently there is overlap in kinetic parameters between "induced" and control populations. This necessitates a paired comparison of subjects studied before and after administration of the drug under investigation, or where this is not possible, the accumulation of data from relatively large numbers of control and test subjects. The method also has the practical disadvantage of requiring multiple venepunctures and plasma drug level analyses. Moreover, a theoretical objection is the possibility of competitive inhibition of the test drug by the potential inducing agent, particularly when the latter has a higher affinity for the non-specific microsomal enzyme system than the test drug (Stitzel et al, 1968; Gillette, 1971; Remmer et al, 1973). In this connection, the time chosen for pharmacokinetic testing in relation to administration of the drug under investigation may be critical (Remmer et al, 1973). In addition, alternative explanations for observed changes in drug half-life (such as alteration in Vd or in protein binding) should also be considered when interpreting the results of such studies.

- alterations in drug Kinetics.

(ii) Urinary metabolite excretion patterns:

Measurement of the total urinary excretion of the oxidative metabolites of a drug, which reflects the activity of the hepatic mixed function oxidase system, may also provide an index of hepatic microsomal activity.

The criteria outlined earlier for the choice of an ideal test drug for plasma pharmacokinetic studies also apply, and an additional requirement in urinary studies which measure absolute amounts of drug or metabolite is that the drug administered should be completely absorbed (or given intravenously) otherwise comparison of urine data is invalid. Special pharmacokinetic techniques are available for deriving drug elimination rate constants from cumulative urinary excretion measurements. This however complicates the collection and analysis of data, and limits its application to co-operative subjects.

A number of animal and human studies have utilised measurement of the urinary metabolites of dipyrone, a soluble derivative of aminopyrine (Remmer, Schoene & Fleischman, 1973; 1973A). In clinical studies this drug was administered orally, and the urinary excretion of its major oxidative metabolite, 4-amino-antipyrine, determined over a six hour period. These studies showed considerable inter-person variability in the excretion of the metabolite (possibly due to individual differences both in absorption and metabolism of the parent drug). As excretion of 4-amino-antipyrine was reasonably constant for a given individual, increased urinary metabolite excretion was regarded as signifying enhanced hepatic drug oxidation. However, little or no augmentation of 4-amino-antipyrine excretion occurred when dipyrone was given concomitantly with an inducing drug, despite significant elevation in the hepatic concentration of cytochrome P450, a central and readily induced component of the mixed function oxidase system. This anomalous finding was considered to result from competition for microsomal enzyme binding sites between dipyrone and the inducing drug, as increased metabolite excretion was found when the test was repeated shortly after withdrawal of the inducing drug. Competition between test drug and inducing agent is thus likely to produce misleading results in pharmacokinetic tests of hepatic enzyme activity, particularly in the investigation of enzyme induction in subjects exposed to environmental inducers or on continuing drug therapy. These considerations, together with the potential risk of agranulocytosis from dipyrone, the critical requirement for accurately timed and complete urine collection, relatively small changes in 4-amino-pyrine excretion, and the necessity for paired studies, would seem to seriously limit the usefulness of this procedure.

Other test drugs may prove more suitable, and pharmacokinetic analysis of cumulative excretion data could provide a more sensitive index of changes in hepatic drug metabolising activity than measurement of the total excretion of metabolites over an arbitrary period. An example of this type of approach was a study of the cumulative urinary excretion over 8 days of metabolites of 14 C Warfarin (as a percentage of the total administered dose) in two subjects, before and during the administration of a inducing drug (antipyrine). There was considerable variation between the two subjects, but the rate of excretion of radioactivity was greater after administration of antipyrine (Breckenridge et al, 1971).

Identification of agents capable of causing enzyme induction and of patients in whom enzyme induction has taken place represents a separate area from attempts to quantify an individual's capacity to eliminate drugs. The preceding sections dealt with the former problem, while the next section is concerned with pharmacological techniques designed to quantitate drug metabolism in individual patients.

ii) Quantitative methods of assessing microsomal activity in vivo

The wide inter-individual differences in rates of elimination of drugs metabolised by the liver, which for some drugs can exceed a thirty fold range, has already been emphasised (Sjoquist & Von Bahr, 1973). For this reason it might be desirable to know the rate of elimination, or more precisely the plasma clearance,of a drug in a given patient. Then, individualised dosage regimens could be designed to attain and maintain any desired blood or plasma concentration of the drug. Ability to precisely individualise drug therapy may be very important for drugs with narrow therapeutic ratios or serious toxicity, or when it is necessary to achieve efficacious blood levels rapidly for a drug with a prolonged half-life and small margin of safety. The most obvious means for determining the disposition of a particular drug in an individual patient is to administer that drug, measure blood levels over time, and determine the kinetic parameters. However, for some drugs this approach may not be possible, either because the drug cannot be measured in plasma, or because insufficient time is available for such a study.

For these reasons it has been felt that it would be useful to have a laboratory test involving the administration of a model drug, the results of which could be used to predict the plasma clearances of other drugs eliminated by microsomal metabolism in an individual patient.

- model compounds

The useful properties of antipyrine have led to the wide use of this drug as a model compound in pharmacokinetic studies. However this approach has met with only limited success. Davies et al (1973) were able to show that although there was a poor correlation between antipyrine half-life and phenylbutazone half-life when both were given as a single dose, the correlation was excellent when phenylbutazone was continued for 5 days. The reason for this discrepancy is not understood. In this same study there was a fairly good correlation in 4 subjects between antipyrine half-life in vivo, and ethylmorphine N-demethylase activity in vitro, but no correlation was apparent between the drug half-life and P450 reductase or cytochrome c reductase activities. Vesell and co-workers were unable to demonstrate a correlation between antipyrine half-life and dicoumarol half-life, although such a relationship appeared to exist between phenylbutazone and dicoumarol (Vesell & Page, 1969). Kadar et al (1973) in a crossover study using four drugs hydroxylated by the liver microsomal enzyme system - antipyrine, amobarbital, sulfinpyrazone and glutethinmide - were also unable to find any correlation between antipyrine half-life and the half-life of any of the other three drugs. However, positive correlations were demonstrated between the remaining pairs of three drugs.

Although some investigators have reported correlations of antipyrine clearance or half-life with clearances or half-lives of other drugs (Symposium on " Assessment of Drug Metabolism in man - methods and clinical applications " Dundee, March 1974) many others have reported poor correlations between antipyrine and other drugs both in normal subjects and in patients with liver disease. It is becoming more apparent that many factors other than specific enzyme activity control the rate of metabolism of drugs in vivo (Chapter I).

Two of the most important of these seem to be alteration in the protein binding of drugs (both tissue and plasma protein binding), and in liver blood flow. Until it is possible to define the rate-limiting steps for certain classes of drugs, it would appear that the use of model compounds will be unsatisfactory.

The blood or plasma half-life of a drug, the index which has been most frequently used in making comparisons between drugs, is itself affected by two other variables - clearance and volume of distribution (Vd). Since the volume of distribution of a drug is independent of the rate of its metabolism, clearance is the preferred index. It should be emphasised however that plasma clearance is the sum of renal and metabolic clearance. Even when metabolic clearance is calculated it may not be entirely hepatic, and it also is an indirect estimate of hepatic microsomal activity since the rate-limiting step in the metabolism of any drug will still determine its clearance.

- ^{14}C labelled drugs and $^{14}CO_2$ breath analysis.

Another approach to the quantitative assessment of hepatic microsomal enzyme activity in vivo is the measurement of radioactive CO_2 in expired air. The technique is analogous to the ^{14}C glycocholic acid breath test used to demonstrate bacterial deconjugation in the gut (James et al, 1973). The basis of the method is the selection of an appropriately labelled drug which liberates $^{14}CO_2$ during metabolism by the liver. In rats the rate of exhalation of $^{14}CO_2$ has been used to calculate demethylation rates of 4- dimethyl - ^{14}C aminoantipyrine (aminopyrine) after intravenous administration. Excellent correlations were found when this method was compared with simultaneously determined plasma disappearance curves. Increased demethylation rates could be detected after induction by phenobarbital and decreased rates after partial hepatectomy (Lauterburg & Bircher, 1973).

Recently a similar but technically simpler technique using ^{14}C aminopyrine has been tested in man. Using interval rather than total CO_2 breath collection, a strong correlation was demonstrated between serum aminopyrine half-life and 12 hour breath $^{14}CO_2$ output.

Differences in plasma aminopyrine half-lives and $^{14}CO_2$ output could also be demonstrated in patients with hepatic dysfunction, and after phenobarbital or disulfiram pretreatment. Correlations were also found between $^{14}CO_2$ output and antipyrine plasma half-lives, and still obtained when tracer rather than usual doses of ^{14}C aminopyrine were used (Hepner & Vesell, 1974). Similar results in normal volunteers and patients with liver disease have been reported using ^{14}C dimethylaminoantipyrine (Gikalov et al, 1975).

The advantage of this technique is that it is non-invasive to the patient and relatively simple to perform. However breath analysis is also subject to the same limitations mentioned earlier for pharmacokinetic tests involving model compounds; namely that different drugs seem to have different rate-limiting factors influencing their hepatic clearance. Moreover it has not yet been reported whether correlations exist between $^{14}CO_2$ output and the hepatic clearance of other drugs. A further disadvantage of the $^{14}CO_2$ breath analysis technique as it has thus far been applied is that it appears to be less quantitative than plasma drug assay techniques. This is most likely due to the use of interval samples, since CO_2 output may vary with time, and the use of estimated rather than measured total CO_2 output, for calculating the ^{14}C specific activity of the expired CO_2. Despite these limitations the technique has much promise and deserves further investigation.

(c) Biochemical techniques

All the techniques to be described in this section have as their primary function the identification of compounds which may act as inducers of hepatic microsomal enzyme activity, or of patients in whom an induced state may exist. Although widely utilised in research studies, none is particularly useful for the clinician in adjusting therapy. They do have both advantages and disadvantages when compared with morphological and pharmacological techniques previously mentioned, and these will be discussed.

i) Measurement of components of the hepatic mixed function oxidase system in vitro.

The most direct test of whether a compound induces drug metabolising enzymes is of course to assay the activity of hepatic drug metabolising

enzymes in treated animals with suitable drugs as substrates. A great many drugs and foreign compounds tested in this way have been foundto stimulate the activity of hepatic microsomal enzymes (Conney, 1967) while others such as components of commercial diets (Brown et al, 1954) insecticides (Hart et al, 1963) and cedar-chip bedding material (Ferguson, 1966) have been discovered fortuitously.

Analysis of the hepatic concentration of cytochrome P450 may be a more generally useful test for enzyme induction than assay of overall drug metabolising activity, since the concentration of this carbon monoxide binding haemoprotein is easily measured in liver microsome suspensions (Omura & Sato, 1964), and increases after treatment with all stimulators of drug metabolism including the polycyclic hydrocarbons which are rather selective inducers of drug metabolising activity.

Direct assay of the components of the hepatic mixed function oxidase system (such as cytochrome P450 concentration) or of overall drug metabolising activity in human liver tissue is clearly limited by ethical and technical considerations. However a number of reports have demonstrated directly the stimulating effect of drugs on human liver microsomal enzymes, using tissue obtained either by surgical or percutaneous needle liver biopsy. Higher values for the hepatic concentration of cytochrome P450 and enhanced activity of bilirubin UDP-glucuronyl transferase were found in patients who had received phenobarbitone or other medication prior to upper abdominal surgery (Billing & Black, 1971). Administration of phenobarbitone 10mg/kg daily for 5 days increased activity of NADPH cytochrome c reductase (that component of the mixed function oxidase system which is considered to be rate-limiting in microsomal drug metabolism) assayed in needle biopsy specimens from 5 of 8 children studied. (Thaler et al, 1972).

Percutaneous needle biopsies generally yield only around 20 to 40 mg. of liver tissue, but recently micromethods have been developed which allow determination of cytochrome P450 content in addition to the activity of NADPH cytochrome C reductase and certain other enzymes in homogenates prepared from less than 20 mgs of liver (Schoene et al, 1972).

Induction of cytochrome P450, as well as increase in the rate of drug (p- nitroanisole) hydroxylation *in vitro* (but not of glucose - 6 - phosphatase or pseudocholinesterase, enzymes which are also located on the endoplasmic reticulum) were found in subjects taking rifampicin and phenytoin in therapeutic doses. Although large inter-individual differences in *in vivo* drug metabolism in man are well recognised, surprisingly small inter-individual differences have been found in the concentration of cytochrome P450 and *in vitro* rates of metabolism of some commonly used substrates using human liver biopsy material (Billing & Black, 1971; Schoene et al, 1972). This finding again emphasises the importance of non-enzymatic factors in drug metabolism, and may partly explain the poor correlation which exists between drug oxidation *in vivo* and *in vitro* (Sjoquist & Von Bahr, 1973).

Apart from the practical and technical difficulties encountered when working with human liver biopsy material, caution must be exercised when enzyme activities measured in milligram amounts of biopsied liver are extrapolated to an organ weighing many grams. In laboratory animals enzyme activities derived from assay of small samples of liver may not be representative of the entire organ, and significant variation has been found when sampling from different lobules (Fouts, 1973). This variation may be caused by structural differences in the composition of the liver biopsy (for example, in the relative amounts of fibrous and parenchymal tissue). In addition, there may be a genetic explanation for sample variation. Under certain circumstances the liver may contain a mosaic of two populations of cells, with one predominating in the liver as a whole or in a certain anatomical area. Thus samples from one area may differ considerably in activity from those from another area (Conn, 1973).

Clearly hepatic enzyme activity measured by a test reflecting the functional capacity of the whole organ would be advantageous. Three such tests, all involving measurement of endogenous products of hepatic metabolism, have been proposed as indirect measures of hepatic microsomal enzyme function.

ii) Measurement of endogenous products of hepatic metabolism.

- Plasma gamma glutamyl transpeptidase activity (GGT)

This enzyme is formed in several tissues including liver, kidney and prostate. Administration of hepatic microsomal enzyme inducing agents has been shown to result in elevation of GGT activity in a number of patients, in some of whom a relationship appears to exist between the extent of alterations in enzyme activity, and changes in rates of drug oxidation (Rosalki et al, 1971; Whitfield et al,1973). However exceptions occur sufficiently frequently to make assay of plasma GGT activity an unreliable index of alterations in hepatic biotransformation. The theoretical basis for these changes in GGT activity is obscure, and furthermore elevated plasma activity of this enzyme occurs with hepatocellular damage, and may also be seen following administration of drugs which do not alter rates of drug oxidation.

- Urinary 6β hydroxycortisol excretion

Endogenous compounds, such as steroids, and bilirubin, as well as drugs, are metabolised by the same non-specific microsomal mixed function oxidase system. Compounds that stimulate microsomal drug metabolism also stimulate the hydroxylation of steroids in animals and man (Conney, 1967; Kuntzman et al, 1968). 6β hydroxycortisol is a polar metabolite of cortisol produced by the microsomal hydroxylation of cortisol in the 6β position. The urinary excretion of this cortisol metabolite is stimulated by various drugs such as phenobarbitone (Burstein & Klaiber, 1965; Brooks et al, 1972), phenytoin (Werk et al, 1964) and phenylbutazone (Kuntzman et al, 1966). In experimental animals these same drugs have been shown to stimulate the hepatic microsomal enzyme system responsible for metabolising cortisol. Chronic administration of a non-hypnotic barbiturate (N - phenylbarbital) to volunteer subjects caused a greater than three-fold increase in the urinary excretion of 6β hydroxycortisol, without affecting the excretion of urinary 17 hydroxycorticosteroids. This would indicate that the increased 6β hydroxycortisol excretion did not result simply from an increased adrenal output of cortisol (Kuntzman et al, 1968).

Increase in the urinary excretion of this cortisol metabolite has been used as a measure of increased hydroxylating capacity of the hepatic endoplasmic reticulum, and thus as evidence of induction of hepatic microsomal enzymes in a number of clinical studies. Significant increases in both 6β hydroxcortisol excretion and phenylbutazone metabolism have been reported in workers exposed to organachlorine insecticides (Poland et al, 1970; Jager, 1970) which are potent enzyme inducers in animals. However no correlation was found between the rate of phenylbutazone disappearance and 6β hydroxycortisol excretion in these subjects. This may not be surprising, since endogenous steroid output probably differs considerably between subjects. In another report a two to three fold increase in 6β hydroxycortisol excretion after administration of therapeutic doses of antipyrine and dichloralphenazone was interpreted as indirect evidence of enzyme induction (Breckenridge et al, 1971). In their study the number of subjects was limited, and no correlation with other tests of activity of drug metabolising enzymes was reported. Smaller but again significant increases in both the total excretion of 6β hydroxycortisol and the ratio of 6β hydroxycortisol to total 17 corticosteroids were reported in an investigation of changes in human drug metabolism after long term exposure to various hypnotics in subjects taking standard doses of amylobarbitone and the non-barbiturate hypnotic diphenhydramine-methaqualone (Mandrax). A significant decrease in the plasma antipyrine half-life was also found with the use of these drugs. Again it was not stated whether a correlation existed between antipyrine half-life and 6β hydroxycortisol excretion (Stevenson et al, 1972). However, Smith et al (1972) were unable to find any correlation between the plasma half-life of antipyrine, warfarin or phenylbutazone, and the daily urinary output of 6β hydroxycortisol or D-glucaric acid. Nor was there any significant relationship found between the excretion of 6β hydroxycortisol and the metabolism of glutethimide, amobarbital, antipyrine or sulfinpyrazone in another clinical study (Kadar et al, 1973).

There is no evidence that the urinary excretion of this endogenous metabolite correlates well enough with an individual's ability to metabolise drugs to be useful as a clinical guide to therapy (Sjoquist & Von Bahr, 1973).

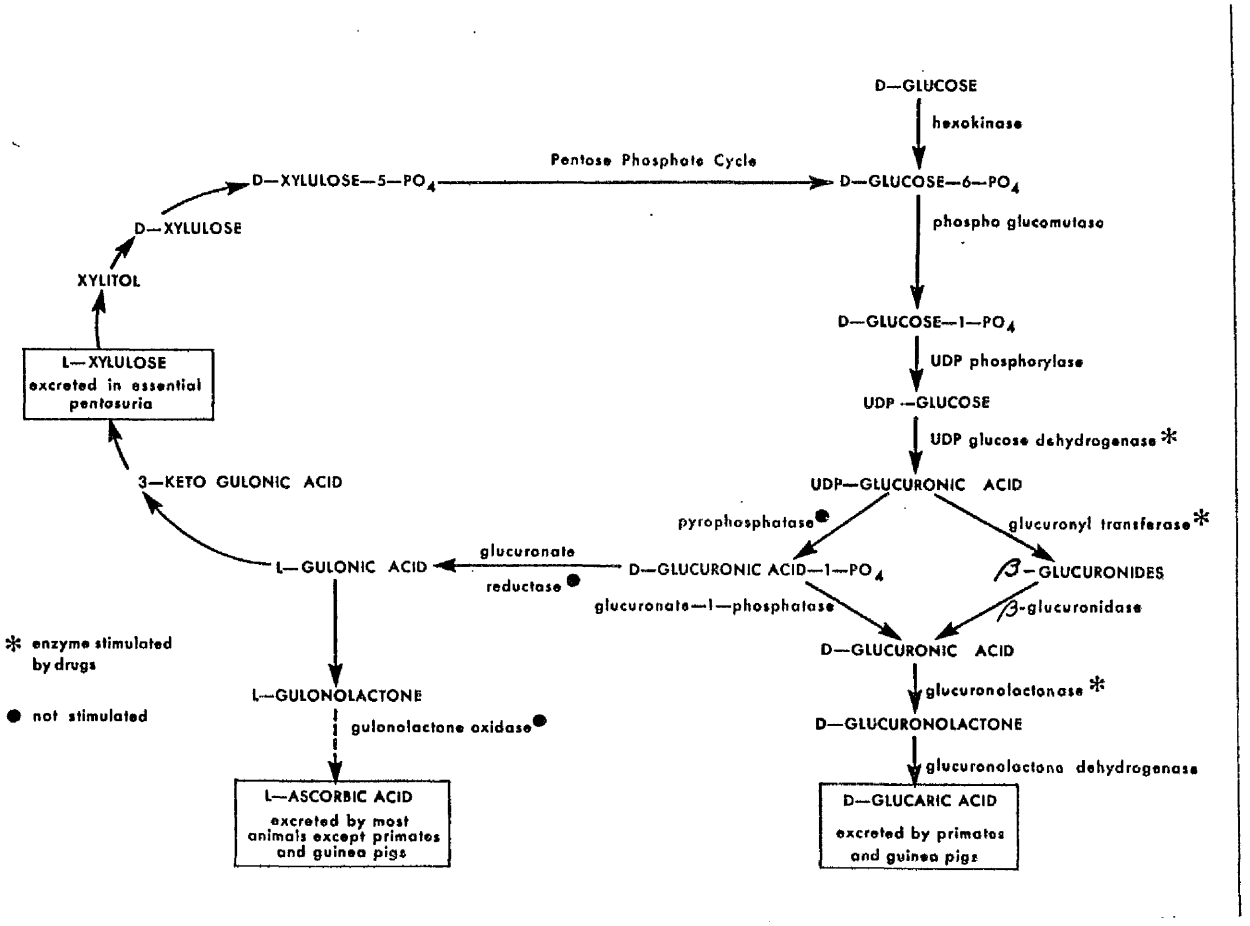
However the theoretical basis of the test as an index of hepatic enzyme induction seems reasonably well established. The absence of change in total urinary oxogenic steroid excretion following administration of inducing drugs suggest that increased 6β hydroxycortisol excretion represents enhanced microsomal cortisol hydroxylation rather than increased cortisol secretions due to drug administration. Unfortunately there is overlap between "induced" and normal values, and measurement of 6β hydroxycortisol is somewhat time consuming as it involves successive extractions of the polar metabolite followed by paper chromatography (Kuntzman et al, 1968; Thrasher et al, 1969). However the test does have the advantage of patient acceptability, as it is non-invasive and avoids the potential hazards associated with tests requiring the administration of drugs.

- Urinary ascorbic acid and D- glucaric acid excretion.

An incidental and unexplained observation reported in the study of the stimulatory effect of phenytoin on 6β hydroxycortisol excretion referred to earlier (Werk et al, 1964) was the finding of significantly increased inhibition of β glucuronidase in vitro by urine from subjects receiving the drug. It is now recognised that this effect is due to the stimulatory action of drugs on another endogenous metabolite, D- glucaric acid, which is an end product of glucuronic acid metabolism in man, and a potent inhibitor of β glucuronidase.

In fact evidence for the stimulatory effect of drugs on the metabolism of carbohydrates via the glucuronic acid pathway in man was first reported almost 30 years earlier when aminopyrine and antipyrine were found to elevate the urinary excretion of L-xylulose in patients with essential pentosuria (Margolis, 1929; Enklewitz & Lasker, 1935). These drugs were also found to stimulate ascorbic acid synthesis in the rat (Longenecker et al, 1940). Now a variety of drugs and foreign compounds which differ widely in chemical structure have been found to be capable of both increasing the activity of drug metabolising enzymes in liver microsomes, as well as of stimulating the biosynthesis of L-ascorbic acid from glucose through the glucuronic acid pathway (Burns,1959; Conney & Burns,1959).

Fig. 2



The Glucuronic acid pathway showing its major end products D-Glucaric acid (synthesised and excreted by primates and guinea pigs) and L-Ascorbic acid (synthesised and excreted by most animals except for primates and guinea pigs). Adapted from Dutton (1966).

* denotes enzymes in the pathway known to be stimulated by drugs.

● denotes enzymes not stimulated by drugs.

The urinary excretion of ascorbic acid may be used as an index of enzyme induction in rats (Conney et al, 1961) but not in primates or guinea pigs. These mammals are unable to synthesise vitamin C, and their end product of glucuronic acid metabolism is D-glucaric acid rather than ascorbic acid, as is shown in Fig. 2

D-glucaric acid ("acid potentiated inhibitor of B-glucuronidase") can be measured quite simply by its specific inhibitory effect on β- glucuronidase (Marsh, 1963; Hunter et al, 1971). Since a number of drugs have been shown to cause a parallel acceleration in the synthesis of D-glucaric acid and in drug metabolism, an increase in the urinary excretion of this endogenous metabolite has been proposed as a clinical test for hepatic enzyme induction (Aarts,1965). Increased excretion of this metabolite in man has been shown after administration of a wide range of drugs known to induce hepatic microsomal enzymes including barbiturates, primidone, phenytoin and organochlorine pesticides (Hunter et al, 1971; Hunter et al, 1972), antipyrine and phenylbutazone (Aarts, 1965), progesterone (Fahim et al, 1969) and high dose oral contraceptives (Mowat, 1968). Interestingly during pregnancy D-glucaric acid excretion is also elevated (Chapter V). A relationship has been demonstrated between the urinary excretion of D-glucaric acid and the total dose of various anticonvulsant drugs (measured by a simple scoring system) which suggests that excretion of this metabolite, like microsomal enzyme induction itself, is a dose-dependent phenomenon. Furthermore excretion of D-glucaric acid strikingly increased (up to ten fold) after treatment with barbiturates, and correlated well with plasma levels of exogenous and endogenous substrates known to be metabolised by microsomal enzymes (Hunter et al, 1971; Hunter et al, 1972; Maxwell et al, 1973).

The urinary excretion of D-glucaric acid parallels natural variations in microsomal enzyme activity, being low in the neonate and rising with maturity (Hanna et al, 1970) and showing a circadion rhythm in the adult similar to that seen in the activity of drug metabolising enzymes (Radzialowski & Bosquet, 1968) with peak excretion occurring in the afternoon and evening. Increased D-glucaric acid excretion in man is detectable as early as 4 to 6 hours after administration of an inducing drug, which is similar to the time of onset of increased microsomal enzyme activity following

administration of phenobarbitone to experimental animals (Marver & Schmid, 1968). In guinea pigs a significant correlation has been found between the urinary excretion of D-glucaric acid and the total hepatic content of cytochrome P450. Pretreatment of these animals with an inhibitor of protein synthesis (Actinomycin D) prevented both the rise in cytochrome P450 concentration and in D-glucaric acid excretion normally seen in response to phenobarbitone, providing evidence that the increased metabolite excretion is due to de novo protein synthesis (Hunter et al, 1973).

The glucaric acid pathway is complex, and both cytosol and microsomal enzymes are involved. Glucuronolactonase, a microsomal enzyme which catalyses the conversion of glucuronic acid to glucuronolactone can be induced (Kawada et al, 1961) but it is unlikely to be the critical step since glucuronic acid synthesis (an earlier event) is also readily induced after drug administration. UDP glucose dehydrogenase which catalyses the formation of UDP glucuronic acid from UDP-glucose can also be stimulated by drugs, but it is not known whether this is the rate limiting enzyme.

Although the precise mechanism is still unknown, it seems likely that drugs which stimulate the activity of microsomal enzymes involved in hepatic biotransformation also increase D-glucaric acid excretion by a similar effect on enzymes of the glucuronic acid pathway. An attractive but at present speculative explanation for enhanced activity of the glucuronic acid pathway following the administration of inducing drugs is that this process is an adaptive response occurring in parallel to the induction of microsomal oxidative drug metabolising enzymes. Increased activity of this pathway may provide additional glucuronic acid for conjugation of drugs after they have undergone initial (Phase 1 or oxidative) metabolism. In addition, the end product of the pathway (D-glucaric acid) provides a natural inhibitor of β-glucuronidase, an enzyme which is widely distributed in liver, kidney, and gut (Chatterjee et al, 1961). D-glucaric acid may thus have a role in preventing deconjugation of drugs (by the ubiquitous β glucuronidase) before they can be excreted.

Neither 6β hydroxycortisol nor D-glucaric acid correlate well with pharmacokinetic tests used to study hepatic drug metabolising activity (Smith et al, 1972). Thus it is unlikely that these biochemical tests will prove to be useful as clinical guides to individualising drug therapy (Sjoquist & Von Bahr, 1973; Kadar et al, 1973). However this does not reflect on their validity as tests of enzyme induction, as they both appear capable of detecting increased activity of hepatic microsomal enzymes, and share the advantages of patient acceptability and safety. Excretion of D-glucaric acid appears more sensitive to changes produced by inducing drugs than does 6β hydroxycortisol, and has the additional advantage of simplicity of assay. More information is needed on the regulation of the glucuronic acid pathway, but measurement of D-glucaric acid excretion seems to have some advantages over other currently available tests in the evaluation of hepatic enzyme activity, particularly in the study of large numbers of subjects on continuing drug therapy, or in the investigation of the possible effects of environmental inducing agents.

INFLUENCE OF CHRONIC LIVER DISEASE (CIRRHOSIS) ON DRUG METABOLISM

CHAPTER III

(1) INFLUENCE OF CHRONIC LIVER DISEASE (CIRRHOSIS) ON DRUG METABOLISM

The liver plays a central role in the detoxication of foreign compounds (Chapter I) and impaired drug metabolism is found in animals with various forms of liver damage. Partial hepatectomy (Masson & Beland, 1945); obstructive jaundice (McLuen & Fouts, 1961); carbon tetrachloride administration (Kato et al, 1962); viral hepatitis (Kato et al,1963) and experimental cirrhosis (Marshall & McLean, 1969) have all been shown to reduce drug metabolism in laboratory studies.

(a) Clinical Studies

Not surprisingly patients with hepatic cirrhosis have been considered to have impaired drug metabolism (Sherlock, 1968). With decreasing liver function a reduced elimination rate of drugs metabolised by the liver might be expected, but the available evidence is conflicting. However, the clinical impression that patients with cirrhosis tolerate drugs, especially hypnotics and sedatives, less well than normal subjects has been confirmed by studies showing that morphine (Laidlaw et al, 1961) and chlorpromazine (Read, Laidlaw & McCarthy, 1969) can cause changes in the clinical state and EEG wave pattern, particularly in patients with impending or overt hepatic coma. As both these drugs are transformed to inactive compounds by hepatic microsomal drug metabolising enzymes (Yoshimura, 1969; Coccia & Westerfeld, 1967) the increased sensitivity of cirrhotic patients might be explained, at least in part, by impairment of drug metabolising capacity. Consequently, sedative drugs that are excreted largely unchanged via the kidneys, rather than as metabolites, have been recommended for these patients (Sherlock, 1968).

Direct investigation of drug metabolism in cirrhotic patients has produced conflicting results (Tables 5 & 8). In many of the earlier studies no difference from normal could be detected with such varied drugs as pentobarbital (Sessions et al, 1954), phenylbutazone (Weiner et al, 1954; Brodie, Burns & Weiner, 1959); chloramphenicol (Kunin et al, 1959); alcohol (Lieberman, 1963; Winkler et al, 1969); tolbutamide (Nelson, 1964) and digoxin (Marcus & Kapadia, 1964).

Table 5

	DRUG	AUTHOR	ASSAY	PHARMOKINETIC MEASUREMENTS
1)	ALCOHOL	Weiner et al (1954)	Micro diffusion	Metabolism gm/h
2)		Winkler et al (1969)		
3)	AMINOPYRINE	Brodie et al (1959)	Spectro	$t\frac{1}{2}$
4)	ANTIPYRINE	Brodie et al (1959)	Spectro	$t\frac{1}{2}$
5)	CHLORAMPHENICOL	Kunin et al (1959)	Biolog.	$t\frac{1}{2}$
6)	CHLORPROMAZINE	Maxwell et al (1972)	GLC	$t\frac{1}{2}$, kel
7)	DICOUMAROL	Brodie et al (1959)	Spectro	$t\frac{1}{2}$
8)	DIGOXIN	Marcus et al (1964)	^{3}H-digoxin	$t\frac{1}{2}$ plasma, urine, faeces
9)	PENTOBARBITAL	Sessions et al (1954)	Spectro	Plasma levels only up to 60 min after infusion.
10)	PHENYLBUTAZONE	Weiner et al (1954)	Spectro	% rate disappearance per day.
11)		Brodie et al (1959)	Spectro	$t\frac{1}{2}$
12)		Hvidberg et al (1974)	Spectro	$t\frac{1}{2}$, kel
13)	SALICYCLIC ACID	Brodie et al (1959)	^{14}C Carboxyl salicyclic acid	$t\frac{1}{2}$
14)	TOLBUTAMIDE	Nelson (1964)	Spectro	$t\frac{1}{2}$

SOME STUDIES SHOWING LACK OF EFFECT OF CHRONIC LIVER DISEASE ON DRUG METABOLISM IN MAN

Table 5 (continued)

	NO.	PATIENT DATA AETIOL.	SEVERITY	BIOCHEM	ALTERED SENSITIVITY?
1)	10 severe	Alcoholic liver disease		?	?
2)	10 severe +	jaundice			
3)	10	?	?	?	?
4)	9	?	?	?	?
5)	11	?	10 bil ↑ 3 ascites	?	?
6)	24	Variable	Compensated	+	Yes
7)	6	?	?	?	?
8)	3	Alcoholic	Jaundiced	+	?
9)	21	Mostly alcoholic	Ascites +	+	?
10)	11	?	"Advanced"	?	?
11)	11	?	"Severe"	?	?
12)	6	Alcohol 4 Idiopathic 2	"Variable"	+	?
13)	8	?	?	?	?
14)	10	?	"Variable"	+	?

Table 5 (continued)

	CONCOMITANT THERAPY DETAILS	COMMENTS
1)	?	Alcohol metabolised largely by non-microsomal systems.
2)		
3)	?	" " "
4)	?	"Results suggest that metabolism of certain drugs is not affected in Laennec's cirrhosis."
5)	?	
6)	?	Factors from jaundiced serum may interfere with biolog. assay. Imperfect correln $t_{\frac{1}{2}}$ with bilirub. Metab. chloramphenicol essentially normal in majority of patients with cirrhosis.
7)	+	Increased sensitivity to sedative effects most apparent in those with previous history of PSE.
8)	?	
9)	?	Unsatisfactory assay - contribution from interfering substances and pharmacologically inactive forms. Crude clinical and pharmacokinetic assessment.
10)	?	Insufficient sampling.
11)	?	"Severe cirrhosis does not significantly alter the rate of phenylbutazone transformation."
12)	?	
13)	+	Correlation between kel and galactose elimination capacity in cirrhotics. $t_{\frac{1}{2}}$ determined after 4 days treatment. "Further pharmacokinetic data needed."
14)	?	Assay measures drug and main metabolite. No controls studied (historical controls.)
15)	?	

Levi, Sherlock and Walker (1968) suggested that the maintenance of normal drug metabolising capacity in cirrhosis might be due to hepatic enzyme induction as a result of concomitant administration of other drugs such as barbiturates, anticonvulsants and corticosteroids. However in their study no distinction was made between patients with cirrhosis and those with acute liver disease. The former group is more likely to be receiving continuing drug therapy, while experimental production of acute hepatitis has been shown to result in definite impairment of drug metabolism.

(b) In Vitro Studies of drug metabolism by cirrhotic liver

Measurement of the activity of drug metabolising enzymes in liver biopsies from patients with cirrhosis has demonstrated a reduction in the content of cytochrome P450, together with a lowering of the demethylating activity of drug metabolising enzymes (Schoene et al, 1972). This approach is limited by the sample variability characteristic of cirrhotic liver (Soloway et al, 1971). Furthermore in vitro studies of drug metabolism do not take into account the total hepatic capacity for drug metabolism, and disregard the influence of tranport processes, hormones and the in vivo availability of NADPH in the intact hepatocyte (Chapter I). Such studies are consequently difficult to interpret, and most effort has been directed to the in vivo study of the effect of chronic liver disease on drug metabolism by examining drug kinetics in patients with cirrhosis.

(2) PLASMA DISAPPEARANCE AND CEREBRAL EFFECTS OF CHLORPROMAZINE IN CIRRHOSIS

In deciding to investigate the problem further chlorpromazine was chosen as the test drug as it is often used for sedation, particularly of those with alcoholic liver disease, and because a method was available for its specific and accurate assay in plasma (Curry,1968). There had been no previous study in cirrhotic patients in which the cerebral effects of a sedative drug had been assessed in parallel with its metabolism, using objective quantitative techniques (Maxwell et al, 1972).

(a) Patients and Methods

Chlorpromazine (25mg) was administered by intravenous injection over

Table 6

CLINICAL DETAILS OF PATIENTS WITH CIRRHOSIS

PATIENT	AGE	DIAGNOSIS	ASCITES	VARICES	PREVIOUS ENCEPHALO
J.B. *	39	Chronic active	0	+	0
E.P.	58	Chronic active	0	+	0
W.L.	48	Chronic active	+	+	+
J.S.	70	Chronic active	0	+	0
E.R.	49	Chronic active	0	+	+
T.J.	25	Chronic active	0	+	0
J.C.	46	Alcoholic	0	+	0
P.R.	64	Alcoholic	+	0	+
J.W.	49	Alcoholic	+	0	0
G.H.*	47	Alcoholic	0	+	0
B.J.	26	Cryptogenic	0	+	0
N.W.	37	Cryptogenic	+	+	0
H.A.	57	Cryptogenic	+	+	+
D.J.	61	Primary biliary	0	+	0
F.G.	60	Primary biliary	+	0	+
G.B.	70	Haemochromatosis	0	0	0
J.S.	71	Chronic active	0	+	0
F.G.	49	Chronic active	0	0	0
C.C.	52	Chronic active	0	0	0
E.S.	58	Primary biliary	0	0	0
D.J.	48	Primary biliary	0	0	0
C.W.*	29	Primary biliary	0	+	0
B.M.	47	Post hepatitic	0	+	0
A.S.	53	Alcoholic	+	0	0

* Not involved in plasma level study

Table 6 (continued)

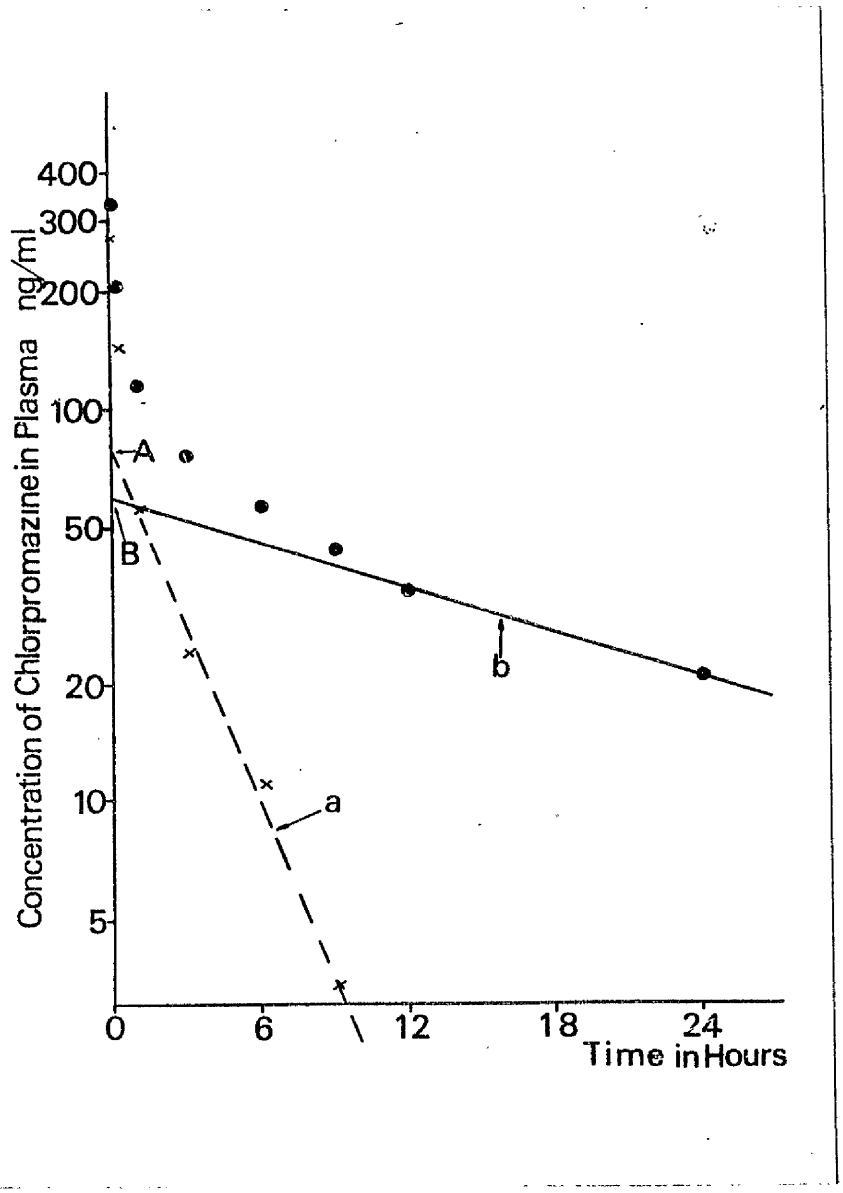
BIOCHEMICAL DETAILS OF PATIENTS WITH CIRRHOSIS

SERUM		PROTHROMBIN TIME	CONCOMITANT THERAPY WITH
ALBUMIN g/100 ml	BILIRUBIN mg/100 ml	SECONDS CONTROL	INDUCING DRUGS
2.5	4.0	9	-
2.4	1.2	3	-
3.1	6.0	1	-
2.3	1.2	4	-
2.2	1.7	0	-
2.9	1.7	4	-
3.1	0.5	2	-
3.0	1.9	5	-
4.0	2.0	4	-
3.4	1.5	6	-
3.3	0.8	3	-
2.9	1.0	4	-
3.6	2.9	4	-
2.5	1.7	1	-
2.4	6.0	3	-
2.6	0.5	1	-
2.1	0.9	4	Prednisone
2.7	2.2	2	Prednisolone
2.8	2.6	5	Prednisone
3.4	1.7	0	Phenobarbitone/Prednisone
2.9	8.5	2	Prednisone
3.5	2.9	1	Phenobarbitone
4.2	0.5	1	Tolbutamide/Phenoformin
4.0	4.0	4	Phenobarbitone

one minute to twenty-four patients aged from 25 to 71 years with cirrhosis (proven histologically or at laparoscopy) of whom eight had been on therapy with enzyme-inducing drugs for at least one week before the study, and were therefore considered to be 'induced'. Clinical and biochemical details of these patients are provided in Table 6 . None had clinical features of encephalopathy at the time of study, although five had a past history of this. The thirteen control patients had various medical conditions not known to affect hepatic or cerebral function and were matched for age, sex and weight. No patients in either group had clinical or biochemical evidence of renal impairment on the basis of history and all had normal blood urea on biochemical screening. Informed consent was obtained in each case after the purpose of the investigation had been explained in detail. Venous blood samples were collected at various times up to 48 hours after administration of chlorpromazine, and plasma concentrations were measured by using a gas-liquid chromatography technique in which unchanged chlorpromazine is completely separated from its metabolites. The method is capable of detecting nanogram amounts of the drug (Curry, 1968).

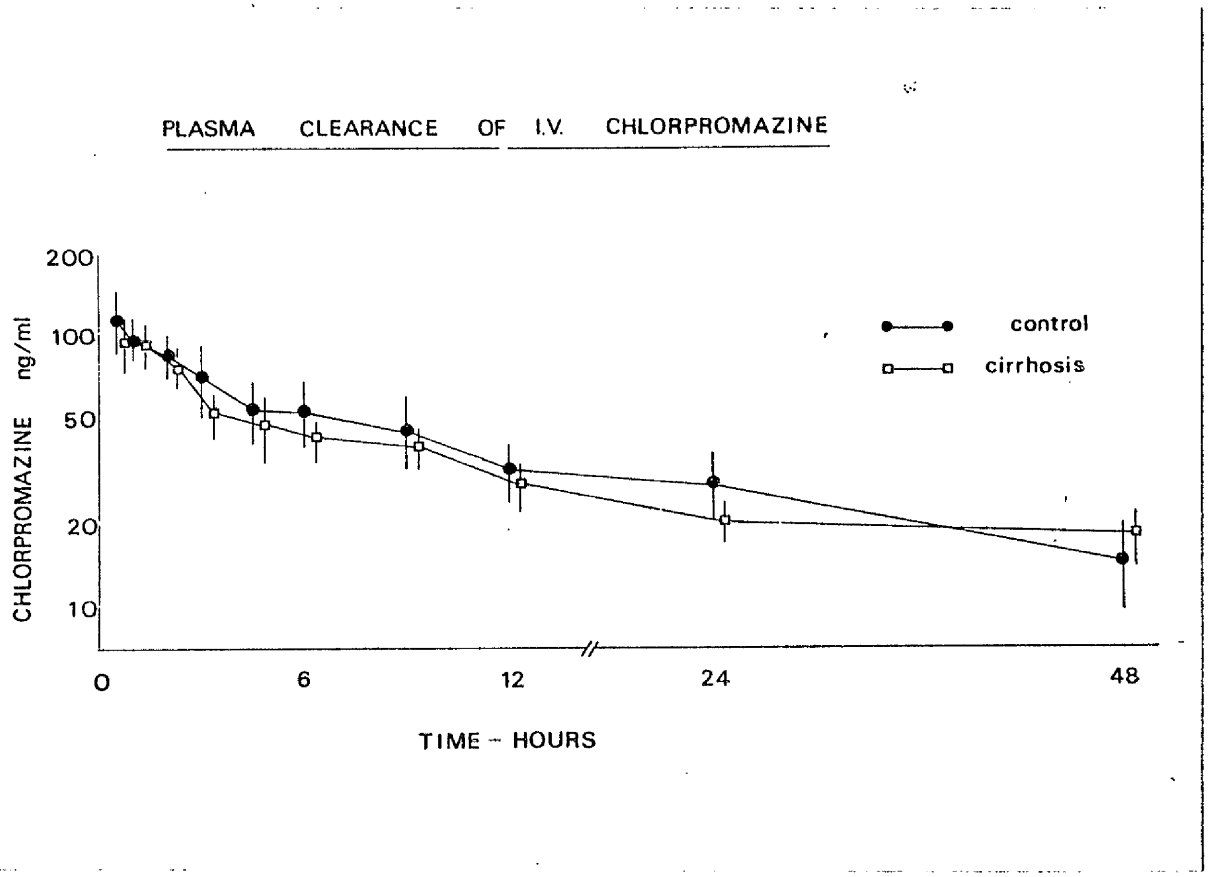
Clinical tests of cerebral function included assessment of alertness and orientation, writing and drawing of a five pointed star and the presence of asterixis. These tests were done before, 30 minutes and 6 hours after administration of chlorpromazine. EEG recordings were also made at these times in eight of those with cirrhosis including four with previous encephalopathy and in four patients without liver disease. Frequency analysis of the EEG recordings was performed with a B.N.1. low-wave form frequency analyser. Epochs (10s) from the right and left parieto-occipital regions were analysed and the skew mean-dominant-frequency (skew MDF) was calculated by a method which takes into account only those frequencies of greater than average abundance (Laidlaw & Aitken, 1966). Usually only three to five frequencies are included in this calculation and thus the skew MDF is less affected by slow and fast frequency artifacts in the recording than is the absolute mean-dominant-frequency. The techniques for EEG recording and automatic wave form analysis have been described by Parkes et al,(1970).

Fig. 3



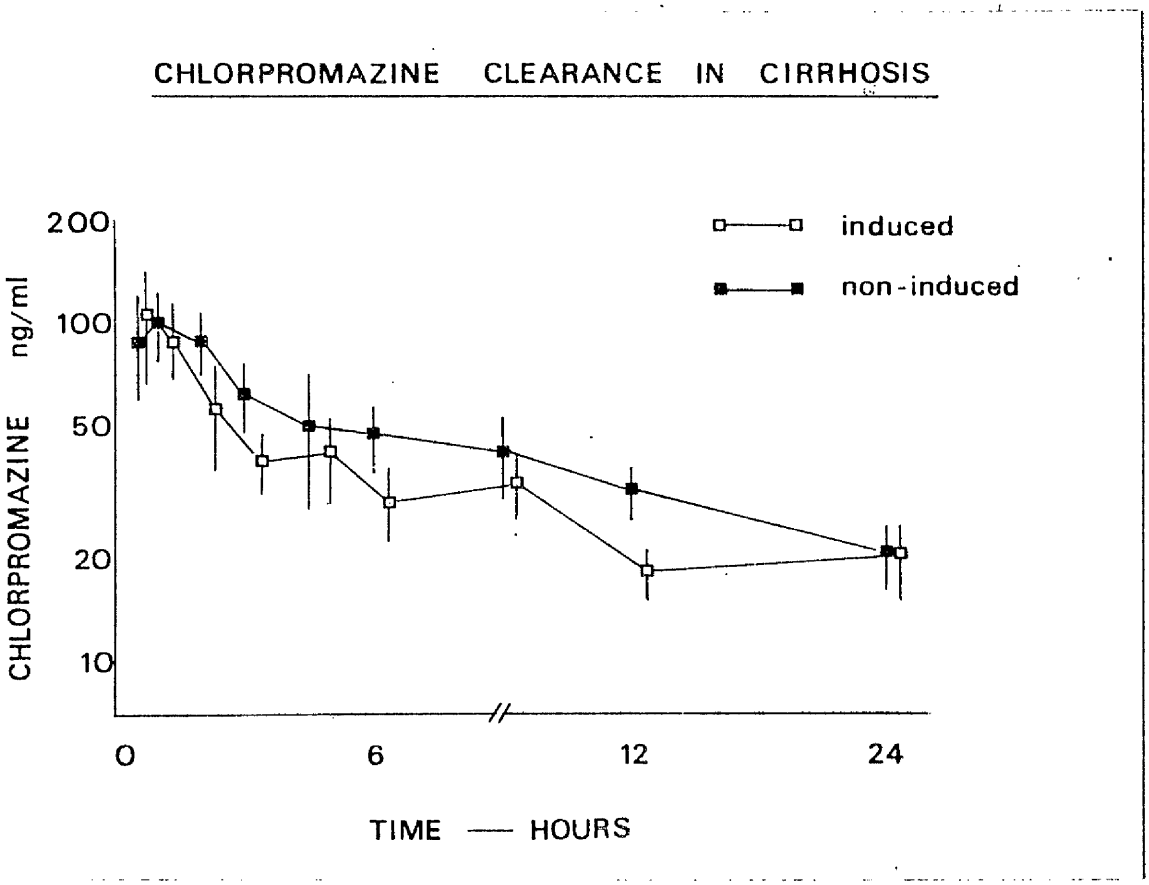
Semi-logarithmic plot for chlorpromazine concentrations in a control subject analysed into two components. Fast component (– – –), time constant $\hat{a}$, intercept A; slow component (———), time constant $\hat{b}$, intercept B.

Fig. 4



Plasma disappearance of chlorpromazine in controls (●) and in patients with cirrhosis (□).

Fig. 5



Plasma disappearance of chlorpromazine in cirrhosis, the patients being subdivided into induced (□) and non-induced (■) groups.

(b) Pharmacokinetics

In general, the plasma drug concentrations in each subject showed a smooth pattern of decline from the highest in the earliest sample to the lowest concentration at the latest time of sampling (Fig. 3) However, in a few subjects a fluctuating pattern was seen with unexpected increases in concentrations of the drug at 6 or even 24 hours. Mean drug concentrations (together with the SEM) at each sample time were determined for the complete group of control and cirrhotic patients (Fig. 4), and for the latter subdivided according to whether they were considered to be induced or not (Fig. 5). When this data was analysed by Student's t test for non-paired comparisons no differences in the drug concentrations in the control and cirrhotic patients, or in the induced and non-induced subgroups were evident at any sampling time.

The fluctuations in plasma concentration in some of the subjects already referred to occurred at various times, and when the values at each time-point from all patients were averaged, the resulting 'mean curve' showed a relatively smooth decline in concentration. It is apparent from these mean curves (Figs. 4 and 5), and from the curve for an individual patient in whom no fluctuation occurred (Fig. 3) that these could not be fitted by a single exponential, and a double exponential model was therefore applied making use of the methods of Riggs (1963). In the patient shown in Fig. 3 such an analysis gave values for the $t_{\frac{1}{2}}$ of the earlier fast component of 2.1 hours (rate constant α = 0.33hours^{-1}) and for the later slow component of 16.0hours (rate constant β = 0.043hours^{-1}). The extrapolated lines of the first and second components gave intercepts of 77ng/ml (A) and 60ng/ml (B) respectively, from which the theoretical drug concentration at time zero (Cp^{O}) was calculated to be 137ng/ml (Cp^{O} = A+B). The plasma concentrations recorded during the first hour could not be accommodated by this double-exponential model, but insufficient early points were recorded in this particular study to allow multiple-exponential analysis.

The true elimination rate constant Kel, which provides the best assessment of the rate of removal of drug from plasma, was calculated by the formula of Riegelman et al (1968) where:

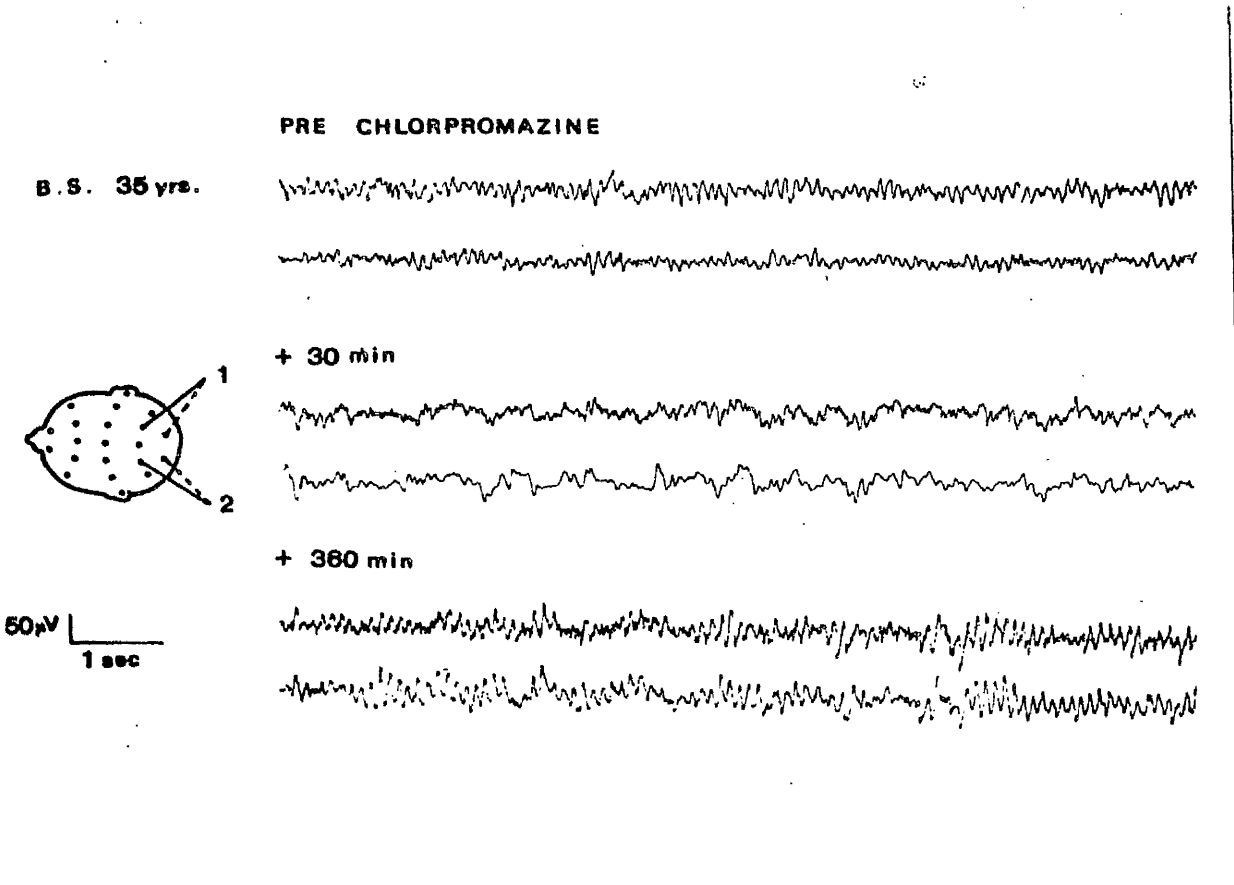
Table 7

COMPOSITION OF CLINICAL GROUPS AND THE CALCULATED KINETIC CONSTANTS FOR CHLORPROMAZINE IN PLASMA

	Controls	Cirrhosis		
		Total Groups	On inducing drugs	No other drugs
No. in sample	13	21	7	14
Wt. (kg)	64	63	64	62
Age	57	51	52	51
Cp^o (ng/ml)	116	121	97	116
A (ng/ml)	72	76	68	50
B (ng/ml)	44	45	29	66
a (h^{-1})	0.364	0.385	0.385	0.554
(h^{-1})	0.022	0.028	0.027	0.047
$t_{\frac{1}{2}}\alpha$ (h)	1.9	1.8	1.8	1.3
$t_{\frac{1}{2}}\beta$ (h)	31	24	26	25
K_{e1} (h^{-1})	0.053	0.067	0.078	0.078

C_p^o = theoretical drug concentration at zero time; A and B = intercepts on ordinate of extrapolated components; α and β = rate constants of two components; K_{e1} = true elimination rate constant.

Fig. 6



Effect of chlorpromazine on electroencephalogram of a patient with cirrhosis and no history of encephalopathy. Tracings taken before and after (30 and 360 min) chlorpromazine.

$$k_{el} = \frac{A + B}{A/\alpha + B/\beta}$$

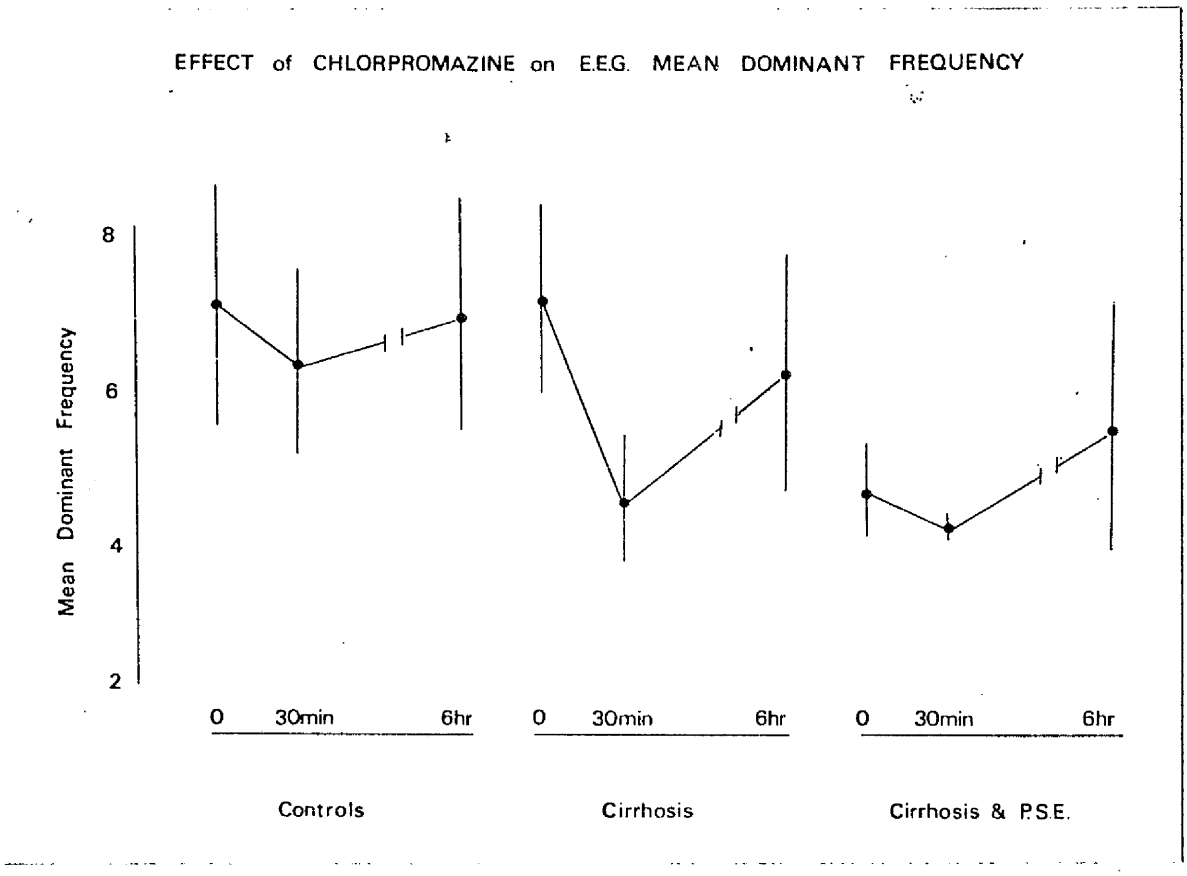
A, B, α and β were derived from analysis of the plasma disappearance curve. This gave a kel value for the patient illustrated in Fig. of 0.084^{-1} hours, and when the mean curves for the various patient groups (Figs. 4 and 5) were analysed in the same way the values obtained varied little (Table 7). This again indicates the lack of any significant difference in drug metabolism between cirrhotic and control patients, although a larger number of samples at later time points might have affected the values calculated for B and β.

(c) Effect of Chlorpromazine on the E.E.G.

Visual assessment of the recordings showed a change after administration of chlorpromazine in all patients with cirrhosis whether or not there was a past history of encephalopathy (Fig. 6). Slowing of the wave pattern was apparent at 30 minutes after administration of the drug, with a return towards normal at 6 hours. The calculated value of the skew mean-dominant-frequency before chlorpromazine in the cirrhotic patients without a history of encephalopathy (7.1 ± 1.29 Hz) was just below the lower limit of normal (9 - 11Hz) and was abnormally low in those with previous encephalopathy (4.5 ± 0.6Hz). In both controls and cirrhotics there was a reduction in mean dominant frequency 30 minutes after administration of the drug, with a return toward normal at 6 hours (Fig. 7). The most marked slowing was noted in those with a past history of encephalopathy, where the mean-dominant frequency decreased to 4.1 ± 0.2 Hz. All subjects were initially alert and well orientated but experienced drowsiness after administration of the drug. Both the controls and those with cirrhosis but without a history of encephalopathy could be readily aroused to an alert state and performed clinical tests well. In three of the four patients with a past history of encephalopathy mild confusion and deterioration in the test of writing and drawing was caused temporarily.

(d) Discussion

Fig. 7



Effect of chlorpromazine on EEG skew mean-dominant frequency (Hz) in control and cirrhotic groups with and without a history of porto-systemic encephalopathy.

The results of the present investigations show no modification of plasma concentrations of chlorpromazine in patients with compensated cirrhosis and are thus in keeping with the majority of reports of drug metabolism in cirrhosis (Table 5). The unexpected fluctuations in plasma concentration of chlorpromazine seen in some of the subjects in the present study have also been observed in dogs (Curry et al.,1970) and the increases are greater than those which could have arisen as artifacts due to analytical error. The double exponential concentration-time curve found from 1 to 24 hours after the dose has also been observed with a number of other drugs. The initial faster component represents the concentration declining in plasma and rapidly perfused tissues such as blood cells, lungs, brain, liver and kidney (distribution phase), whereas the slower component may represent the declining concentration in the whole body (elimination phase), and predominates when distribution equilibrium with all tissues has been fully achieved. This slower component has often been taken as a measure of the rate of metabolism of a drug. However, the half-life obtained from this portion of the plasma disappearance curve is a poor measure of the activity of processes removing the drug from plasma as this slope is affected not only by metabolism and excretion of the drug, but also by its redistribution between plasma and tissue compartments. The elimination constant (kel) provides a better assessment of the processes of drug-metabolism and/or excretion, as it takes into account the major contribution to plasma concentrations made by continual re-entry of drug into blood from tissue stores. However, even this parameter showed only minor differences between the patients with cirrhosis and those with normal liver function.

In the present study, only the disappearance of unchanged chlorpromazine was measured and we have no information as to any possible changes in the pattern of metabolism in cirrhosis. Chlorpromazine is metabolised by microsomal drug-metabolising enzymes to more polar metabolites such as chlorpromazine sulphoxide and various demethylated and hydroxylated derivatives (Coccia & Westerfeld, 1967).

(3) POTENTIAL MECHANISMS FOR ALTERATION OF DRUG KINETICS IN PATIENTS WITH CIRRHOSIS.

These can best be appreciated from a consideration of formulae previously presented in Chaper II for calculation of the elimination

half-life $t_{\frac{1}{2}}$ for a given drug.

$$t_{\frac{1}{2}} = \frac{0.693. \quad Vd \text{ (Apparent volume of distribution)}}{Q(\text{blood flow}).E \text{ (Extraction ratio)}}$$

From this derivation it can be seen that alteration of a number of different variables in patients with cirrhosis could affect the $t_{\frac{1}{2}}$ of an administered drug.

(a) Altered Hepatic Blood Flow

This factor is important for drugs which have a high hepatic extraction ratio ($\simeq$ 1) and whose metabolism is thus flow limited, but has little effect on the majority of drugs which have a low extraction ratio (Chapter I).

(b) Altered Hepatic Extraction

In contrast, this variable can be expected to affect the half-life of drugs with low hepatic extraction ratios which are capacity (rather than flow) limited. Such an effect may be brought about in a number of ways.

i) Decreased serum albumin binding of the drug. If hepatic metabolism is dependent upon the concentration of free (unbound) drug in the plasma, accelerated clearance of drug from plasma might be expected in situations in which the percentage of unbound drug is greater than normal (Rowland, 1972). The reduction in plasma protein levels seen in advanced cirrhosis could provide such a situation. Alternatively displacement of drug from protein could result as a consequence of hyperbilirubinaemia in chronic liver disease, as competition for albumin binding sites between bilirubin and drugs occurs in this situation (Hooper et al, 1974).

ii) Altered hepatic uptake and/or binding characteristics of the drug.

iii) Reduced hepatic drug metabolising activity. As the majority of drugs are metabolised by the hepatic microsomal mixed-function oxidase system (Chapter I) their rate of elimination is a function of the activity of this enzyme system.

(c) Altered Drug Distribution

Alteration in the apparent volume of distribution of a drug (Vd) may also be expected to alter its half-life, since these kinetic variables are directly correlated, as demonstrated by the above derivation.

In patients with cirrhosis the net effect of the disease process on the elimination of capacity limited drugs will thus reflect a balance between any increase in the percentage of unbound (free) drug, which will result in accelerated plasma clearance of the drug; and factors diminishing the rate of drug elimination such as impaired synthesis of microsomal proteins, reduction in the capacity of the liver microsomal enzyme system to metabolise the drug, or an increase in apparent volume of distribution of the drug.

(4) WHY IS METABOLISM OF SOME DRUGS UNAFFECTED IN COMPENSATED CIRRHOSIS ?

The maintenance of relatively normal drug metabolism in patients with impairment of other aspects of liver function is surprising, and is still unexplained. The contribution of compensatory alterations in drug disposition in cirrhosis already referred to may be important, but a number of additional or alternative explanations should be considered.

The possibility that significant extra-hepatic metabolism of drugs occurs in cirrhotic patients cannot be discounted, although this seems unlikely for chlorpromazine as in vitro studies have provided no evidence for metabolism of this drug by heart, muscle, brain, lung, kidney or spleen (Gillette & Kamm, 1960).

Electron microscopic appearances of the hepatocyte in certain types of experimental cirrhosis show proliferation of the endoplasmic reticulum (Stenger, 1970) but these appearances have not been

described in human cirrhosis (Steiner et al, 1965). Our data do not support the suggestion that maintenance of relatively normal drug metabolism in cirrhotic patients is due to concomitant therapy (Levi et al, 1968), but it may represent a form of adaptive or compensatory response, possibly due to endogenous induction of the non-specific microsomal enzymes by natural substrates such as steroids or terpenes. However, patients with chronic liver disease are capable of responding to treatment with enzyme inducing drugs with enhanced in vivo drug metabolism (Branch, Herbert et al, 1973).

(5) ALTERED SENSITIVITY TO SEDATIVE DRUGS IN PATIENTS WITH CIRRHOSIS

Increased sensitivity to the CNS effects of morphine has been conclusively demonstrated in patients with cirrhosis with a history of impending or overt hepatic coma (Laidlaw et al, 1961). However, there are conflicting reports as to the effects of barbiturates in this setting. Shideman et al (1949) showed that the duration of action of a standard dose of thiopentone was significantly prolonged in a group of 6 patients with abnormal liver function (only one of whom had unequivocal cirrhosis) compared to controls. In a clinical study Dundee (1952) reported that 21 patients with "severe liver damage" of unspecified type required substantially less thiopentone to produce prolonged anaesthesia than did normal subjects. However Sessions et al (1954) found that patients with chronic liver disease were not more affected by pentobarbitone than were healthy controls when responses were assessed using a crude clinical scoring system. With a similar clinical scoring system which assessed the presence of lateral nystagmus, impaired co-ordination and altered level of consciousness at intervals after injection of the drug, Mawer et al (1972) demonstrated that although the response of cirrhotic patients with slow amylobarbitone metabolism was increased after I.V. administration of this barbiturate, it was not significantly greater than that seen in a group of cirrhotic patients with normal amylobarbitone metabolism. However, sedative effects were not assessed in a control group of patients, and none of the studies using barbiturates stated whether the cirrhotic patients examined included any with a history of encephalopathy.

In the present study using chlorpromazine more sensitive and objective criteria were available to assess the sedative effects of the test drug. Despite the apparently normal clearance of chlorpromazine from the plasma, increased sensitivity of patients with cirrhosis to the sedative effects of this drug was apparent, particularly in those with a previous history of encephalopathy. Chlorpromazine caused a comparable fall in the EEG skew MDF in both control subjects and patients with cirrhosis, but in the latter the initial EEG activity was already depressed, particularly in the subgroup of patients with a history of porto-systemic encephalopathy (Fig. 7).

In these patients chlorpromazine resulted in a fall in the skew MDF to around 4.0 Hz, which is comparable with values found in patients with overt hepatic encephalopathy (Hawkes et al, 1970). In the present study these EEG changes were accompanied by severe drowsiness and deterioration in ability to perform graphic tests. Thus the susceptibility shown by some patients with cirrhosis to chlorpromazine (and presumably to other sedatives which have also been reported to cause EEG changes both in cirrhosis (Laidlaw et al, 1961) and in normal subjects (Malpas et al, 1970) appears to be related to an underlying abnormality of cerebral function, resulting in an increased sensitivity to the drug, rather than impaired drug metabolism. However enhanced clinical effects due to greater concentrations of free (unbound) drug cannot be excluded. All central nervous system depressant drugs may therefore be potentially dangerous to patients with cirrhosis, even where there is only minor evidence of encephalopathy either at the time or in the past. Thus there may be no rational basis for advocating sedatives that are eliminated by renal, rather than hepatic, mechanisms. Indeed there is probably no ideal sedative for patients with cirrhosis, but theoretically a drug which is minimally protein bound, with a short half-life, and given in small doses, would appear to be the safest compromise.

(6) CRITIQUE OF CLINICAL STUDIES OF DRUG METABOLISM IN CIRRHOSIS

Although patients with liver disease are said to have impaired detoxication of drugs (Sherlock, 1968), many of the earlier studies

Table 8

	DRUG	AUTHOR	ASSAY	PHARMACOKINETIC MEASUREMENTS
1)	AMYLOBARBITONE	Mawer et al (1972)	GC	Vd, $t\frac{1}{2}$, Cl and urinary exern metab.
2)	ANTIPYRINE	Branch et al (1974)	?	$t\frac{1}{2}$, Cl
3)		Forrest et al (1975)	GC	$t\frac{1}{2}$
4)	AZATHIOPRINE	Whelan and Sherlock (1972)	Bioassay	"Serum immuno-suppressive activity."
5)	DIAZEPAM	Avant et al (1974)	GC	$t\frac{1}{2}$, Vd, Cl
6)	DIPHENYLHYDANTOIN	Kutt et al (1964)	Spectro	Plasma levels with constant dosing; urinary metabolite (HPPH) output
7)	LIGNOCAINE	Thomson et al (1973)	GC	Vd, $t\frac{1}{2}$, Cl
8)		Forrest et al (1975)	GC	$t\frac{1}{2}$
9)	PHENYLBUTAZONE	Levi et al (1968)	Spectro	$t\frac{1}{2}$
10)	TOLBUTAMIDE	Ueda et al (1963)	Spectro	$t\frac{1}{2}$

SOME STUDIES SUGGESTING IMPAIRED DRUG METABOLISM IN PATIENTS WITH CIRRHOSIS

Table 8 (continued)

	NO.	PATIENT DATA AETIOL.	SEVERITY	BIOCHEM	ALTERED SENSITIVITY?
1)	10	Varied, 2 not cirrhotic	5 hypoalbuminaemia	+	No
2)	20	?	Stable	?	?
3)	15	Varied, 2 not cirrhotic	?	?	?
4)	20	10 ACH 6 PBC 4 miscellaneous	Variable	+	?
5)	9	Alcoholic	?	-	?
6)	15	Variable 1 not cirrhotic	?	+	?
7)	8	Alcoholic	Advanced	+	?
8)	16	Varied, 2 not cirrhotic	?	?	?
9)	10	?	?	?	?
10)	10	?	?	+	?

Table 8 (continued)

	CONCOMITANT THERAPY DETAILS	COMMENTS
1)	+	2 of 10 patients with chronic liver disease did not have cirrhosis. Crude clinical assessment of drug effect. ↓ drug metab. seen in patients with ↓ serum albumin where $t_{\frac{1}{2}}$ and cl reduced to half.
2)	?	Increase in $t_{\frac{1}{2}}$ most marked in patients with severe liver disease. Albumin + bilirubin correlated with $t_{\frac{1}{2}}$. Clearance not reduced if albumin normal.
3)		Abnormal in 9 with decompensated liver disease. $t_{\frac{1}{2}}$ correlated with albumin and PT ratio.
4)	?	Not known whether impaired immuno suppressive activity in chronic liver disease is due to impaired absorption, hepatic biotransformation or endorgan response.
5)	+	$t_{\frac{1}{2}}$ in cirrhotics more than double that in controls due primarily to decrease in cl.
6)	Yes	Uncontrolled study. 4 of 15 patients (1 with hepatitis) showed lowered clinical tolerence to standard doses DPH, with plasma drug accum + low urinary output of metab (HPPH).
7)	?	Significant fall in $t_{\frac{1}{2}}$ and Cl but lignocaine is flow limited drug - results could reflect changes in hepatic blood flow rather than impaired metabolism.
8)	?	
9)	+	Arbitrary distinction between significant pretreatment and no pretreatment. No distinction made between acute and chronic liver disease.
10)	?	Non-specific assay measures tolbutamide + major metabolite. $t_{\frac{1}{2}}$ normal in 5, reduced in 5 who had renal impairment which may have been responsible for increased $t_{\frac{1}{2}}$.

of drug metabolism in patients with cirrhosis (as in the present investigation of chlorpromazine) have failed to show any effect of chronic liver disease (Table 5). In contrast a number of studies have claimed to demonstrate such a distinction (Table 8). A critical analysis of these various papers suggests that a number of factors may have contributed to the current controversy over the influence of liver disease on drug elimination.

(a) Study Design

Certain reports can be criticised on the basis of faulty or inadequate experimental design. For example the paper by Kutt et al, (1964) is widely quoted as evidence that phenytoin metabolism is impaired in cirrhosis. However this conclusion was based on the anecdotal reports of four out of fifteen patients who showed lowered clinical tolerance to standard doses of the drug. Moreover one of these four patients had acute hepatitis and not cirrhosis, and the study was completely uncontrolled. Again no controls were included in Nelson's (1964) study of tolbutamide metabolism in cirrhosis. Instead his results were compared with control values taken from the literature.

(b) Patient Selection

The varying aetiology of cirrhosis, and the difficulty in defining its stage and severity has made evaluation and comparison of different studies difficult if not impossible. Some studies have included patients with non-cirrhotic chronic liver disease (such as hepatic fibrosis or chronic cholestasis), while in the paper by Levi et al, (1968) no distinction was made between patients with acute (hepatitis) and chronic (cirrhosis) liver disease - a crucial point when inferences are made concerning the possible effects of concomitant drug therapy. In some reports clinical and laboratory details regarding the severity of liver disease in patients studied have been completely omitted. Where such information has been provided it is apparent that some investigators have studied relatively well patients with a compensated cirrhosis, while others have investigated ill, poorly nourished subjects with decompensated cirrhosis accompanied by ascites, hypoalbuminaemia, and frank jaundice.

That the severity of the underlying liver disease and its accompanying biochemical derangements is important in determining the effect on drug metabolism in patients with liver disease is suggested by several studies (Lieberman, 1963; Mawer et al, 1972; Hvidberg et al, 1974; Branch et al, 1974).

(c) Analytical Methodology

Earlier studies relied on non-specific and insensitive spectrophotometric or biological techniques to assay drugs in plasma and urine. The former method is not entirely satisfactory because of the possibility of non-specific absorption by interfering substances and by pharmacologically inactive forms of the drug with a consequent inability to separate parent drug from its metabolites. For example the spectro-photometric assay used by Ueda et al, (1963) measured not only tolbutamide but its major metabolite carboxytolbutamide. The latter compound, which is normally rapidly eliminated by the kidneys, accumulates in renal impairment and may have spuriously resulted in a prolonged $t_{\frac{1}{2}}$ for tolbutamide in several patients with cirrhosis accompanied by renal impairment in Ueda's study (Nelson,1964). Some biological assays for drugs are also readily affected by factors present in jaundiced serum (Kunin et al, 1959). Improved assay methods have eliminated some of these problems, and it might be of interest to repeat some of the earlier studies using more sensitive and specific techniques.

(d) Choice of test drug

In interpreting these studies the distinction between capacity and flow limited drugs has rarely been made. It should be remembered that certain drugs such as lignocaine and propranolol (Thomson et al,1973; Branch et al, 1974) have a high hepatic extraction ratio and their elimination is limited by hepatic blood flow rather than the metabolic capacity of the liver. Thus the impaired elimination of such drugs seen in patients with cirrhosis is more likely to be due to reduction in effective hepatic blood flow (for example by porto-systemic shunting) than to impaired drug metabolising activity.

(e) Drug Interactions

Most of the earlier studies did not document the drug therapy patients were receiving at the time of study, nor was the possible significance of hepatic microsomal enzyme induction appreciated in these patients. Thus some investigators may have failed to demonstrate impaired drug metabolism as a result of the masking effect of enzyme induction (Levi et al, 1968) which has been shown experimentally to enhance drug metabolism in patients with chronic liver disease (Branch et al, 1973).

(f) Pharmacokinetic considerations

A general criticism of many of the earlier studies is that they utilised only the crudest pharmocokinetics parameters, such as drug elimination half-life. (In some studies not even this simple parameter was calculated. For example the investigation by Sessions et al (1954) on phenobarbitone elimination in cirrhosis analysed blood levels of the drug for only 60 minutes after its infusion, a time interval too short to allow calculation of the elimination half-life of the drug).

As discussed previously (Chapter II) plasma drug clearance (Cl) provides a better index of processes eliminating a drug from the body than does the half-life ($t\frac{1}{2}$). Moreover it is now appreciated that factors other than changes in hepatic metabolism may alter the $t\frac{1}{2}$, and that hepatic cirrhosis may produce a number of disposition changes, often acting in opposite directions, whose net effect may be an unaltered $t\frac{1}{2}$. These changes in disposition and hepatic metabolism are difficult to identify by simple pharmacokinetic measurements.

(g) Future studies

Future investigation of the effect of liver disease on drug metabolism should take account of the experimental and methodological criticisms of previous studies discussed above. Such studies should aim to clearly define and quantify the precise changes in drug disposition and response occurring in patients with liver disease of well defined type, aetiology and severity. Ideally both flow and capacity limited drugs should be used as investigative tools.

In addition to the drug elimination half-life, the apparent volume of distribution, plasma clearance changes, changes in protein binding and urinary excretion profile of both parent drug and metabolite(s) should be examined, and objective criteria used to assess patient response. Ultimately such studies should lead to the development of useful predictive tests which would permit easier and more rational evaluation of correct dosing regimes in patients with liver disease.

CHAPTER IV

EFFECT OF HEPATIC ENZYME INDUCING DRUGS ON PLASMA BILIRUBIN AND BILE FLOW

(1) EFFECT OF PHENOBARBITONE ON PLASMA BILIRUBIN IN JAUNDICED PATIENTS.

(a) Clinical Studies

An excellent example of the application of advances in the basic sciences to medical practice has been provided by the use of phenobarbitone and other enzyme inducing agents in the treatment of various types of hyperbilirubinaemia. The stimulus for this therapeutic advance came from a better understanding of bilirubin metabolism (Billing & Black, 1971), and from the recognition that the non-specific hepatic microsomal enzyme system responsible for the biotransformation of drugs and other xenobiotics was also concerned with the metabolism of endogenous substances such as bilirubin and steroids, and could be stimulated by a large number of different compounds (Conney, 1967; Chapter II).

Bilirubin is the nonpolar tetrapyrrole pigment derived from the breakdown of haem. It is produced mainly by the catabolism of effete circulating red cells, but a small contribution (early labelled bilirubin) is derived from non-haemoglobin haem from the liver, bone marrow and spleen. Bilirubin is transported in the plasma bound to albumin, and is normally excreted by the liver into bile after undergoing a series of processes which are not fully understood. These include:

(i) Dissociation of unconjugated bilirubin from plasma albumin, and entry into the liver cell (either by active transport of passive diffusion), followed by binding to at least 2 cytoplasmic anion acceptor proteins (Levi, Gatmaitan & Arias, 1969).

(ii) Transfer to the endoplasmic reticulum (microsomes) where glucuronide conjugation occurs, catalysed by the enzyme bilirubin UDP - glucuronyl transferase (UDP-GT).

(iii) Finally active transport of the conjugated and now water soluble bilirubin diglucuronide takes place across the canalicular membrane into the bile ducts.

Brown & Zuelzer (1958) first demonstrated that the microsomal enzyme responsible for conjugation of bilirubin could be stimulated by drugs in studies in guinea pigs. Later phenobarbitone was shown to be capable of increasing the hepatic bilirubin conjugating capacity of mice and rabbits (Catz & Yaffe, 1962; 1968). The activity of glucuronidating enzymes is low in foetuses and newborns of several species including man (Pelkonen et al, 1973). Thus immaturity of this enzyme system may provide a partial explanation for the transient or "physiological" jaundice observed in newborn and premature infants, and impaired glucuronidation may contribute to the hyperbilirubinaemia associated with other causes of jaundice such as sepsis and haemolytic anaemia. Furthermore, patients with certain forms of congenital non-haemolytic unconjugated hyperbilirubinaemia have been shown to have a deficiency of this enzyme (Arias et al, 1969; Black & Billing, 1969). Consequently this novel pharmacological approach for enhancing bilirubin UDP-GT activity has been widely tested in the treatment of various types of jaundice.

Initial clinical reports described the use of phenobarbitone in the treatment of infants with non-haemolytic unconjugated hyperbilirubinaemia (Crigler & Gold, 1966, 1967; Yaffe et al, 1966) and in neonatal hyperbilirubinaemia (Trolle, 1968A; 1968B). Subsequent studies have also demonstrated the success of this form of therapy in reducing bilirubin levels (and requirement for exchange transfusion) in haemolytic jaundice in the newborn, and in obstructive jaundice due to intra hepatic cholestasis. Some of the more recent reports are summarised in Tables 9 to 11. Interestingly, epileptic patients treated with phenobarbitone and other anticonvulsant drugs have also been shown to have significantly lower plasma bilirubin levels than control subjects (Thompson, Eddleston et al, 1969), and phenobarbitone has also recently been shown to reduce bilirubin levels in patients with the conjugated hyperbilirubinaemia of the Dubin-Johnson syndrome (Shani et al, 1974).

(b) Effect of drugs on bilirubin Kinetics

The plasma concentration of unconjugated bilirubin is determined by a balance between two processes: the rate at which newly synthesised bilirubin enters the plasma pool (bilirubin turnover) and the rate of irreversible bilirubin removal by the liver (hepatic bilirubin clearance).

Table 9

SOME STUDIES ON THE EFFICACY OF PHENOBARBITONE IN CONGENITAL NON-HAEMOLYTIC HYPERBILIRUBINAEMIA

DIAGNOSIS	REFERENCE	SUBJECTS	PHENOBARBITONE DAILY DOSE	COMMENTS
Crigler-Najjar Syndrome (unconjugated hyperbilirubinaemia)	Arias et al (1969)	Type 1 - 4 patients Type 2 - 9 patients treated 2 - 32 weeks	90 mg	Type 1 - no response Type 2 - substantial reduction in bilirubin levels
	Hunter et al (1973)	Type 2 - 3 patients	Variable	All responded to PB with reduction in bilirubin.
Gilbert's Syndrome (unconjugated hyperbilirubinaemia)	Black & Sherlock (1970)	13 patients treated for 2 weeks	180 mg	Significant reduction in plasma bilirubin in all cases associated with increase in bili UDP-GT activity in liver biopsies
	Hunter et al (1971)	11 patients Double blind 4 week comparison with PB and non-hypnotic barbiturate	60 mg	Significant reduction in plasma bilirubin with both drugs but non-hypnotic barbiturate preferred.

DIAGNOSIS	REFERENCE	SUBJECTS	PHENOBARBITONE DAILY DOSE	COMMENTS
Gilbert's Syndrome (unconjugated hyperbilirubinaemia)	Maxwell et al (1973)	12 patients	60-100 mg	Bilirubin levels fell in all subjects and correlated with D-glucaric acid excretion
Dublin-Johnson Syndrome (conjugated hyperbilirubinaemia)	Shani et al (1974)	13 patients	300 mg	PB caused significant decreased in total & conjugated bilirubin levels and elevation of Tm for BSP.

Table 9 (continued)

Table 10

SOME STUDIES ON THE EFFICACY OF PHENOBARBITONE IN HYPERBILIRUBINAEMIA OF NEONATAL JAUNDICE

DIAGNOSIS	REFERENCE	SUBJECTS	PHENOBARBITONE DAILY DOSE	COMMENTS
"Physiological" Jaundice	Ramboer et al 1969	24 mothers treated from 32nd week of pregnancy	60 mg	High significant reduction in total bilirubin in infants by third day.
	Ramboer et al 1969	33 infants treated immediately after birth	10 mg	No significant effect of therapy either in normal or low birth weight infants.
	Vest et al (1970)	56 new born infants	3-15 mg	Significant reduction in bilirubin (from day 4) and exchange transfusion requirement.
	Sinniah et al (1971)	41 jaundiced new-born Malay/Chinese/ Indian		a) significant reduction in bilirubin and exchange transfusion requirement
		a) normal weight	15 mg	b) no effect.
		b) low birth weight	6 mg/kg	

DIAGNOSIS	REFERENCE	SUBJECTS	PHENOBARBITONE DAILY DOSE	COMMENTS
"Physiological" Jaundice	Valdes et al (1971)	23 predominantly Negro infants treated for 3 days ± phototherapy	5 mg/kg	PB treated group had significant reduction in bilirubin on days 3 - 5, but drug less effective than continuous photo-therapy.
	Yeung et al (1971)	a) 44 mothers treated nightly for 2 weeks prior to delivery.	30 mg	a) All mothers Grp. O. Significant reduction in neonatal hyperbilirubin-aemia.
		b) 44 infants treated for 3½ days after birth.	15 mg	b) Treatment of infants more effective.
	Halpin et al (1972)	96 mothers treated from 32nd week gestation	20 mg	Significant reduction in total bilirubin 24-96 hours after birth.
Premature Infants	Carswell et al (1972)	21 pairs of preterm infants gestational age 36 weeks	8 mg/kg	Significant reduction in peak serum bilirubin levels achieved after 21 pairs studied.
Rhesus Haemolytic Disease	McMullin et al (1970)	30 Coombs +ve infants treated until sustained fall in bilirubin	6 mg/kg	Significant reduction in need for exchange transfusion.

Table 10 (continued)

DIAGNOSIS	REFERENCE	SUBJECTS	PHENOBARBITONE DAILY DOSE	COMMENTS
G-6-PD deficiency	Meloni et al (1973)	33 infants with documented RBC G-6-PD deficiency	7 mg/kg	Reduction in hyper-bilirubinaemia and need for exchange transfusion.

Table 10 (continued)

Table 11

SOME STUDIES ON THE EFFICACY OF PHENOBARBITONE IN HYPERBILIRUBINAEMIA OF OBSTRUCTIVE JAUNDICE

DIAGNOSIS	REFERENCE	SUBJECTS	PHENOBARBITONE DAILY DOSE	COMMENTS
Intra hepatic Cholestasis	Thompson & Williams (1967)	4 adults with chronic intra-hepatic cholestasis treated for 50 days	180 mg	Varying aetiology. Plasma bilirubin lowered by up to 50%
	Stiehl et al (1972)	2 children treated for 4 days for intra-hepatic biliary atresia	10 mg/kg	Bilirubin reduced to 20-50% pretreatment values Diminished serum bile salts and increased faecal Rose Bengal excretion
	Sharp & Mirkin (1972)	3 children treated chronically. Variable aetiology.	3-5 mg/kg	Reduction in bilirubin in only 1 child (intra hepatic biliary atresia) associated with increased volume duodenal juice.
Extra hepatic Cholestasis	Stiehl et al (1972)	1 child treated for 14 days	10 mg/kg	No effect.

Early studies analysing plasma bilirubin curves following a tracer dose of C^{14} radiolabelled bilirubin were interpreted as suggesting a defective uptake of bilirubin in patients with Gilbert's syndrome (Billing & Black, 1971). In this condition (even in subjects in whom serum bilirubin levels were normal) plasma disappearance curves for radiobilirubin showed a much slower decline than in normal controls. Approximately 25-40% of the administered dose was retained after 4 hours, compared to less than 10% in the normal subject. Moreover, when patients with Gilbert's syndrome were treated with phenobarbitone or glutethimide, the plasma disappearance curve for bilirubin returned to normal.

More sophisticated studies of bilirubin kinetics have been carried out by Berk et al, (1975). Plasma radiobilirubin disappearance curves were fitted by computer analysis, and hepatic bilirubin clearance and bilirubin turnover derived from calculations which were independent of any hypothetical compartmental model of bilirubin metabolism. Glutethimide (500mg/day) and phenobarbitone were shown to increase hepatic bilirubin clearance in both patients with Gilbert's syndrome and in normal controls (Blaschke et al, 1974). In addition, a reduced bilirubin turnover was shown to contribute to the reduction in plasma bilirubin concentration, but administration of these drugs had no effect on haem catabolism or bilirubin production. Although these elegant pharmacokinetic techniques demonstrated increased bilirubin clearance as a result of drug therapy, they could not indicate which of the various theoretically possible mechanisms (see below) was responsible for this effect.

A more recent independent study of radiobilirubin kinetics in Gilbert's syndrome has confirmed that phenobarbitone accelerates bilirubin clearance, without affecting bilirubin production (Schmid, 1976). However in this study, drug administration did not appear to alter bilirubin turnover. Again this pharmacokinetic model provided no information on the physiological basis for drug enhanced increase in hepatic bilirubin clearance.

(2) REDUCTION OF PLASMA BILIRUBIN BY PHENOBARBITONE: SOME POSSIBLE MECHANISMS

The previous section described some of the studies which have now clearly established that administration of phenobarbitone results in reduction in the concentration of (unconjugated) bilirubin both in normal subjects and in patients with unconjugated hyperbilirubinaemia due to several types of hepatic dysfunction.

This effect has been shown to be due to enhanced hepatic bilirubin clearance, while total bilirubin production is unaffected by the drug. (Berk et al, 1975; Schmid, 1976).

Since phenobarbitone is a powerful enzyme inducing agent (Conney, 1967) and has been shown to stimulate the activity of bilirubin UDP-GT in animal studies (Catz & Yaffe, 1968; Blaschke & Berk, 1972) the implication in these clinical and experimental studies has been that bilirubin conjugation is enhanced as a result of induction of UDP-GT. Indeed phenobarbitone has been reported to increase the activity of bilirubin UDP-GT in Gilbert's syndrome (Black & Sherlock, 1970) and in patients who had received the drug prior to laparotomy (Billing & Black, 1971).

However, an increasing number of additional alterations in hepatic physiology and cellular composition attributable to phenobarbitone could provide alternative explanations. Phenobarbitone and other enzyme inducing drugs enhance functional liver mass by stimulating liver cell growth and protein content (Platt & Cockrill, 1969; Pilcher et al, 1972), and increase the concentration of specific cytoplasmic acceptor proteins which bind bilirubin (Reyes et al, 1969). They also produce an increase in canalicular bile flow (Klaasen, 1969) which is independent of bile salt secretion (Berthelot et al, 1970) as well as an increase in hepatic blood flow (Ohnhaus et al, 1971). Furthermore, phenobarbitone and other drugs may have an indirect effect on plasma bilirubin by stimulating the secretion of D-glucaric acid, an end product of glucaric acid metabolism which is a potent inhibitor of β glucuronidase (Chapter II). This could theoretically diminish biliary and/or intestinal deconjugation of bilirubin glucuronides and thus reduce the entero-hepatic circulation of bilirubin.

Experimental studies have shown that when large quantities of bilirubin are administered by intravenous infusion to animals with normal liver function, saturation of the transport system for excretion of conjugated bilirubin into bile, rather than bilirubin conjugation, is the rate limiting step in the overall rate of transport of the pigment from blood to bile (Arias et al, 1961). Since phenobarbitone lowers the bilirubin levels of children with intra-hepatic biliary atresia (Sharp & Mirkin, 1972; Stiehl et al, 1972) and in other forms of intra-hepatic cholestasis (Thompson & Williams, 1970) it has been suggested that drug induced choleresis,

rather than an increase in activity of hepatic enzymes, may be the major factor in lowering bilirubin levels in man (Klaasen, 1969; Boyer, 1971). Moreover, phenobarbitone therapy has recently been shown to reduce bilirubin levels in the Dubin-Johnson syndrome, a rare inherited form of conjugated hyperbilirubinaemia which is thought to result from a congenital defect in hepatic excretory function without other associated hepato-cellular abnormality (Shani et al, 1974). This observation provides further support for the suggestion that phenobarbitone, in addition to inducing hepatic enzymes involved in the metabolism of drugs and endogenous substrates, may enhance biliary excretion of various compounds.

(3) EFFECT OF PHENOBARBITONE ON BILE FLOW AND BILIRUBIN METABOLISM: EXPERIMENTAL STUDIES IN MAN AND THE RAT.

In an effort to determine the relative importance of stimulation of bilirubin conjugation and of choleresis in the reduction of plasma bilirubin levels observed after phenobarbitone, the effects of this drug on bile flow, bilirubin metabolism and enzyme activity in the Wistar rat were studied. Experiments were also carried out in the homozygous Gunn rat which completely lacks bilirubin UDP-GT activity. This experimental animal can therefore be used as a model to determine changes in the biliary excretion of bilirubin independent of any effects on conjugation. Parallel studies were also carried out in different groups of subjects (Maxwell, Hunter et al, 1973). But since in these clinical studies direct assay of hepatic enzymes and quantitative measurement of bile flow was not possible, these were assessed indirectly. The urinary excretion of D-glucaric acid was used to provide a quantitative index of hepatic microsomal enzyme activity (Chapter II) and in a group of patients with non-haemolytic unconjugated hyperbilirubinaemia (Gilbert's Syndrome) the maximum excretory rate of bromosulphthalein (TmBSP), which is known to be affected by changes in bile flow (O'Maille et al, 1966; Klaasen & Plaa,1968; Gronwall & Cornelius, 1970) was also measured.

(a) Methods

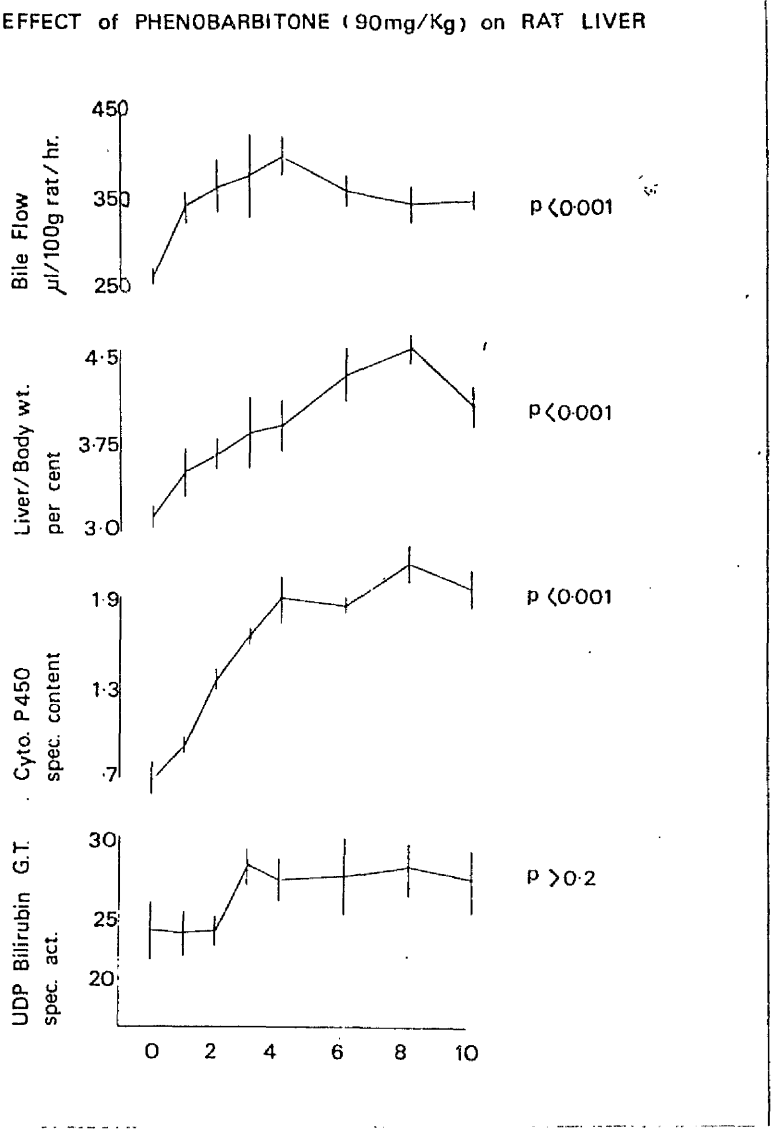
(i) Wistar Rats: Phenobarbitone 90mg/kg body weight/day was administered intraperitoneally for up to 10 days to 60 male Wistar rats (350-450g) on

a standard Medical Research Council laboratory diet with water ad libitum. Twelve control rats received saline. Under light ether anaesthesia, the common bile duct was cannulated with polyethylene tubing (Portex PE 10) 1cm proximal to the duodenum. Animals were placed in restraining cages, and after recovery from anaesthesia bile was collected in the dark on ice for 3h. Room temperature was maintained at 22°C and rectal temperature of the rats at 36.5 + 0.5°C. At the end of each experiment the rats were stunned and exsanguinated, and the livers rapidly removed, placed on ice, blotted and weighed. Microsomes were prepared from a 25% (W/V) homogenate of 2.5 - 3.0g portions of liver taken from the centres of hepatic lobes, in 0.25M sucrose, centrifuged at 10,000g for 20min, and the pellets rehomogenised with a volume of sucrose 4 X (V/W) the weight of the portion of liver and recentrifuged. The pooled supernatants (now 12.5% W/V) were centrifuged at 105,000g for 60min and the microsomal pellets suspended in 4.5ml buffered sucrose solution (0.25M sucrose 0.1M tris-HCl buffer (ph 7.4) 1:2 V/V) and stored in aliquots at - 18°C. The content of cytochrome P450 was measured by the method of Omura & Sato (1964) and the activity of glucuronyl transferase using bilirubin as substrate by the method of Van Roy & Heirwegh (1968). Results were related to microsomal protein concentration measured by the method of Lowry et al (1951). Plasma and biliary bilirubin concentration were measured by a modification of Michaelsson's method (Thompson 1969A).

(ii) Gunn Rats: Phenobarbitone was administered intraperitoneally (90mg/kg body weight/day) to four homozygous Gunn rats for 10 days, while paired controls received saline. The bile ducts were cannulated, body temperature maintained and the bile collected for 15min from the rats while under light ether anaesthesia. A bolus injection of conjugated bilirubin (1ml bile containing 60-80ug conjugated bilirubin freshly collected from a Wistar rat) was given via the femoral vein to each of a pair of treated and control Gunn rats and four 15min collections of bile obtained.

(iii) Investigations in Man: Nine adult males and three females with mild unconjugated hyperbilirubinaemia of Gilbert's syndrome (plasma bilirubin 1-4mg/100ml) were studied before and after 1 month's treatment with 60-100mg phenobarbitone daily (1-1.5mg/kg body weight).

Fig. 8



Effect of phenobarbitone on bile flow (ul/100 g rat/h), ratio of liver weight to body weight (%), specific content of cytochrome P 450 (umol/mg protein), and specific activity of bilirubin glucuronyl transferase (O.D. units/mg protein) in the male Wistar rat.

Fifty-six subjects without liver disease were also investigated; 23 hospital personnel (14 males and 9 females) not receiving drugs, and 33 epileptic patients (14 males and 19 females) on anti-convulsant drug therapy. Blood samples were taken between 9.00 and 10.00am after a light breakfast, protected from light, and plasma bilirubin measurements performed as described within 4h. D-glucaric acid excretion was measured in 24 hour urine collections. Aliquots of urine were heated at 100°C at ph 2.0 to convert glucaric acid to its lactone, and this was then measured by its specific inhibitory effect on a standard β glucuronidase assay by the method of Marsh (1963) with minor modifications. The maximal excretory rate (Tm) and relative storage capacity (S) for BSP were determined by a standard technique (Wheeler et al, 1960; Preisig et al, 1966). The same infusion rates of dye were used for each subject before and after phenobarbitone treatment.

(b) Results

(i) Animal Studies:

In Wistar rats given phenobarbitone, bile flow reached a peak after 4-6 days with values about 50% above the control bile flow of 250ul/100g body weight/h (SEM 0.03) (Fig. 8). There was a progressive rise in the ratio of liver to body weight and an early and striking increase in the specific content of cytochrome P450 from a baseline of 0.70 umol/mg protein (SEM 0.05) to about 2.5 times control values. The specific activity of bilirubin glucuronyl transferase was increased only slightly (9%) and the plasma bilirubin levels in these animals (0.19mg/100ml SEM 0.03) did not differ significantly from values in the control animals (0.17mg/100ml SEM 0.02), nor did excretion of endogenous bilirubin differ between the two groups:- 0.69mg/kg body weight/3h (SEM 0.05) in controls and 0.68mg/kg body weight/ 3h (SEM 0.07) after phenobarbitone.

The effect of pretreatment with phenobarbitone in the Gunn rats on bile flow and on the excretion of an injected load of conjugated bilirubin is shown in Table 12 . In the first 15min after administration 30% more bilirubin was excreted than in controls, but the numbers studied were small, and this difference did not achieve statistical significance. Total recovery of bilirubin in 1h after administration of the exogenous bilirubin was slightly increased (Table 12).

Table 12

EFFECT OF PHENOBARBITONE ON BILE FLOW AND ON EXCRETION OF AN INFUSED LOAD OF CONJUGATED BILIRUBIN IN THE HOMOZYGOUS GUNN RAT

	CONTROL	TREATED GROUP	SIGNIFICANCE
Bile flow, ml/kg body weight / 15 min	0.74 ± 0.60	0.91 ± 0.05	n.s.
Bile flow, ml/kg liver / 15 min	1.88 ± 0.26	1.80 ± 0.09	n.s.
Biliary excretion of bilirubin, ug/kg body weight / 15 min			
0 - 15 min	70.1 ±19.6	91.0 ±16.5	n.s
16 - 30 min	70.7 ±10.1	56.1 ± 9.7	n.s.
31 - 45 min	14.7 ± 2.0	15.3 ± 3.3	n.s.
46 - 60 min	9.2 ± 1.8	11.5 ± 3.2	n.s.
Recovery of infused bilirubin in 1 h, %	85.6 ± 2.8	92.7 ± 3.9	

All values given as mean ± SEM

(ii) Clinical Studies:

In the patients with Gilbert's syndrome there was a mean reduction of 60% in the plasma bilirubin from a level of 1.92mg/100ml (SEM 0.28), to 0.74mg/100ml (SEM 0.11) following 4 weeks treatment with phenobarbitone ($P.<0.001$). This was accompanied by a large increase in urinary D-glucaric acid excretion from 9.9µmol/day (SEM 1.3) to 37.2µmol/day (SEM 7.4) ($P.<0.01$). BSP Tm was unchanged with mean values of 9.24mg/min (SEM 0.6) and 9.02mg/min (SEM 0.4) before and after phenobarbitone. There was no significant change in BSP S, the mean values being 43.6mg/mg% (SEM 6.4) and 51.3mg/mg% (SEM 8.5) before and after phenobarbitone respectively (Table 13), and Fig. 9).

There was a significant correlation between plasma bilirubin levels and glucaric acid excretion in patients with Gilbert's syndrome ($r= -0.517$; $P.<0.05$, Fig. 10) and a similar correlation was also observed in the 56 subjects without liver disease ($r = -0.56$ $P.<0.001$).

(c) Discussion

(i) Drug dosage in comparative studies:

Enzyme induction is a dose dependent phenomenon (Blaschke & Berk, 1972; Breckenridge et al, 1973) and enzyme inducing drugs may only increase the activity of hepatic microsomal enzymes when given in doses which exceed the immediate capacity of the liver to metabolise them (Hoffman et al, 1970; Remmer, 1970). As experimental animals generally metabolise drugs much more rapidly than man (Burns, 1968), it is not possible to calculate equivalent inducing doses in comparative studies. We therefore chose for the rat a dose of phenobarbitone which readily induces the activity of various enzymes in this animal (Conney, 1967; Pilcher et al, 1972). In man a standard therapeutic dose of the barbiturate (1.0 - 1.5mg/kg) was selected, as this has been shown to reduce plasma bilirubin levels in normal subjects and those with Gilbert's syndrome.

(ii) Effects on hepatic enzyme activity:

In the Wistar rats, phenobarbitone caused an early and striking rise

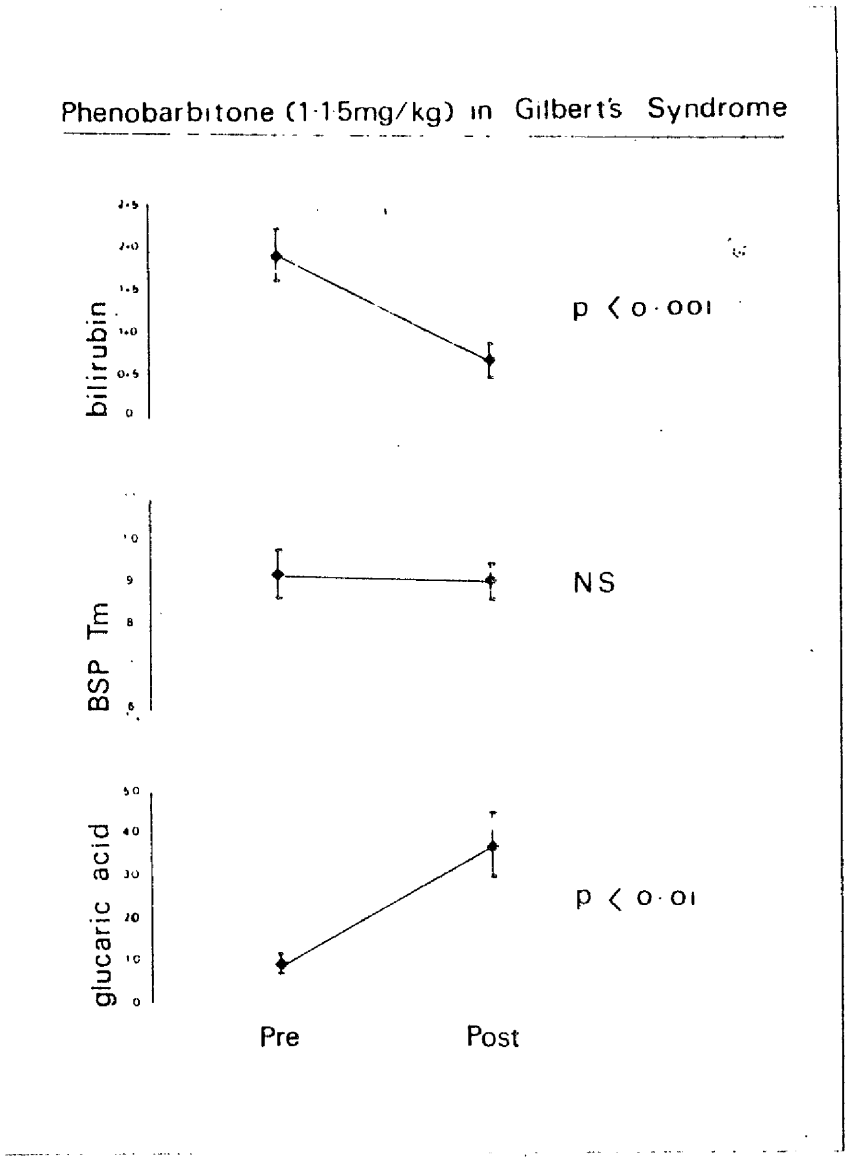
Table 13

EFFECT OF PHENOBARBITONE THERAPY IN PATIENTS WITH GILBERT'S SYNDROME

	CONTROL PERIOD	AFTER PHENOBARBITONE	SIGNIFICANCE
Plasma bilirubin, mg/100 ml	1.92 ± 0.28	0.74 ± 0.11	$p < 0.001$
D-Glucaric acid excretion, umol/24h	9.9 ± 1.3	37.2 ± 7.4	$p < 0.01$
BSP Tm, mg/min	9.2 ± 0.6	9.0 ± 0.4	n.s.
BSP S, mg/mg%	43.6 ± 6.4	51.3 ± 8.5	n.s.

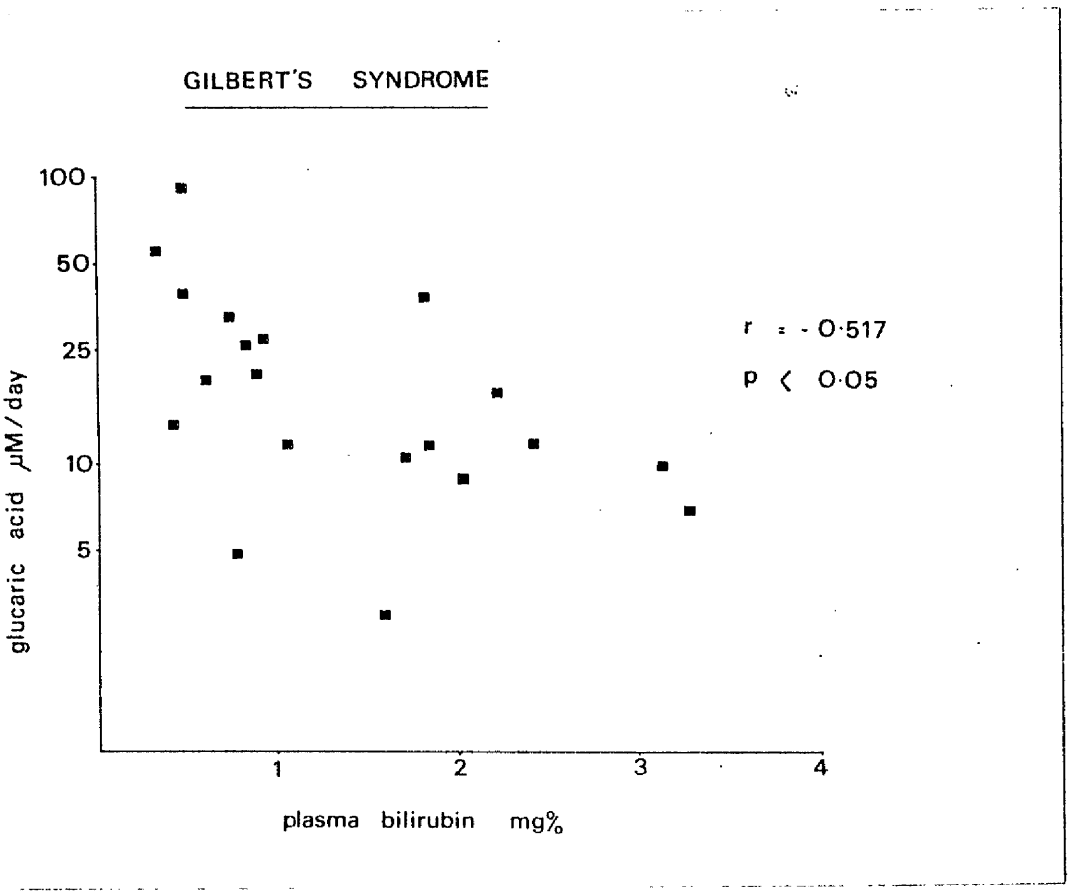
All values given as mean ± SEM

Fig. 9



Effect of Phenobarbitone Therapy In Patients With Gilbert's Syndrome

Fig. 10



Relationship between plasma bilirubin and urinary D-glucaric acid excretion in patients with Gilbert's syndrome.

in the specific content of cytochrome P450. However, there was no significant change in the specific activity of bilirubin UDP-GT, although the total hepatic activity of this enzyme increased as a result of liver enlargement. This finding is in agreement with some previous reports (Potrepka & Spratt, 1971; Pilcher et al, 1972), but at variance with others which have demonstrated an increase in the specific activity of bilirubin UDP-GT after drug therapy (Catz & Yaffe, 1968; Thompson, 1969B; Blaschke & Berk, 1972). These conflicting reports on the response of bilirubin UDP-GT to drug administration in laboratory animals may be due to species variation and differences in enzyme assay procedures employed (Thompson, 1969B; Winsnes, 1971).

In patients with Gilbert's syndrome, Black & Sherlock (1970) demonstrated small increases in the activity of bilirubin UDP-GT in needle biopsy specimens of liver after 2 weeks treatment with phenobarbitone 180mg daily, but found no correlation with the reduction in bilirubin levels. The significant rise in the urinary excretion of D-glucaric acid in our patients receiving the drug suggests that even with the low dose used, there was increased activity of hepatic enzymes. Furthermore, the significant correlation between glucaric acid excretion and plasma bilirubin levels suggests that the reduction in plasma bilirubin may be related to increased activity of hepatic microsomal enzymes. A similar correlation was found in subjects with normal liver function. In animal studies we have reported a correlation between total microsomal cytochrome P450 concentration and D-glucaric acid excretion, (Hunter, Maxwell et al., 1973), but the possibility cannot be excluded that these are parallel but unrelated phenomena.

(iii) Effects on bile flow:

There is no method for directly measuring bile flow rates in man under physiological conditions. Estimates of bile flow from aspiration of duodenal juice are likely to be highly inaccurate, and measurements obtained from T-tube studies are affected by the underlying pathology and by depletion of bile salts. More recently the biliary clearance of ^{14}C mannitol has been utilised as an indirect measure of canalicular bile secretion in man (Boyer et al, 1973).

The method used in the present study, measurement of BSP Tm, again provides only an indirect estimate of bile flow, but several animal studies have shown that it is increased when canalicular bile flow is increased, for example following infusion of bile salts (Goresky & Kluger, 1969; Gronwall & Cornelius, 1970) or after the non-bile salt dependent choleresis which follows phenobarbitone administration (Berthelot et al, 1970) although this relationship may not be obtained with all agents which stimulate canalicular bile flow (Erlinger & Dumont, 1972).

Despite a significant reduction in plasma bilirubin, the patients with Gilbert's syndrome showed no change in BSP Tm following phenobarbitone administration. This finding suggests that , with the doses used, the drug caused no change in bile flow. Our results differ from those of Cartei et al (1971) who reported a significant increase in BSP Tm in patients with Gilbert's syndrome, but not in normal subjects, after 100mg phenobarbitone/day for 1 month. The discrepancy is difficult to explain particularly since these workers found a significant fall in bilirubin in both their patients and normal subjects. There is no good evidence that phenobarbitone given in usual therapeutic doses affects bile flow in man. Using the biliary clearance of ^{14}C mannitol as an estimate of canalicular bile secretion Boyer et al,(1973) showed only small increments in canalicular bile flow in 2 of 4 subjects after administration of massive doses of phenobarbitone (8-10 x greater than used in the present study). Sharp & Mirkin (1972) treated a child with intrahepatic biliary atresia with large doses of phenobarbitone (5mg/kg) and showed an increase in the volume of duodenal aspirate. In another report treatment of two further children with intra-hepatic cholestasis, again using large doses of phenobarbitone (10mg/kg), resulted in a fall in serum bilirubin and bile salt concentrations, and an increase in ^{131}I Rose Bengal faecal excretion (Stiehl et al, 1972). It was speculated that these findings might have resulted from barbiturate stimulation of bile secretion, but other explanations are not excluded.

Our animal studies confirm previous reports that administration of phenobarbitone will produce a hypercholeresis in the rat (Klaasen,1969; Mok et al, 1974), rabbit (Berthelot et al, 1970) and monkey (Redinger & Small, 1973).

Liver hypertrophy after phenobarbitone is well recognised but it is of interest that in the present study changes in the ratio of liver to body weight did not parallel the increase in bile flow. This is further evidence that choleresis may not be related simply to liver cell mass, since compounds such as 3-methyl cholanthrene and 3,4-benzpyrene greatly increases liver weight, but have no effect on bile flow (Klaasen, 1969).

No detectable change in plasma bilirubin was observed in the rats after phenobarbitone, which was in contrast to the striking reduction found in patients with Gilbert's syndrome in this and other studies (Black & Sherlock, 1970; Hunter et al, 1971). The lack of any change in the total biliary excretion of bilirubin in the Wistar rat is further evidence that bilirubin metabolism in this animal was unaffected by phenobarbitone. In the homozygous Gunn rat, however, some increase in the excretion of a large exogenous load of conjugated bilirubin after phenobarbitone pretreatment was observed. Although the numbers studied were small, these results are in keeping with previous reports that phenobarbitone administration increases the maximal biliary excretion of bile in the rat (Roberts & Plaa, 1967). Studies in the dog (Goresky & Kluger, 1969) and sheep (Upson et al, 1970) demonstrated that such an increase in Tm for bilirubin may be due to an increase in bile flow.

(iv) Species variation in response to phenobarbitone:

The importance of species variation in the hepatic responses to inducing drugs is well known (Conney, 1967). In the present studies in the Wistar rat, the lack of any effect of phenobarbitone on plasma bilirubin or biliary excretion of bilirubin, despite marked increases in bile flow, liver size and total content of bilirubin conjugating enzyme, suggests that this animal has an efficient apparatus for the disposal of endogenous bilirubin. However under the non-physiological conditions prevailing when a large exogenous load of bilirubin is administered or infused at Tm, biliary excretion is enhanced by pretreatment with phenobarbitone.

In contrast, in man the reduction in bilirubin levels following administration of therapeutic doses of phenobarbitone appears to be associated with increased activity of hepatic microsomal enzymes, without any accompanying change in bile flow.

These studies emphasise the need for caution before extrapolating the results of experiments in animals to man. However, our understanding of the precise mechanism whereby phenobarbitone and other enzyme inducing drugs increase hepatic bilirubin clearance and lower plasma bilirubin levels in man still remains incomplete. Unless bilirubin conjugation is rate-limiting, it is not clear that these data can explain the effect. Phenobarbitone does not seem to lower plasma bilirubin levels by displacing unconjugated bilirubin from its binding sites on plasma protein (Khanna et al, 1969). However, the other documented effects of the drug may equally be responsible. These include an increase in functional hepatic mass and intrahepatic binding proteins either alone or together with stimulation of bilirubin conjugation, and possibly also enhancement of hepatic excretory function. Further information about the kinetics of the individual steps in the bilirubin transport process, together with an understanding of the rate limiting step in bilirubin excretion under normally prevailing conditions in man, is necessary before this phenomenon can be satisfactorily explained.

CHAPTER V

CLINICAL IMPLICATIONS OF ENHANCED HEPATIC BIOTRANSFORMATION

(1) DRUGS AND LIVER HYPERTROPHY: ADAPTIVE OR TOXIC RESPONSE?

It has long been recognised that many foreign compounds, including drugs and food additives, cause enlargement of the liver in experimental animals which is usually accompanied by induced synthesis of microsomal processing enzymes, but without histologically detectable damage. (CHAPTER II). Platt and Cockrill (1969) suggested that when a compound elicited increases in size, protein content and processing enzyme activity, but no frank pathological damage to the liver, the hepatic enlargement could be regarded as a physiologically adaptive response to exposure to the foreign agent. Earlier however, Barka & Popper (1967) in a discussion of liver enlargement due to drugs, speculated that the anabolic reaction, although beneficial in principle, might become detrimental, and proposed the concept of "anabolic liver cell injury." Subsequent investigation has failed to confirm their suggestion that drug mediated hypertrophy accompanied by stimulation of enzyme activity is toxic to the hepatocyte per se, but has justified their concern for the possible adverse consequences of hepatic enzyme induction. There is now an increasing awareness that stimulation of hepatic microsomal enzyme in man is a "double-edged sword", with potential for both good and ill. Both aspects of this adaptive response will be discussed in this chapter.

(2) STIMULATION OF HEPATIC BIOTRANSFORMATION IN MAN

(a) Drug mediated hepatic enzyme induction

Over 200 drugs and foreign chemicals have been shown to stimulate the activity of hepatic microsomal enzymes in laboratory rodents, and a great deal of information is available about the factors regulating this phenomenon in the experimental animal. In laboratory studies many factors including species, strain, sex, age, diet, ambient temperatures, hormonal status and circadian rhythmn have been demonstrated to play a role in defining the response of microsomal enzymes to potential inducers. (CHAPTER II).

However, surprisingly little is known of the extent, regulation or significance of drug induced changes in hepatic biotransformation in man, and the clinical implications of hepatic enzyme induction have yet to be fully evaluated. In his review on "Pharmacological implications of microsomal enzyme induction," Conney (1967) devoted only 5 of 47 pages to studies in man. However, comparison of animal and human data is difficult, and extrapolation of results from the laboratory to the clinical situation may be misleading.

Methods for the clinical assessment of drug mediated alterations in hepatic enzyme activity, and examples of their application have been discussed in CHAPTER II. Microsomal enzyme induction in man, as in the experimental animal has been shown to be dose-dependent (Breckenridge, Orme et al., 1973) under genetic control (Vesell, 1972), and affected by age and sex (O'Malley, Crooks et al., 1971). Those drugs which have been reported in clinical studies to stimulate hepatic biotransformation have recently been reviewed (Sher, 1971). However, this list is almost certainly incomplete.

(b) Non-drug stimulants of human microsomal enzymes

In man, as in the experimental animal, stimulation of microsomal enzymes may result not only from drug administration, but also in response to other environmental inducing agents such as chlorinated hydrocarbon insecticides (Kolmodin, Azarnoff et al., 1969; Poland, Smith et al., 1970; Smith et al., 1970; Hunter, Maxwell et al., 1972) and cigarette smoke (Welch, Harrison et al., 1969). It is not certain whether food additives, other anutrients, or even char broiled steaks (CHAPTER II) - all of which are capable of inducing microsomal enzyme activity in animals - may also have this effect in man. In addition endogenous substrates such as steroids may have an important regulatory role in the control of hepatic enzyme induction in animals (Marshall, 1971) and it has been speculated that they might contribute to the maintenance of relatively normal drug metabolism in patients with compensated chronic liver disease (CHAPTER III).

There is also some evidence that the massive increase in secretion of gestational steroids during human pregnancy is accompanied by enhanced activity of hepatic enzymes (see later section of this chapter).

(3) POSSIBLE THERAPEUTIC APPLICATIONS OF ENZYME INDUCTION

Recognition that hepatic microsomal enzyme activity in man could be manipulated has led to the investigation of the therapeutic possibilities of "enzyme induction" in a number of clinical situations.

(a) Jaundice

The use of enzyme inducing drugs in the treatment of various forms of hyperbilirubinaemia has been detailed in CHAPTER IV. The validity of this approach seems best established in the treatment (or prophylaxis) of neonatal hyperbilirubinaemia. Although controversy remains over the most appropriate therapeutic regimens, and the precise mode of action of these drugs, there is little doubt that they are effective in reducing bilirubin levels (and the need for exchange transfusion) in various forms of neonatal jaundice. However, paediatricians remain cautious about the use of these drugs until the ultimate effects of this form of treatment have been established. (Behrman & Fisher, 1970; Sisson, 1971). Long term controlled prospective studies of phenobarbitone treated infants are necessary before the assets and liabilities of therapeutic "enzyme induction" can be accurately gauged and compared with alternative forms of treatment such as conventional exchange transfusion or phototherapy. Until this has been done its widespread adoption cannot be recommended (Wilson, 1971).

The efficacy of enzyme inducing drugs in lowering bilirubin levels in patients with non-haemolytic unconjugated hyperbilirubinaemia is also well documented (CHAPTER IV); but again the indications for such treatment are unclear.

Gilbert's syndrome appears to be a harmless biochemical anomaly, and in most patients with this disorder mild jaundice is discovered incidentally on laboratory screening. Under these circumstances it is debatable whether a "therapeutic white-wash" with powerful and potentially harmful drugs is appropriate (Hunter, Thompson et al., 1971). On the other hand where jaundice is clinically apparent and socially embarrassing (as in the more pronounced hyperbilirubinaemia associated with the Crigler - Najjar Syndrome) the cosmetic improvement achieved with the use of chronic "enzyme induction" would seem to justify the risks involved. In this setting the use of enzyme inducing agents with a prolonged half-life and duration of effect, such as dicophane, may offer an advantage (Thompson, Stathers et al., 1969).

(b) Cushing's Syndrome

There is considerable information concerning the effects of enzyme inducing drugs on normal steroid metabolism (CHAPTER II). Kuntzman, 1969). Laboratory studies have provided evidence that the in vitro hydroxylation of various steroids including testosterone, oestradiol, oestrone, progesterone, cortisol corticosterone and cortisone is increased in phenobarbitone treated animals. Furthermore in vivo studies have shown that phenobarbitone pretreatment decreases the effects of various steroids including the anaesthetic action of progesterone and the uterotrophic action of oestradiol in immature female rats.

The stimulatory effect of various enzyme inducing drugs on the urinary excretion of 6 βhydroxy cortisol has already been discussed (CHAPTER II) and this has suggested a possible therapeutic application for "enzyme induction" in cases of steroid overproduction. Clinical studies have suggested that the increased metabolism of cortisol following administration of phenytoin or o, p-DDD may be useful in the treatment of Cushing's syndrome. Amelioration in the biochemical and clinical signs of this disease was observed following treatment of two subjects with 300 to 400 mg per day of Phenytoin (Werk et al., 1964).

o, p-DDD administration was also reported to reduce the symptoms of Cushing's syndrome before the drug produced any effect on adrenal cortisol secretion (Southren, Tochimoto et al., 1966). In epileptic subjects, with presumably normal adrenal function, the enhanced cortisol metabolism produced by inducing drugs (Phenytoin) was accompanied by increased cortisol secretion (Werk, Thrasher et al., 1971). However, Southren and coworkers have suggested that the increased amounts of hydroxylated cortisol metabolites which are formed following treatment with these compounds may ameliorate the symptoms of Cushing's syndrome by inhbiting the action of cortisol.

In summary, although hepatic enzyme induction has proved to be a theoretically attractive approach to the medical treatment of Cushing's syndrome, its practical application has been limited to a number of anecdotal reports, and its use appears to have been eclipsed by the introduction of selective inhibitors of adrenal secretion.

(c) Cholelithiasis

The recent major advances in the physiology of bile salt secretion have underlined the role of the liver in the pathogenesis of cholesterol gall stones, and have raised the intriguing possibility that hepatic enzyme induction may have a place in the treatment or prophylaxis of this disorder. The size of the bile acid pool is of fundamental importance in the production of gall stones, since a reduced bile acid pool is one of the major metabolic defects in patients with cholesterol gall stones, and bile from such patients is usually supersaturated with cholesterol. Re-expansion of the bile acid pool size improves cholesterol solubility in bile and promotes gall stone dissolution. As enzyme inducing drugs may stimulate cholesterol metabolism to bile acids, there has been considerable interest in the possibility of using these drugs in the treatment or prevention of human cholelithiasis.

There is evidence both for and against the induction by phenobarbitone of the microsomal enzyme, cholesterol 7 hydroxylase, which is the rate limiting step in the conversion of cholesterol to bile salts (Kuntzman, 1969; Redinger & Small, 1973). However both in the monkey (Redinger & Small, 1973) and in the rat (Mok, Perry et al. 1974) phenobarbitone significantly enhances bile acid synthesis and pool size. As there is no evidence that phenobarbitone affects intestinal bile acid absorption (Ostrower et al, 1973) it seems likely that the expanded bile acid pool and increased bile acid synthetic rates are due to a direct effect of phenobarbitone on hepatic bile acid synthesis. Whether or not phenobarbitone enhances the specific activity of cholesterol 7 hydroxylase, the increase in liver size after phenobarbitone would alone adequately explain the net increase in synthesis and expansion of the bile acid pool in these animal studies.

There is disagreement as to whether an increase in bile acid pool size will in fact reduce the saturation of bile with cholesterol in man. Furthermore, Mok et al. (1974) were unable to show in two patients with gall stones that therapeutic doses of phenobarbitone had any effect on bile lipid composition. However, in a controlled study of 36 patients with gall stones both chenodeoxycholic acid and phenobarbitone (180 mg/day) significantly decreased biliary cholesterol saturation. Unfortunately, the change in lithogenic index induced by phenobarbitone alone was still above the line of cholesterol saturation and none of the patients in this group showed reduction in gall stone size after one year. Combined therapy did not significantly augment the effect of chenodeoxycholic acid alone. (Coyne et al, 1975).

(d) Enhancement of drug efficacy

Where a drug's effects are due not to its parent compound, but to a metabolite (CHAPTER II) enhanced metabolism may result in increased efficacy or toxicity. The latter effect will be discussed in a subsequent section of this chapter, together with other potential adverse effects of enzyme induction.

Azathioprine requires to be metabolised by microsomal mixed function oxidases before it is active as an immunosuppressive agent (Whelan & Sherlock, 1972) and there is also evidence that biotransformation activates cyclophosphamide (Sher, 1971). Experimental studies have demonstrated increased efficacy of chlorambucil after phenobarbitone pretreatment, which suggests that metabolic activation may be responsible for the anti-tumour activity of this compound (Hill, 1972). However, whether hepatic enzyme induction as a means of enhancing the activity of these or other similar drugs will have any useful clinical application remains to be established.

(e) Elimination of toxic drugs and chemicals

Chronic administration of enzyme inducing agents accelerates the elimination not only of the compound itself, but of toxic drugs and chemicals subsequently administered. King Mithridates' pioneering application of the principles of drug tolerance in an attempt to thwart would-be poisoners was mentioned in CHAPTER II. A more recent advocate of "Mithridatism" has suggested that occasional doses of organochlorine insecticides such as dicophane (DDT) which are very powerful and persistent inducers of microsomal enzymes (Hart, Shultice et al, 1963; Thompson, Stathers et al., 1969) might be given to patients who make repeated self-poisoning attempts (Prescott, 1969). Unfortunately, enzyme induction does not seem to occur rapidly enough to be use in the treatment of an established drug overdose, and could be dangerous as a form of prophylaxis as it may enhance the effect of certain toxins whose activity is due to a metabolite (see subsequent section of this chapter on adverse effects of enzyme induction). The removal of pesticide residues in man is accelerated by barbiturates and phenytoin (Davies, Edmundson et al, 1969), an observation which has been used in veterinary medicine to accelerate the removal of dieldrin residues from contaminated dairy cattle (Braund et al, 1971). Prolonged treatment of rats with phenobarbitone has been shown to accelerate the tissue depletion of radiolabelled vitamin D (Silver et al, 1974), and this approach has been suggested for the clinical management of patients with vitamin D intoxication.

Since the first experiments 20 years ago demonstrated that induction of experimental animals with 3-methyl cholanthrene could inhibit the carcinogenicity of amino azo dyes (Conney, Miller & Miller, 1958) a number of studies have confirmed that it is possible to protect against the carcinogenic effects of a variety of chemical carcinogens by inducing increased activity of the microsomal mixed function oxidases (Wattenberg, 1972). The relevance of these studies to human carcinogenesis has yet to be established but it is possible, for example, that resistance to pulmonary neoplasms may depend on the body's ability to increase detoxication of inhaled or ingested carcinogens present in cigarette smoke, such as benzpyrene. It is conceivable that a deliberate long-term increase of this detoxicating ability might protect against environmental carcinogens whose metabolic products are less toxic than the parent compound (Welch, Harrison et al, 1969). Interestingly, recent Danish data on cancer morbidity in epileptic patients treated with (enzyme inducing) anticonvulsant drugs for over 10 years showed a lower than expected incidence of carcinoma for a number of organs (Clemmesen et al, 1974). Thus despite the potential of an induced microsomal mixed function oxidase system to produce some adverse effects (see subsequent section of this chapter) from a biological stand point the system can be regarded as having essential survival value for the organism confronted with foreign organic compounds. This could be of considerable importance in our increasingly contaminated environment.

(f) Treatment of inherited enzyme deficiency states

The use of enzyme inducing drugs in the treatment of the jaundice of Gilbert's Syndrome and the Crigler-Najjar syndrome (type 2) has already been discussed (CHAPTER IV). In these disorders the hyperbilirubinaemia is considered to be at least partly related to reduced hepatic bilirubin UDP glucuronyl transferase activity, and reduction in bilirubin levels following drug therapy to be due to stimulation of the activity of this microsomal enzyme. Stimulation of the activity of hepatic enzymes by a large variety of drugs appears to be limited to the microsomal mixed function oxidases and conjugases.

However, certain enzymes located in other sites in the cell such as ALA-synthetase (mitochondria) or mouse kidney β glucuronidase (lysosomes) may be synthesised on the endoplasmic reticulum and then transferred to their site of action. In view of this, attempts have been made to stimulate the activity of certain non-microsomal enzymes with drugs, but without therapeutic benefit. Phenobarbitone had no effect on the activity of hexosamidase, a lysosomal enzyme deficient in the Sandhoff variant of Tay-Sachs disease (Applegarth & Dunn, 1972). Similarly no clinical benefit or reduction in red cell galactose was observed after progesterone was administered to children with congenital galactosaemia (Pesch, et al., 1960), or after attempted enzyme induction using phenobarbitone in children with alpha - 1 - anti trypsin deficiency (Porter et al, 1972). In a child with glycogen storage disease, glucose - 6 - phosphatase was induced, but again without improvement of the more distal metabolic lesion (Moses, et al., 1966). In the hereditary hepatic porphyrias the primary genetic defect is considered to be a partial enzymatic block in haem synthesis. However, far from correcting this enzyme deficiency, phenobarbitone and other enzyme inducing drugs are known to be capable of precipitating life threatening acute attacks of the disease. Studies on the mechanism of the sensitivity to such drugs in this pharmacogenetic disorder are presented in CHAPTER VI.

(4) ADVERSE CONSEQUENCES OF HEPATIC ENZYME INDUCTION

(a) Altered drug pharmacokinetics

Except in the rare instances where a drug metabolite is more active than the parent compound or another drug interaction predominates (CHAPTER II), administration of enzyme inducing drugs accelerates the rate of drug inactivation and excretion and results in reduced pharmacological and toxic effects. Surprisingly little is known of the extent or significance of this interaction in man, but an up to date review of clinical reports describing the effects of one drug on the metabolism of a second drug or on its own metabolism has been provided by Sher (1971).

The most clinically significant of these interactions occur between enzyme inducing drugs and anticoagulants. The hydroxylation of coumarin anticoagulants is apparently stimulated in man if barbiturates or other compounds are given at the same time (Macdonald, Robinson et al., 1969).

The hypoprothrombinaemic effect of the anticoagulant is reduced over a period of one to two weeks and the dose may have to be doubled to maintain the desired effect on the prothrombin time. Consequently the patient is at risk from bleeding when inducing drugs are withdrawn, and fatal haemorrhage has been reported after the withdrawal of chloral hydrate and phenobarbitone in patients on oral anticoagulants (Cuccinell, Odessky et al, 1966; Robinson & Macdonald, 1966).

Phenylbutazone, a potent enzyme inducing drug, paradoxically potentiates the effect of Warfarin. This effect has been ascribed to displacement of the anti-coagulant drug (Aggeler et al., 1967) a suggestion supported by the finding that in vitro phenylbutazone interferes with the binding of many drugs to albumin and markedly increases the concentration of free drug. Warfarin is a mixture of R and S isomers, and recent studies suggest that interaction between this anticoagulant and other drugs might be more subtle and complex than previously suspected. Indeed phenylbutazone has been demonstrated to have a differential effect on the metabolism of the two isomers. Depression of the rate of clearance of the S isomer masked the stimulation of the clearance of the R isomer - while the overall rate of clearance of the racemic Warfarin mixture was unaffected (Lewis, Trager et al., 1974). Since S Warfarin is 5 times more potent an anticoagulant than R Warfarin, inhibition of the metabolism of S Warfarin provides one mechanism for augmented anticoagulation which follows phenylbutazone.

Administration of inducing drugs may also have deleterious effects on steroid therapy. Phenobarbitone has been shown to decrease the half-life and increase the clearance rate of dexamethasone in asthmatic patients.

Prednisone dependent asthmatic patients showed clinical and spirometric deterioration after phenobarbitone, and these changes were reversed after withdrawal of the barbiturate. Since dexamethasone and prednisone seem to be metabolised more rapidly when barbiturates are also administered, care is necessary when prescribing enzyme inducing drugs to asthmatic patients treated with steroids (Brooks, Werk et al., 1972). More recently attention has been drawn to the possibility that enzyme inducing drugs may also impair the effectiveness of oral contraceptive steroids. Rifampicin, a potent enzyme inducing drug in animals and man (Jezequel et al., 1971; Remmer, Schoene et al., 1973) has been reported to result in an increased occurrence of pregnancy in tuberculous patients taking this drug together with oral contraceptives (Nocke-Finck et al., 1973). The reduced effectiveness of oral contraceptives in these patients may be explained by the finding that the metabolism of oestradiol in liver microsomes from patients receiving rifampicin was increased more than fourfold. (Bolt et al., 1974; Mumford, 1974). Rifampicin has also been shown to lower plasma methadone levels and precipitate withdrawal symptoms in narcotic addicts (Kreek, et al., 1976). However, it is not known whether these effects are due to enhanced hepatic microsomal drug-metabolising activity.

(b) Enhanced drug toxicity

The microsomal mixed function oxidases are usually thought of as detoxicating enzymes, but some otherwise inert substances are activated by these processing enzymes to active or toxic molecules (CHAPTER II). The administration of enzyme inducing agents such as phenobarbitone or DDT greatly increases the hepatotoxic and lethal effects of carbon tetrachloride in animals, while the feeding of a low protein diet (which diminishes drug metabolising activity) almost abolishes this effect. The toxicity of chloroform, retrorsine (Judah et al., 1970) bromobenzene (Mitchell et al., 1973) and cyclophosphamide (Sher, 1971) are similarly enhanced by prior enzyme induction. Interestingly, diethylnitrosamine which exerts its hepatotoxic effect through a metabolite, is more toxic to pregnant than to non-pregnant rabbits (Texler et al., 1970), although there is disagreement about the effect of inducing drugs on the toxicity of this chemical (Magour & Nievel, 1971).

Of more immediate clinical significance has been the recognition that the fulminant hepatic and renal tubular necrosis which can follow overdose with paracetamol is also mediated by a toxic metabolic (Mitchell et al., 1973, 1973A). Subjects whose hepatic microsomal enzymes are induced are thus theoretically at great risk from the hepatoxic effects of this analgesic drug, although this factor could not been implicated in a recent report of 49 patients with paracetamol induced hepatic necrosis (Clark et al., 1973).

The hepatotoxicity of these relatively stable substances appear to be due to the formation by hepatic microsomal enzymes of chemically reactive metabolites which bind covalently to vital hepatic macromolecules (Brodie et al., 1971; Jollow et al., 1973). These findings have stimulated speculation that similar mechanisms might explain the occasional hepatotoxicity of halogenated aromatic hydrocarbon anaesthetic agents (Dollery 1972).

An unusual example of enhanced drug toxicity after administration of enzyme inducing drugs was reported by Shahidi (1968) who studied two sisters who readily developed methaemoglobinaemia after exposure to phenacetin, and who were shown to excrete excessive 2-hydroxy derivatives of phenacetin, but relatively little of the normal de-ethylated metabolites. Pretreatment with phenobarbitone worsened the condition and the findings were interpreted as suggesting that in the relative absence of the usual de-ethylation pathway, alternative metabolites responsible for methaemoglobin formation were excreted in increased amounts.

Another concern is that hepatic enzyme induction may enhance the toxicity of environmental carcinogens. From animal studies it appears that aflatoxin B1 and possibly other aflatoxins are themselves directly carcinogenic. They are converted to non-toxic metabolites by hepatic microsomal drug metabolising enzymes, and enzyme induction with phenobarbitone has been shown to diminish their carcinogenicity. Similarly, the carcinogenicity of benzpyrene is greater than that of its metabolites (Welch et al., 1969), and the carcinogenicity of a number of polycyclic hydrocarbons has been shown in animal studies to be diminished by induction of aryl hydrocarbon hydroxylase activity (Wattenberg, 1972).

In contrast, the toxicity and carcinogenicity of diethylnitrosamine seems to depend on a metabolite and not the parent compound. Its toxicity is not altered however by phenobarbitone or DDT, but is enhanced by benzpyrene which induces aryl hydrocarbon hydroxylase activity and a different spectrum (cytochrome P-448) of microsomal enzyme activity (McLean & Magee, 1970). In laboratory studies enzyme inducing drugs such as phenobarbitone (Walker et al., 1973; Thorpe & Walker, 1973) and DDT (Kemeny & Tarjan, 1966) have been reported to produce tumours in rodents but the clinical relevance is not clear. Reassuringly, a recent retrospective study from Denmark of patients receiving long term therapy with enzyme inducing drugs (anticonvulsants) showed no evidence of an oncogenic effect (Clemmesen et al, 1974). However long term prospective studies from various different regions are required, as different environmental carcinogens may exist elsewhere whose toxicity may be enhanced by inducing drugs. (Maxwell, 1976A).

(c) Metabolic disturbances related to vitamin deficiency

There is increasing concern over the possible adverse effects of chronic drug therapy. In few diseases are drugs administered in such quantities and over such prolonged periods as in epilepsy, and the untoward metabolic effects of anticonvulsant therapy are beginning to attract attention. Administration of enzyme inducing drugs such as the anticonvulsants has been associated with clinical deficiencies of vitamin D, folate and vitamin K. It now appears highly likely that anticonvulsant osteomalacia is a consequence of hepatic enzyme induction, but the evidence that this is the mechanism for folate deficiency, or the coagulation disturbances reported after the administration of these drugs is still circumstantial and incomplete.

i. Vitamin D - ANTICONVULSANT OSTEOMALACIA

This condition has only recently been described, but is of considerable interest as it is the first metabolic disorder recognised to result from chronic therapy with enzyme inducing drugs, and because of its relevance to our understanding of vitamin D metabolism.

The earliest indication of the existence of this disorder was provided when biochemical abnormalities were noted in patients taking part in a clinical study of Trinuride, a potent combination anticonvulsant. Wright (1965) reported that 10 of 50 patients in a colony for adult epileptics treated with this drug over a period of one year developed raised serum alkaline phosphatase levels, but this abnormal finding was also present in 7 of 44 control patients on phenytoin and phenobarbitone. Moreover, alkaline phosphatase activity remained elevated for a prolonged period after withdrawal of the drug. No explanation could be provided for this finding, although it was not considered to be due to disturbance of liver function, as BSP was normal, and there was no other abnormality in standard liver function tests. As a result of this interesting observation a survey of calcium metabolism was iniated in the epileptic patients resident at the centre. Before this was completed, however, the first reports that rickets might be associated with epilepsy in children appeared from Germany, and suggested that the bone disease might be related to long term drug treatment (Schmid, 1967; Kruse, 1968). The survey of adult epileptic patients at the Chalfont St Giles colony later provided support for this hypothesis, as an elevated alkaline phosphatase was found in 29% of patients, and separation of the iso-enzymes revealed a raised bone fraction in many cases. Serum calcium levels below 9.0 mg/10 ml. were found in 22.5%, and frank bone disease was present in a few patients (Richens & Rowe, 1970). Using a simple scoring scheme, a quantitative assessment of anticonvulsant therapy was made for each patient. Analysis of drug treatment in this way demonstrated a strong correlation between the presence of biochemical osteomalacia and the dose of anticonvulsant drugs administered to the patients. It was postulated that anticonvulsant osteomalacia might be due to induction of liver enzymes responsible for enhancing hydroxylation of vitamin D to inactive metabolites, and thereby greatly increasing the requirement for this vitamin (Dent, et al, 1970).

Lingfield Calcium Study

The opportunity to test this hypothesis was provided by the availability of an assay for urinary D-glucaric acid which was being evaluated as an index of hepatic microsomal activity in man (Hunter, Maxwell et al, 1971; Chapter II). Accordingly a study of the urinary excretion of D-glucaric acid in children with epilepsy at a residential school (Lingfield, Surrey) was planned. We were particularly interested in investigating whether a relationship existed between hepatic microsomal enzyme activity (as measured by the test) and the level of serum calcium and other biochemical criteria of osteomalacia in these children on anticonvulsant drug therapy.

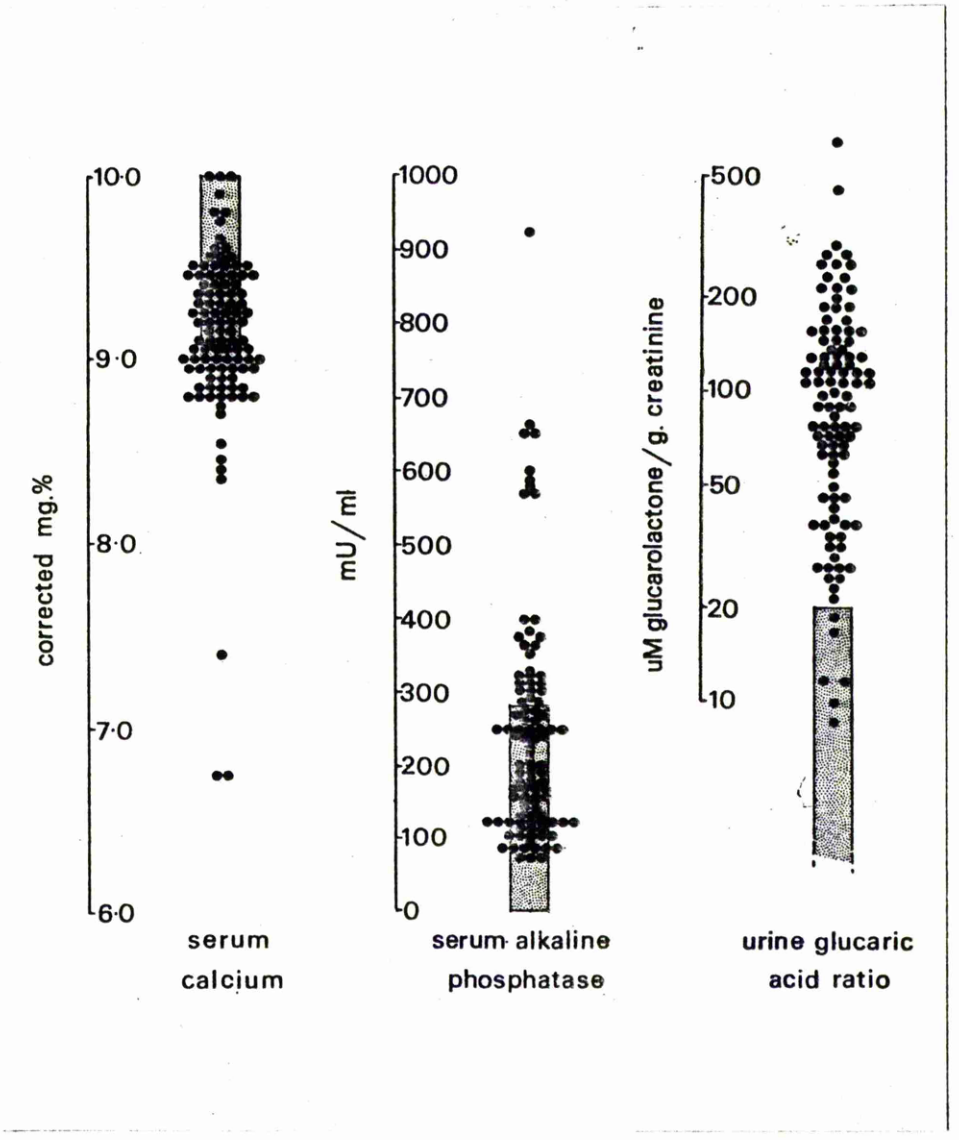
Subjects and Methods:

105 children with epilepsy were investigated, aged between 10 and 16 years. They included 60 boys and 45 girls. The children studied were all resident at the school, and thus the population was controlled for diet and sunshine exposure. A further 15 children aged 10 - 15 years who were resident in a nearby home for the mentally retarded, and were on no drug therapy, provided urine samples as controls for the epileptic patients.

Serum calcium, phosphate, and alkaline phosphatase values were determined by means of the SMA 12/60 Auto Analyzer in non-fasting blood samples taken between 9 and 11 a.m. If the serum specific gravity differed from 1027 the value for the serum calcium was corrected accordingly (Dent, 1962). The isoenzymes of alkaline phosphatase were separated by electrophoresis on polyacrylamide gel (Canapa-Anson & Rowe, 1970), the relative proportions of bone and liver alkaline phosphatase being determined visually.

The concentration of D-glucaric acid in a sample of urine obtained at the same time was determined by the inhibitory effect of glucarolactone to which D-glucaric acid is converted by heating at pH2, on β-glucuronidase as described by Marsh (1963).

Fig. 11



Values for serum calcium, alkaline phosphatase and urinary glucaric acid excretion (expressed as umol of glucarolactone/g of creatinine) in 105 children with epilepsy shown in relation to the normal range (hatched area).

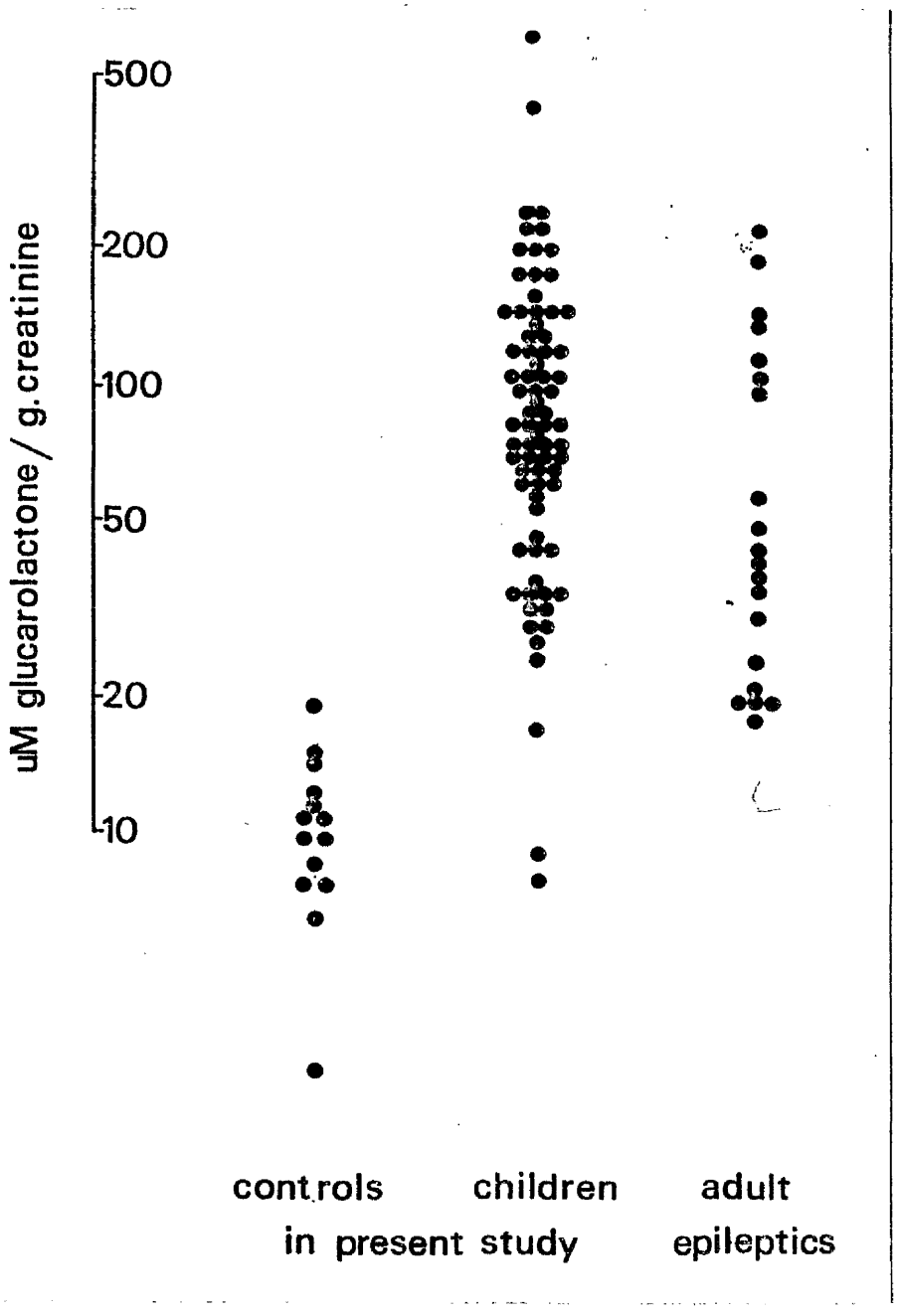
The result was related to the concentration of creatinine determined in the same urine sample - a ratio which was previously found both in epileptic and in normal subjects to correlate closely with the total daily D-glucaric acid excretion when measured in a complete 24-hour urine collection (r=0.97). The results were compared with values found in the control series of urine samples obtained from 15 children who were resident in a home for the mentally retarded, and not on any drug therapy.

Results:

Dietary assessment. Many of the children investigated were of subnormal intelleigence, and it was not possible to obtain individual dietary histories, but according to their supervisors none had noticeable food fads, and calculation of the vitamin D content of their diet over two separate weeks indicated an intake of 140-290 IU daily.

Biochemical screening. This showed that the serum phosphate value was within the normal range in every instance, the mean value of the group being 3.8 mg/100 ml (range 2.5 - 4.8). Serum alkaline phosphatase levels were raised in 25 children to above 295 mU/ml, the upper limit of normal for this age group (Bauer and De Vino, 1969). The highest level was 910 mU/ml, and nine children had levels above 500 mU/ml (Fig. 11). In every patient with values above 295 mU/ml electrophoresis showed that the percentage of bone isoenzymes was very high, comprising 85 - 100% of the total amount of alkaline phosphatase present. In only nine patients was the liver isoenzyme the dominant one, and in all these the total serum level fell within the normal range. In 31 children (14 boys and 17 girls) the serum calcium was below 9 mg/100 ml, which is regarded as the lower limit of the normal range for the SMA 12/60 Auto Analyzer (G. Walker, personal communication, 1971). Six children had a serum calcium of less than 8.5 mg/100 ml, and in four of these the calcium phosphate product (Ca X P) was below 30 mg/100 ml the lower limit of normal (Fraser & MacIntyre, 1970). In three of these children, as well as in the two with a normal Ca X P product, the serum alkaline phosphatase was markedly raised (298-640 mU/ml). These six children were considered to have biochemical osteomalacia.

Fig. 12



Glucaric acid excretion (expressed as umol of glucarolactone/g of creatinine) in 15 control and 75 epileptic children, and in 20 epileptic adults.

All had been on anticonvulsant therapy for at least eight years and though most of the pupils in the school are of British descent, this group included two West Indians and one Italian. Two of the others had been resident at the school for less than 12 months.

Drug therapy. A detailed record of the present anticonvulsant regime and of the duration of therapy was taken in each case. The length of drug treatment varied widely from 2 to 15 years, as did the current dosage of drugs. In many of the children control of fits had finally been achieved only by the use of several drugs in combination. With the simple scoring scheme of Richens and Rowe (1970) an index of the total daily dose of drugs was calculated for each child, but no correlation could be detected between this and the serum calcium level ($r = -0.14$, $P > 0.1$).

Relationship to Urinary D-glucaric Acid. Increased concentrations indicating a raised total urinary excretion of D-glucaric acid were found in 99 of the 105 children. Four of the six children with values within the normal range had stopped anticonvulsant drugs several months previously because they had been free of fits for some years, and two were currently on small doses only. The increase in D-glucaric acid excretion were often considerable, the highest being 631 uM/g creatinine as compared with a mean in control subjects of 12 UM/g (Figure 12). The six children with biochemical evidence of osteomalacia all had a raised urinary D-glucaric acid, though the range of levels was wide (66-279u/M/g. Statistical analysis showed a significant correlation between the concentration of D-glucaric acid and the serum calcium level ($r = -0.275$, $P < 0.01$). This correlation was slightly higher when those on anticonvulsant drugs for more than eight years were considered separately ($n=69$, $r = -0.312$, $P < 0.01$) and an analysis of those on treatment for a shorter period than this failed to show a significant correlation ($n=33$, $r = -0.087$, $P > 0.1$). No correlation could be detected between urinary D-glucaric acid and serum alkaline phosphatase levels ($r = -0.040$, $P > 0.1$).

Discussion:

Recent major advances in the understanding of vitamin D metabolism have been reviewed by Stamp (1973) and Kodicek (1974). It is now established that ingested vitamin D undergoes hepatic biotransformation to form an active metabolite, 25 hydroxycholecalciferol (25HCC) which is the major circulating form of the vitamin (De Luca, 1969; Ponchon et al 1969A; 1969B). Further hydroxylations produce more polar metabolites of vitamin D. One such metabolite, 1,25 dihydroxycholecalciferol (1,25 DHCC) which is synthesised only by the kidney, was later found to have an immediate and powerful effect in promoting intestinal calcium absorption (Kodicek, et al, 1970), and is now regarded as the principal active hormonal form of the vitamin. Other polar metabolites of cholecalciferol have been isolated, but their physiological and clinical significance have yet to be evaluated.

Regulation of the synthesis of these active vitamin D metabolites appears to be subject to feed-back control mechanisms. The synthesis of 25HCC has been shown to be product inhibited and the accumulation of 1,25DHCC in intestine and other organs is regulated in part by the plasma calcium concentration (Boyle, et al, 1972), so that a low calcium diet and associated hypocalcaemia in vitamin D deficient rats results in rapid accumulation of 1,25DHCC. It is now established that control of the synthesis of this major active metabolite of vitamin D is effected by the parathyroid gland (Stamp, 1973).

The frequency of biochemical abnormalities in our Lingfield study of epileptic children - namely 30% with hypocalcaemia and 24% with a raised alkaline phosphatase, is very similar to that reported by Richens & Rowe (1970) in their earlier survey of 160 adult epileptics, and in a later biochemical and radiological study of adult epileptic patients from Finland, 30% of whom had low serum calcium levels and 32% an elevated alkaline phosphatase (Sotanieni, et al, 1972). Additional studies showing reduced bone mineral content in epileptics without biochemical signs of bone disease suggest the occurrence of latent osteomalacia in a high proportion of patients taking anticonvulsants (Christiansen, et al, 1972). Interestingly, there have been few reports of frank anticonvulsant

osteomalacia from the United States, and biochemical abnormalities have been found less frequently (Hahn, Hendin et al, 1972). Indeed some American clinicians have questioned the existence of this condition (Livingston, Behrman et al., 1973). However, the rarity of anticonvulsant osteomalacia in North America is almost certainly explained by the universal practice of fortifying milk and other foodstuffs with vitamin D. This provides a large daily intake, occasionally as much as 2000 - 3000 I.U. (American Academy of Paediatrics, 1963).

Using Richen and Rowe's simple scoring system for drug intake we were unable to confirm their observation of a correlation between serum calcium and the total daily dose of anticonvulsants that the children were receiving. A possible explanation for this discrepancy was that many of the children were taking combinations of drugs including some less frequently used, such as carbamazepine and sulthiame, whose inducing properties are unknown. However, the high excretion of urinary D-glucaric acid found in virtually all the treated epileptic children in our survey was indicative of pronounced induction of hepatic microsomal enzymes. Furthermore the significant correlation found between urinary D-glucaric acid and serum calcium is strong supporting evidence, although indirect, for the suggestion that enzyme induction was the primary cause of the hypocalcaemia associated with long term anticonvulsant therapy. The report of a case of anticonvulsant osteomalacia which was cured simply by discontinuation of the drugs also supports the view that merely by altering vitamin D metabolism in the liver, significant deficiency of its active metabolites can occur and result in the osteomalacia or rickets. (Aponte & Petrelli, 1973).

After hydroxylation vitamin D is excreted largely as an inactive glucuronide. There is no evidence that activity of the 25-hydroxylase can be specifically induced. However, enhanced activity of the non-specific microsomal hydroxylase system might result in a change in the normal pattern of hepatic biotransformation of ingested or endogenous vitamin D, with diminished production of active metabolite. Alternatively, accelerated production of more polar, less active, derivatives would result in shortening of the half life of the active metabolite and thus also in lower effective levels of anti-rachitic cholecalciferol derivatives.

The increased catabolism of cholecalciferol appears to be a major factor in the production of the lower levels of 25HCC observed in subjects on chronic anticonvulsant therapy. As discussed earlier, the biological activity of 25HCC in turn depends on its conversion to certain dihydroxy derivatives, especially the renal synthesis of 1,25DHCC, now regarded as the principal biologically active hormonal form of the vitamin. These further hydroxylations may also be disturbed, but the effects of anti-convulsant therapy on the formation of such additional metabolites have not been defined. Furthermore the effect of drugs on other aspects of the kinetics of vitamin D such as its absorption, protein-binding, and elimination, have yet to be evaluated. Although genetically determined differences in enzyme inducibility have been recognised from twin studies (Chapter II) it is not known whether these play a significant role in determining the severity of anticonvulsant osteomalacia. Hahn (1973) has suggested that the most important factors influencing serum levels of 25HCC in patients on anticonvulsant therapy (and hence the clinical severity of the disorder since serum calcium levels and presumably target organ concentration of active vitamin D metabolites vary directly with the serum 25HCC concentration) appear to be:

(a) total drug intake,
(b) vitamin D intake,
(c) sunlight exposure,
(d) race.

In the institutionalised children who took part in our study exposure to sunlight and dietary vitamin D (estimated from the content of the school diet to be 140 - 290 IU/day) were comparable, and it is of interest that 3 of the 6 epileptic children with frank biochemical evidence of osteo-malacia were dark skinned immigrants. Dent & Smith (1969) have suggested that 75Iu is the minimum daily adult requirement of vitamin D in the United Kingdom, although Lumb et al (1971) estimated that many people in the north west of England have a dietary intake of only 30 to 60 IU. Thus in Britain, with relatively little exposure to sunlight to compensate for those with marginal dietary intake of vitamin D, it is not surprising that deficiency arises when demand for the vitamin increases after hepatic enzyme induction.

Confirmation of this theory has been assisted by the demonstration of abnormally low levels of 25HCC in plasma from patients on anticonvulsant therapy, and by the demonstration of alterations in vitamin D metabolism induced by phenobarbitone.

In an American study of 48 adults epileptic outpatients receiving chronic combined therapy with phenobarbitone and phenytoin significantly decreased 25HCC levels were found in 33% of the epileptic patients, while similar but less marked changes were seen in a group of patients given chronic therapy with either phenobarbitone or phenytoin alone (Hahn, Hendin et al, 1972). A positive correlation was found between serum calcium and serum 25HCC levels in both patients and control groups. Although the serum 25HCC level also correlated with the dietary intake of vitamin D, at any given intake the serum level was lower in the epileptic patients than in controls. In a parallel British study the levels of 25HCC in control and epileptic patients were lower than in their American counterparts, even although the estimations were performed in the same laboratory. This discrepancy is likely to be due to differences in vitamin supplementation (Stamp, Round et al, 1972).

Study of the metabolic fate of tritiated vitamin D_3 in human subjects taking enzyme inducing drugs (a child with anticonvulsant rickets, and phenobarbitone - treated normal volunteers) demonstrated a marked reduction in the half-life of plasma vitamin D, with increased formation of more polar, apparently biologically inactive, vitamin D metabolites. This increased catabolism appears to occur in the liver since the <u>in vitro</u> incubation of tritiated vitamin D with liver microsomes from phenobarbitone treated rats resulted in rapid conversion of tritiated D_3 to metabolites chromatographically similar to those seen in the plasma of phenobarbitone treated humans after tritiated vitamin D_3 administration (Hahn, Birge et al, 1972). These results are supported by the studies of Silver et al, (1974) who showed a significant increase in the rate of synthesis of the more polar (peak iv) metabolites of cholecalciferol in rats given enzyme inducing drugs, and by the clinical observations of Schaefer, et al (1972) on the metabolism of labelled cholecalfierol in epileptic patients on anticonvulsant therapy, and in normal subjects.

As one of the practical implications of these studies it would seem prudent to consider prophylactic vitamin D supplementation in appropriate individuals on chronic anticonvulsant therapy. It has been estimated that the "average" adult epileptic on usual clinical doses of phenobarbitone and phenytoin may require over 600 IU vitamin D per day to maintain normal 25HCC levels, an intake almost ten times the theoretical basal requirement (Hahn, 1973). Patients on multiple drug regimes with dark skins, or with limited exposure to sunlight may require greater amounts, although supplements should always be carefully titrated to individual needs (Hahn et al, 1975). A controlled trial of vitamin D supplementation has been shown to cause a small but significant increase in the bone mineral mass (measured by photon absorptiometry) in epileptic patients on anticonvulsants (Christiansen, et al, 1973), but there is still no agreement as to which patients should be selected for prophylactic therapy, or to the appropriate dose or form of vitamin D (Rodbro & Christiansen, 1975; Silver, 1975).

In additon to the anticonvulsants a wide range of therapeutic agents are capable of inducing hepatic microsomal enzyme activity (Chapter II), and thus drug induced acceleration of vitamin D biotransformation may theoretically occur in a variety of clinical situations. For example in the occasional patient with post-operative hypoparathyroidism who is resistant to vitamin D, concomitant therapy with sedatives should be considered as a possible contributory factor. A further practical application of these studies is the possibility that anticonvulsant or barbiturate drugs might provide effective and relatively non-toxic therapy for vitamin D intoxication.

In a subsequent section of this chapter some data is presented suggesting that hepatic enzyme induction may occur during normal human pregnancy, and in further studies, we have shown a significant reduction in 25HCC levels in coloured women at this time (Turton et al, 1977).

ii Folate - DRUGS AND FOLATE DEFICIENCY: AN EFFECT OF HEPATIC ENZYME INDUCTION?

Anaemia occurring after treatment with anticonvulsant drugs was first reported in 1952 (Mannheimer, et al), and it is now recognised that the cause is folate deficiency resulting in a high incidence of macrocytosis (11 - 53%) and megaloblastic changes in the marrow (Reynolds 1968; Reynolds et al, 1969). Although the role of Phenytoin has been most extensively investigated, folate deficiency may follow treatment with any of the commonly used anticonvulsants, and this complication has also been recognised after therapy with a number of other drugs. Such changes occurring with methotrexate and trimethoprim, which are folate analogues and act as competitive inhibitors of enzymes required for utilisation of folate are well understood (Bertino, et al, 1964; Kahn, et al, 1968). However, the mechanism of folate deficiency following chronic alcohol ingestion, (Eichner & Hillman, 1973) or prolonged therapy with anticonvulsant drugs, or oral contraceptives (Shojania, et al, 1971; Franklin & Rosenberg, 1971) where any structural relationship with folate is slight or absent, is still unclear.

Analysis of the possible mechanisms by which drugs such as the anticonvulsants might influence folate stores requires not only a knowledge of the chemistry and pharmacology of the drug, but also of the availability, absorption, distribution and functions of the vitamin. Understanding of the physiology of even the simplest folate compound, unreduced pteroyl monoglutamic acid (PGA), is still incomplete, but much has been learned since this compound was synthesised chemically and conjugated folate isolated from yeast, cystallised, and its structure identified. A comprehensive review of the digestion, absorption and biochemistry of folate has been provided by Rosenberg & Godwin (1971).

Approximately 75 to 90% of folate in a mixed diet is in the form of polyglutamate, with generally six or more glutamic acid residues attached to the pteroyl moiety. Pteroylheptaglutamic acid is the principal molecular form in yeast (and possibly other plant and animal tissues) while pteroyl triglutamic acid appears to be the major folate form in bacteria.

Methyl, formyl and methenyl derivatives of tetrahydrofolate make up 90% of naturally occurring monoglutamates. The latter are generally absorbed much more readily than natural polyglutamic conjugates which require to be deconjugated to free or monoglutamate forms before intestinal transport. Current evidence points to the intestinal mucosa, rather than gastrointestinal secretions as the source and location of hydrolase enzymes which deconjugate food folate, and most of this conjugase activity is located intracellularly in the lysozymes (Perry & Chanarin, 1972). Monoglutamate folate (either present as such in food, or released from conjugates after hydrolysis by intestinal enzymes) is the form in which folate is transferred across the intestinal lumen. Clinical and experimental evidence suggests that preferential absorption of folate occurs in the proximal jejunum. Whether pteroylmonoglutamic acid, a relatively large (MW 441) negatively charged water soluble molecule, and its derivatives cross the intestinal wall by simple passive diffusion, driven in part by the pH differential inside and outside the call, or by a more efficent structure - specific energy-dependent process (active transport) is still uncertain. Recent studies in the rat suggest that the intestinal absorption of 5 methyl tetra-hydrofolic acid, the principal dietary and circulating monoglutamate, does not depend on an energy dependent carrier mediated system, but occurs by diffusion. (Strum, et al, 1971). During the absorption metabolic conversion of reduced monoglutamates to the methyl form occurs in the intestinal wall prior to release into the portal circulation. Folate circulates in the serum bound to plasma proteins (Markannen et al, 1972; Elsborg, 1972) and is stored in the liver (which is capable of further metabolic conversions) before release into bile or hepatic vein. Injected folic acid is initially concentrated in the hepatic microsomes where it is reduced before conversion to polyglutamates (the major form in which folate is stored in the liver) takes place in the cell sap and/or mitochondria (Corrocher & Hoffbrand, 1972). However, the free (monoglutamate) form of folate is preferentially (perhaps exlusively) used as a cofactor for cellular enzymes, and reduction to tetrahydro folic acid (accomplished in vivo by dihydrofolate reductase in the presence of TPNH) is a basic requirement for coenzyme function.

The various enzymatic reactions in which folate coenzymes are known to participate include:

(a) certain aspects of purine and pyrimidine synthesis

(b) metabolism of various amino acids including the formation of methionine from homocysteine (Rosenberg & Godwin, 1971).

(c) hydroxylation reactions including those involving phenylalanine (Viukari, 1968; Lloyd, et al, 1971); batyl alcohol and other glyceryl ethers (Tietz, et al, 1964); progesterone (Hagerman, 1964) and other steroids (Lehoux, et al, 1972).

The preceeding outline of the digestion, absorption and biochemistry of folic acid suggests a number of possible mechanisms which might be responsible for drug related folate deficiency. The following hypotheses have been proposed.

1. EFFECT ON DIET. Anticonvulsants could conceivably effect the intake of folate. However, there is no evidence that folate deficiency and megaloblastic anaemia occur in patients on anticonvulsant therapy because the drug or disease state alters the dietary intake of folic acid (Hawkins & Meynell, 1958).

2. IMPAIRED FOLATE ABSORPTION. Anticonvulsant drugs might impair the absorption of either polyglutamate or monoglutamate forms of folate.

(a) Polyglutamate absorption: It has been proposed that phenytoin inhibits intestinal conjugase activity and so might prevent enzymatic release of monoglutamate folate from its conjugated form, and thus result in decreased folate absorption. However, the evidence is conflicting. Decreased blood levels of folate have been observed in normal subjects after feeding conjugated folate derived from yeast together with phenytoin and the *in vitro* deconjugation of polyglutamyl folate has been reported to be inhibited by phenytoin (Rosenberg et al, 1968; Hoffbrand & Necheles, 1968).

However, Baugh & Krumdieck (1969), and Bernstein, et al, (1970) using purified preparations of human intestinal mucosa as the source of enzyme, and synthetic labelled polyglutamate as substrate, found that phenytoin did not inhibit conjugase activity.

More recently Perry & Chanarin (1972) using a different test system (utilising the reaction between plasma conjugase and folate polyglutamate as substrate) were also unable to show any inhibitory effect of phenytoin on enzyme activity in vitro, nor did the drug interfere with the absorption of yeast polyglutamate in their clinical studies. Similar results were reported by Houlihan, et al (1972) and Fehling, et al (1973) who investigated absorption of pure synthetic pteroyl triglutamate in normal volunteers and epileptic patients on anticonvulsant drugs.

Chanarin, Anderson et al, (1958) have shown that it is essential to give a loading dose of folic acid in order to saturate tissues if reproducible results are to be obtained from tolerence tests. The discrepant results obtained by earlier workers may have been explained, at least in part, by the use of impure folate preparations (yeast polyglutamates may contain variable amounts of monoglutamates as well as natural inhibitors of folate absorption), and the failure to saturate tissue stores prior to performing absorption studies. Thus specific inhibition of conjugase activity by phenytoin is unproven, and in any case would not explain the folate deficiency seen after a variety of other structurally unrelated drugs. Definitive studies of the effects of drugs on the absorption of folate polyglutamates will require the use of pure radiolabelled synthetic pteroyl polyglutamates to avoid dependence on microbiological assays, and the problems involved in the use of impure extracts of yeast.

(b) Monoglutamate absorption. Whether drugs might effect the absorption of monoglutamates is possibly of greater relevance to the problem of drug related folate deficiency, as these compounds constitute up to 20% of dietary folate and could, if efficently absorbed, provide all normal folate requirements. If anticonvulsants such as phenytoin were capable of blocking absorption of dietary monoglutamate, they might also affect the absorption of monoglutamates released from dietary conjugates. Unfortunately analysis of the effects of phenytoin on intestinal absorption of folate monoglutamates has been restricted by the still incomplete understanding of the mechanisms of folate absorption, and by the limitations of investigative techniques.

Again the results of experimental studies have been conflicting. Virtually all have utilised synthetic unreduced pteroyl monoglutamic acid (PGA) which represents less than 10% of dietary monoglutamate even after release from con jugates and the relevance of these findings to absorption of the more abundant forms of monoglutamate in a normal diet is unknown.

Benn, et al (1971) suggested that folic acid (PGA) absorption might be impaired as a consequence of the elevated intestinal pH they observed in patients on phenytoin therapy. Since folic acid is a weak acid whose dissociation and hence absorption might be expected to be influenced by intestinal pH, conditions resulting in low pH in the intestinal lumen would theoretically favour folic acid absorption. Conversely, drugs such as phenytoin which are alkaline, might be expected to impair folate absorption on physico-chemical grounds. However, Doe, et al (1971) were unable to demonstrate any change in intra luminal ph in normal subjects by feeding phenytoin. Gerson, et al (1972) were also unable to detect any change in intra luminal pH after the administration of 24 mg of phenytoin (which resulted in an intra luminal concentration of 20 ug/ml) although larger concentrations (100 ug/ml) did raise the intra luminal pH. However, Perry & Chanarin (1972) who directly examined the effect of alteration of intestinal pH on folate absorption, found paradoxically that the presence of bicarbonate in fact enhanced the absorption of PGA as judged by serum folate levels.

Meynell (1966) and Dahlke & Mertens-Roessler, (1967) measuring serum levels after oral ingestion of synthetic PGA in human subjects and Hepner (1969) who measured the disappearance of HPGA from rat intestine suggested that phenytoin had an inhibitory effect on pteroyl monoglutamate absorption. More recently Gerson, et al (1972) measured the absorption of synthetic radiolabelled monoglutamate by calculating the disappearance of ^{3}HPGA from the intestinal lumen using direct intestinal perfusion studies in normal subjects. These workers reported a significant reduction in the absorption of tritiated folic acid when phenytoin was added to the perfusion solution in a concentration of 20 Ug/ml, a concentration lower than that attained after ingestion of a 100 mg tablet.

No other drugs were examined for their effect on folate absorption. However, normal absorption of folic acid in patients on anticonvulsant therapy was reported by Klipstein (1964). Rosenberg, et al (1968) were also unable to show that phenytoin (100 mg) had any effect on the intestinal absorption of free folate (PGA) as measured by a rise in serum folate activity, and similar results were reported by Fehling, et al (1973). Furthermore, Perry & Chanarin (1972) were also unable to find any difference in the peak serum folate levels or urinary folate excretion after an oral dose of PGA given with phenytoin (100 mg) and concluded that the action of phenytoin on folate metabolism did not appear to be via an effect on absorption, but required some alternative explanation.

3. DISPLACEMENT OF FOLATE FROM PLASMA PROTEINS. Another mechanism which might theoretically account for folate deficiency in patients taking anticonvulsant drugs is the displacement of folate from its carrier plasma proteins(s) by these drugs. However, although this might contribute to the lowering of serum folate levels it would not explain the associated reduction in tissue folate levels and accompanying deficiency syndrome (Klipstein, 1964).

4. INCREASED FOLATE EXCRETION. A further possible explanation for the depletion of tissue folate by anticonvulsant drugs might be that urinary excretion of folate is enchance by these drugs. This aspect of drug-folate interaction has not received much attention. Perry & Chanarin (1972) investigated the 6 hour urinary excretion of folate after an oral load of pteroyl monoglutamate with and without phenytoin, and found no statistical difference in excretion.

5. ALTERED TISSUE UTILISATION OF FOLATE. It has been suspected for some time that anticonvulsants might produce folate deficiency by causing a block in folic acid metabolism, although no evidence as to the site of such a postulated block has ever been presented. Girdwood & Lenman (1956) noted the structural similarities between phenobarbitone and folic acid, both of which contain a 6 membered pyrimidine ring. However the growth of L casei <u>in vitro</u> is not inhibited by anticonvulsant drugs when added to culture media, containing folic acid, or by serum folate from patients on phenytoin therapy with normal or low folate concentrations (Klipstein, 1964; Hamfelt & Wilmanns, 1965).

No is there any evidence that anticonvulsants interfere with the uptake of folate by cells in the marrow (Corcino, et al, 1971).

It is known that folate is concerned with certain hydroxylations, and Olesen & Jensen (1970) suggested that folic acid might be necessary for the action of hepatic drug metabolising enzymes. The metabolism of phenytoin might thus cause an increased demand for the vitamin, and consequently decrease folate stores. Richens & Waters (1971) speculated that the disturbance in folate metabolism caused by phenytoin could be due to induction by the drug of liver enzyme systems which might be concerned in folate metabolism. However, no precise hypothesis or experimental support for these suggestions was provided.

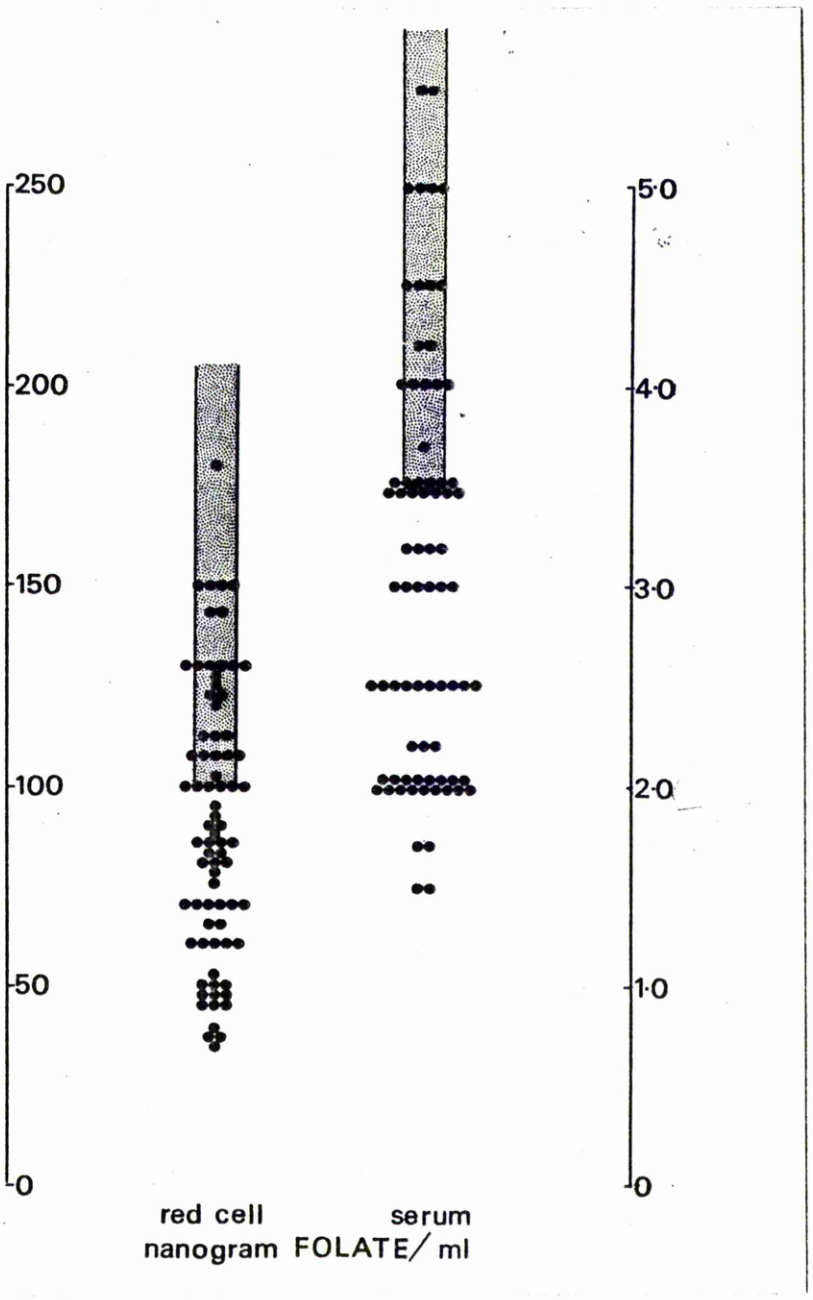
Lingfield Folate Study:

Anticonvulsant drugs are powerful enzyme inducing agents (Conney, 1967). As folate is a cofactor in certain hydroxylations performed by microsomal enzymes, we considered the possibility that folate deficiency occurring after anticonvulsant therapy might result from increased metabolic requirements for the folate confactor by the liver as a consequence of increased activity of drug metabolising enzymes (Maxwell, Hunter, et al, 1972). Using the measurement of the urinary excretion of D-glucaric acid as a quantitative index of microsomal enzyme activity in man (Chapter II), this hypothesis was tested by assessing the relationship between folate levels and microsomal enzyme activity in children with epilepsy receiving treatment with anticonvulsant drugs.

Subjects and Methods:

Seventy-five children (39 boys and 36 girls) aged 12 to 16 years were examined with parental consent. Many were retarded and it was not possible to obtain a reliable dietary history but all were receiving the standard school diet and had been resident for at least three months at the time of study. None were on folic acid or other vitamin supplements.

Fig. 13



Serum and red cell folate levels in 75 epileptic children. Hatched areas are normal ranges for our laboratory.

Some of these children were investigated at the same time as they were being screened for osteomalacia, (Hunter, Maxwell et al, 1971). (See earlier section of this chapter).

Serum and red cell folate concentration were measured in non-fasting blood taken between 9 and 11 a.m. by using a microbiological assay with Lactobacillus casei as the test organism. (Hoffbrand et al, 1966; Chanarin, 1969). The concentration of D-glucaric acid in a urine sample collected at the same time was determined from the inhibitory effect of glucarolactone, to which it is converted by boiling at pH2, on β-glucuronidase. This was related to the concentration of creatinine in the same sample and expressed as umol of glucarolactone/g of creatinine. Previous studies in epileptic and normal subjects showed that this ratio was closely related to the total 24-hour excretion of D-glucaric acid. (Hunter, Maxwell et al, 1971).

The results of D-glucaric acid excretion were compared with values obtained in urine samples collected from a control group of 15 children of the same age in an institution for the mentally retarded, none of whom were on drug treatment. The values for serum, and red cell folate were compared with the established normal range for these tests in our laboratory.

Results:

Serum folate was reduced below 3.5 ng/ml the lower limit of normal in 44 (59%) of the 75 children investigated. The lower limit of normal for red cell folate is 100 ng/ml, and values lower than this were present in 42 (58%) of the 72 children in whom measurements were made (Fig. 13). There was a significant correlation between serum and red cell folate ($r=0.39$, $P<0.002$). Both sexes had a similar frequency of reduced red cell and serum folate levels.

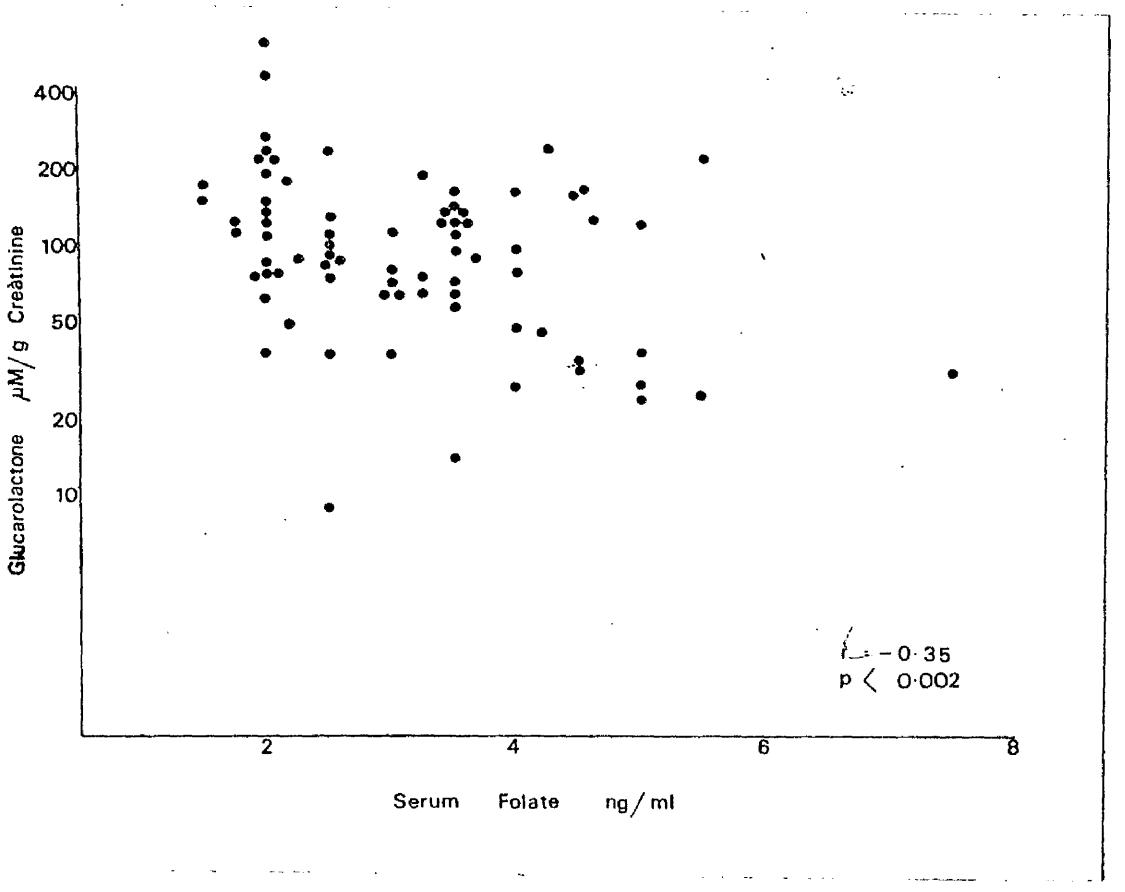
Increased concentrations of glucaric acid were found in 69 (90%) of the epileptic children (Fig. 12). Four of the six children with normal D-glucaric acid values were not on anticonvulsants as they had not had fits for some time, and the other two were on very small doses. The range of D-glucaric acid values in the epileptic children (9-631 umol/g) was comparable to that found by us in a study of adult epileptics (Fig.12).

Children with high D-glucaric acid values were found to have reduced folate levels, and a linear relation was found to exist between D-glucaric acid concentration when plotted logarithmically and the serum folate and red cell folate values. Statistical analysis disclosed a significant inverse correlation between log glucaric acid and serum folate ($r=0.35$, $P<0.002$) and red cell folate ($r=0.34$, $P<0.002$). There was also a significant correlation between the levels of serum and red cell folate in the individual children and the total daily dose of anticonvulsant drugs when expressed in units (Richens & Rowe,1970) ($r= -0.41$, $P<0.001$ and $r= -0.55$, $P<0.001$, respectively). (Fig. 14).

Discussion:

The finding of a significant association between the extent of microsomal enzyme induction and the levels of both serum and red cell folate is strong evidence, though not direct proof, for the hypothesis that folate deficiency may result from accelerated metabolism of folate after the induction of hepatic drug metabolising enzymes caused by anticonvulsant drug therapy. This would also explain the reported occurrence of folate depletion in women on oral contraceptives (Streiff, 1970; Shojania, Hornady & Barnes, 1968 and 1971) and the effect of chronic ethanol ingestion in accelerating the development of serum and tissue folate depletion in subjects on folate deficient diets (Eichner, Pierce & Hillman, 1971), for despite considerable differences in chemical structure ethanol and oestrogens, like the anticonvulsants, share the property of inducing the activity of microsomal enzymes (Conney, 1967; Mowat, 1968; Rubin & Lieber, 1971).

Fig. 14



Relationship between serum folate and urinary glucaric acid excretion (expressed as umol of glucarolactone/g of creatinine) in 75 epileptic children.

In most reported cases patients with folate deficiency occurring after anticonvulsant therapy have been on combinations of drugs and the effect appears to be dose related (Reynolds, 1968). In the cases reported by Hawkins & Meynell (1958) macrocytosis occurred in 27% of patients on phenytoin alone and 34% of those of phenobarbitone alone, while macrocytosis occurred in 45% of those receiving both drugs. There is little evidence that any single anticonvulsant drug is particularly apt to produce these changes, although pheneturide may be more potent in this respect than phenobarbitone, phenytoin or primidone (Latham et al, 1973).

This hypothesis also provides a logical explanation for the finding that administration of folic acid to epileptic patients on phenytoin results in a lowering of the blood phenytoin levels (Baylis et al, 1971), and an increase in the urinary excretion of its principal metabolite (Kutt, et al, 1966) and for the clinical observation that deterioration in fit control may ensue (Reynolds, 1968). This is based on evidence from in vitro studies suggesting that folates may be required as cofactors in the hydroxylation of drugs as well as certain natural substrates. Tietz et al, (1964) showed that liver microsomes lost their ability to oxidise batyl alcohol and other glyceryl ethers after repeated washing but that activity was fully restored and even augmented by the addition of folate or certain pteridines. These compounds have also been shown to be required in the hydroxylation of phenylalanine (Lloyd et al, 1971) and progesterone (Hagerman, 1964; Lehoux et al, 1972). Furthermore, the clinical studies of Kutt et al, (1966) and Baylis et al, (1971 suggested that the folate depletion may limit the rate of metabolism of phenytoin which like other inducing agents is metabolised by microsomal enzymes.

Recent studies by Latham, et al, (1973) have supported our hypothesis that prolonged administration of any drug capable of inducing the activity of microsomal drug metabolising enzymes is likely to result in an increased demand for folate. As body stores are limited (Chanarin, 1970) and dietary intake is usually sufficient only for normal requirements, folate levels will fall until they reach a level at which lack of cofactor, rather than enzyme activity per se, rate limits drug metabolism. If this limitation is removed by administration of folic acid supplements, an increase in the rate of drug metabolism may be expected to occur with

enhanced drug clearance, reduction in plasma steady state levels of the drug (as has been shown for phenytoin) and hence possible deterioration in fit control.

Thus folate deficiency may have to be added to anticonvulsant osteomalacia as another possible adverse metabolic consequence of prolonged induction of hepatic microsomal enzymes. Further clinical and laboratory investigation of the role of folic acid in hepatic drug metabolism is required before this hypothesis can be established and the place of dietary folate supplements in patients at risk also requires further study.

iii Vitamin K: NEONATAL HAEMORRHAGE FOLLOWING MATERNAL ANTICONVULSANT THERAPY

In a prospective study Mountain et al, (1970) performed coagulation factor assays on 16 neonates born to 16 unselected epileptic mothers who were being treated with anticonvulsant drugs. Two of the neonates had clinical evidence of a bleeding tendency and a coagulation defect similar to that in vitamin K deficiency was found in 8 of the infants. These results suggested that anticonvulsant drugs, especially barbiturates, when given to the mother during pregnancy, decreased the levels of vitamin K dependent clotting factors in the infant during the early neonatal period. A further five cases were reported by Evans et al, (1970). In all cases the mothers were established epileptics receiving therapy with 2 or more anticonvulsants. There was no undue birth trauma, and the infants were of sufficiently high birth weight to make severe coagulation defects due to prematurity improbable. More information on the association of maternal anticonvulsant therapy and neonatal haemorrhage has come from a controlled retrospective study of pregnancy and its outcome in a group of 18 epileptic and 180 non-epileptic women. (Speidel & Meadow, 1972). There were five still births and 10 neonatal deaths in the off-spring of epileptic women, and all the perinatal deaths occurred in babies whose mothers took anticonvulsants during pregnancy. The higher death rate in the off-spring of epileptic mothers (almost twice that of controls) was due mainly to spontaneous haemorrhage (4 deaths) and congenital malformations (4 deaths) which together accounted for more than half the perinatal mortality.

Although dietary deficiency of the vitamin is thought to be exceptionally rare, the capacity of the body to store vitamin K is extremely limited, and deficiency as evidenced by hypoprothrombineaemia may be produced in experimental animals by diversion of intestinal lymph or by biliary fistulas (Woolf & Babior, 1972). Vitamin K is metabolised in the liver to glucuronide and sulphate conjugates (Losito et al, 1968; Thompson, Gerber et al, 1972) The combined effects of enzyme inducing drugs and pregnancy (see later section of this chapter) could conceivably accelerate this process, and hepatic enzyme induction with accelerated biotransformation of vitamin K provides a theoretically plausible explanation for the neonatal coagulation defect. This remains speculative and further studies are required.

(d) Enzyme induction and teratogenesis:

An increased incidence of congenital malformations in the children of epileptic women has been noted. The malformations most commonly reported include cleft lip and palate, though other defects, especially cardiac anomalies have also been found. Such an association could be the result of either a direct or a genetic effect of maternal epilepsy on the foetus, or alternatively could be due to the anticonvulsant drugs used to treat epilepsy.

In retrospective study covering a period of 20 years Speidel & Meadow (1972) compared the incidence of malformations in offspring of 186 epileptic women with that in 180 controls in Leeds. Major congenital malformations occurred with twice the frequency, and mental subnormality was 7 to 8 times commoner in offspring of epileptic mothers. Phenobarbitone, phenytoin and Primidone, all potent enzyme-inducing agents, were the drugs most commonly prescribed in this survey. Interestingly, malformations were confined to offspring of epileptic women taking these drugs.

In a more recent study from Boston the frequencies of various malformations in over 50,000 pregnancies were compared between groups of children born to epileptic mothers, and those born to non-epileptic mothers. The highest malformation rate (61/1000) was observed in children exposed during the early months of pregnancy to daily phenytoin use, while the rate was lowest (25/1000) in children born to non-epileptic women. There appeared to be a trend between increasing exposure to the drug and risk of malformation, but the differences observed between children exposured regularly to phenytoin during early gestation, and children born to non-epileptic mothers could still reflect an effect of epilepsy rather than drug toxicity (Monson et al, 1973). However, Visser et al (1976) compared the malformation rate in offspring of a group of epileptic mothers in the pre-anticonvulsant era with that in a more recent group receiving medication. A substantial increase in malformations in offspring of treated mothers suggested an effect of anticonvulsant drugs.

Although not proven, the possibility of a causal relationship between enzyme inducing drugs and congential birth defects is suggestive. This effect does not seem to be unique to any single anticonvulsant (Speidel & Meadow, 1972) and animal studies provide evidence for a causal association (Harbison & Becker, 1969) with enzyme inducing agents. However, such an association requires to be established by a prospective study (Barry & Danks, 1974). Ideally, this should provide information not only on the prevalence of malformations, but also on blood levels of anticonvulsants attained during pregnancy, and some measure of alterations in hepatic enzyme activity in the mother, such as changes in 6 β hydroxycortisol or D-glucaric acid excretion (Chapter II).

5. INDUCTION OF HEPATIC ENZYMES DURING NORMAL HUMAN PREGNANCY

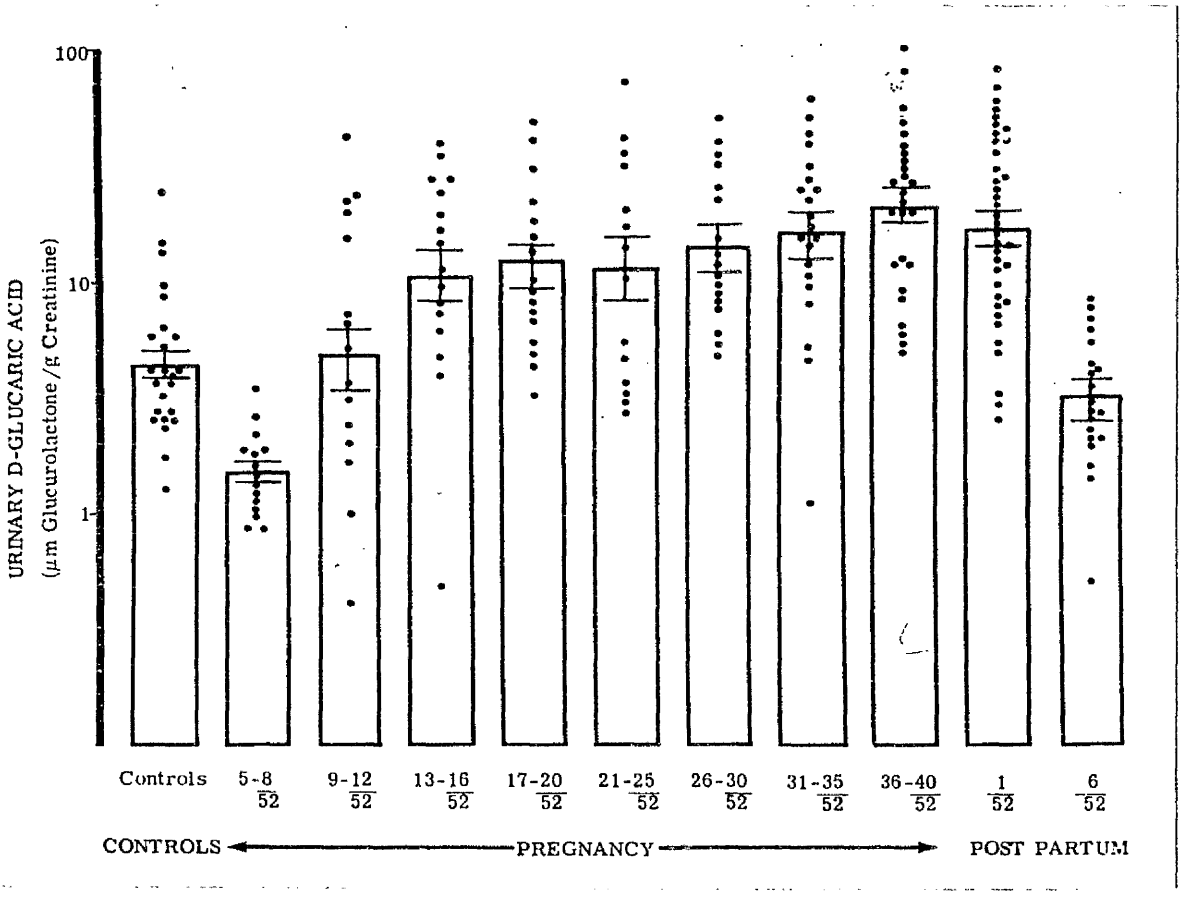
The activity of hepatic microsomal enzymes may be stimulated in animals by high doses of naturally occurring steroids, including oestrogens and progestogens (Kuntzman, 1969). In normal human pregnancy there is a large and progressive increase in placental steroid production with an abrupt return to normal at delivery (Amoroso & Porter, 1970). Growth of gastric mucosa (Crean & Rumsey, 1971) and an increase in liver weight of up to 50% (Song & Kappas, 1968) have been found during the course of pregnancy in the rat. Enhanced hepatic microsomal enzyme activity has been demonstrated in the rat following high doses of progesterone (Hall, Fahim et al, 1971) but it was not known whether such changes occurred in the course of human pregnancy. Accordingly, we measured the urinary excretion of D-glucaric acid (Chapter II) in an attempt to assess possible changes in the activity of hepatic enzymes in pregnant women. (Davis et al, 1973).

Pregnancy Study:- Patients and Methods

Spot urine samples were obtained from 142 healthy pregnant women attending the ante-natal clinics at King's College Hospital. In patients with amenorrhoea of less than 8 weeks duration, pregnancy was confirmed by immunological testing with "Pregnosticon." Urine samples were obtained from a further 58 women at one and 6 weeks post partum. The control group comprised 24 women using intra-uterine contraceptive devices. Subjects were excluded from the study if they were taking any drugs other than routine haematinics.

D-glucaric acid was assayed as its lactone (glucuro - 1,4 lactone) by the method previously described (Chapter II). The concentration of the lactone present in the urine after boiling at pH 2 UM was determined by the degree to which the urine inhibited the activity of β-glucuronidase. Results are expressed as umol glucurolactone/g of creatinine, which was shown to correlate well with the total 24 hours urinary excretion of d-glucaric acid. (r=0.97, Hunter, Maxwell et al, 1971).

Fig. 15



Glucaric acid excretion (expressed as umol of glucarolactone/g creatinine) through pregnancy, and in non-pregnant controls. Note progressive increase in excretion during later pregnancy, with return to normal levels by the sixth week post-partum.

Results:

The distribution of results was markedly skewed in each of the groups studied, but was normalised by logarithmic transformation. Consequently statistical analysis of the data was performed using logarithims of the results.

Figure 15 shows the variation in D-glucaric acid excretion during normal human pregnancy and in the post-partum period. Following a significant ($P < 0.005$) decrease from the control value at 5 to 8 weeks of gestation, there was a return to normal by 12 weeks and thereafter a progressive increase in D-glucaric acid excretion with a maximum mean excretion of 18.7 umol/g of creatinine by 36 - 40 weeks of gestation. One week post partum the level of D-glucaric acid had fallen to 15.6 umol/g of creatinine, a value not significantly different from control ($P > 0.1$).

Discussion:

Electron microscopic studies of liver biopsies taken in the course of normal human pregnancy have shown proliferation of the smooth endoplasmic reticulum (Perez et al, 1971). The present studies suggest that enzyme activity increases progressively throughout pregnancy after the 12th week of amenorrhoea, paralleling the rise in endogenous oestrogen and progesterone excretion (Amoroso & Porter, 1970). The delayed fall in D-glucaric acid output after delivery when the levels of steroids abruptly drop, has been seen when exogenous inducing agents, such as phenobarbitone, are withdrawn (Hunter, Maxwell et al, 1971). Our findings are in keeping with animal studies which have shown increased activity of δ-ALA synthetase (Kappas, Song et al, 1968) in response to administration of oestrogens and progestogens. This enzyme is considered to be rate limiting in the production of haemoproteins involved in microsomal drug metabolism and its activity can be stimulated by enzyme inducing drugs. (Chapter VI).

Neale and Parke (1969), have shown that the capacity for hepatic drug metabolism increases in the pregnant rat, but that this is due more to an increase in liver size than to a rise in specific activity of the enzyme involved. Information on changes in liver size during normal human pregnancy is lacking although the storage capacity for bromsul-phthalein (BSP), which may reflect functional liver capacity, is reported to be increased in pregnant women (Wheeler, Epstein et al, 1960; Wheeler, Meltzer & Bradley, 1960). Although the highest levels of D-glucaric acid output attained during pregnancy were substantially lower than those previously documented after phenobarbitone treatment (Hunter, Maxwell et al, 1971) the greater than four fold increase observed during the third trimester is unlikely to be due simply to an increase in liver mass.

The significant drop in D-glucaric acid excretion below control levels between the fifth and eighth week of pregnancy might be explained by the initial inhibitory effect of certain inducing agents on microsomal enzyme activity. Some progesterone metabolites are inhibitory to glucose - 6 - phosphate dehydrogenase (Marks & Banks, 1960) and glucuronyl transferase (Hsia, et al, 1963), enzymes both involved in synthesis of D-glucaric acid from glucose (Chapter II). These enzymes are both induced by gonadal steroids (Shibata, et al, 1966; Higgins & Yao, 1959) and during early pregnancy the effects of enzyme inhibition could outweigh those of induction.

The factors controlling the pharmacokinetics of drugs administered during pregnancy are complex, and the effect of increased hepatic enzyme activity has yet to be fully evaluated. Although induction of hepatic microsomal enzymes per se might be expected to enhance drug metabolism in pregnancy, the effect on biotransformation of drugs and other foreign substances in vivo will also be determined by other factors such as the relative affinities of drugs and gestational steroids for common binding sites on microsomal processing enzymes, and changes in protein binding and volume of distribution of the drug. Both oestradiol and progesterone have been shown to be competitive inhibitors of microsomal oxidases for drugs such as ethylmorphine and hexobarbital (Tephly & Mannering, 1967). Thus the in vivo clearance of some drugs may actually be reduced during pregnancy

as has been shown for hexobarbital in the rat (Quinn, et al, 1958). However, the metabolism of dicophane (DDT) is said to be increased in pregnancy (Kroger, 1972) and recent studies suggest that the metabolism of organochlorine pesticides may be accelerated in the immediate post partum period (Rowan, Hunter & Maxwell, 1975). Thus there is some evidence that increased oestrogen and progesterone production during pregnancy may lead to enzyme induction but that any effect on drug metabolism may be slight as steroids are also competitive inhibitors of microsomal enzymes. However, after delivery steroid levels fall, but the induced state persists for a few days, temporarily increasing the rate of drug metabolism (Hunter, 1976).

Induction of hepatic microsomal enzymes during normal pregnancy has a number of clinical implications. Administration during pregnancy of those drugs or chemicals which are metabolised by hepatic microsomal enzymes to produce active or toxic metabolites could have deleterious effects on both mother and foetus. It has been shown, for instance, that dimethylnitrosamine which exerts its hepatotoxic effect through an activated mexabolite, is more toxic to pregnant than non-pregnant rabbits (Texler, et al, 1970). It is interesting to speculate whether this mechanism might also explain the occasional hepatotoxicity observed after administration of large doses of tetracycline to pregnant women. Folic acid deficiency is pregnancy might also be explained, at least in part, by increased activity of hepatic processing enzymes at this time (Maxwell, Hunter et al, 1972). We have also recently reported that the well known fall in plasma calcium levels during pregnancy, is accompanied by a significant reduction in plasma 25 hydroxycholecalciferol (Turton et al, 1977). Although multiple factors may contribute to the pathogenesis of hypocalcaemia observed at this time, it may in part be related to increased microsomal enzyme activity during pregnancy.

CHAPTER VI

DRUG SENSITIVITY IN HEREDITARY HEPATIC PORPHYRIA

(1) HEREDITARY HEPATIC PORPHYRIAS

(a) Pharmacogenetics

Sir Archibald Garrod (1902), who pioneered the study of inborn errors of metabolism, was the first to suggest that unusual reactions to drugs might be caused by genetically determined aberrations in metabolic pathways. This far-sighted observation was neglected for many years. However, with the recognition in the 1950's that several "idiosyncratic" drug reactions such as primaquine-induced haemolytic anaemia and suxamethonium-induced prolonged apnoea could be explained on the basis of genetically determined enzyme deficiencies, pharmacogenetics was established. Initially the term referred only to hereditary disorders involving adverse reactions to drugs that were uncovered by administration of the particular agents. This definition seemed too restrictive, as it excluded from consideration genetically determined differences in drug-metabolism or disposition (pharmacokinetics) and drug response (pharmacodynamics) between healthy non-medicated subjects (Vesell, 1975). When it became apparent that the interplay between genetics and pharmacology influenced the action of many drugs, the scope of pharmacogenetics was extended to include all studies at the interface of these two sciences. Pharmacogenetics now incorporates all clinically important hereditary variations in drug metabolism and response.

This chapter is devoted to a consideration of the hereditary hepatic porphyrias - a group of classical pharmacogenetic disorders. (Maxwell & Meyer, 1977). Recent clinical and laboratory studies will be presented which suggest that many of the previously unexplained features of this group of disorders can be understood on a mechanistic basis, and that the "idiosyncratic" reaction to many common drugs, which is such a characteristic feature of hereditary hepatic porphyrias, is a direct consequence of the primary genetic defect in porphyrin and heme biosynthesis.

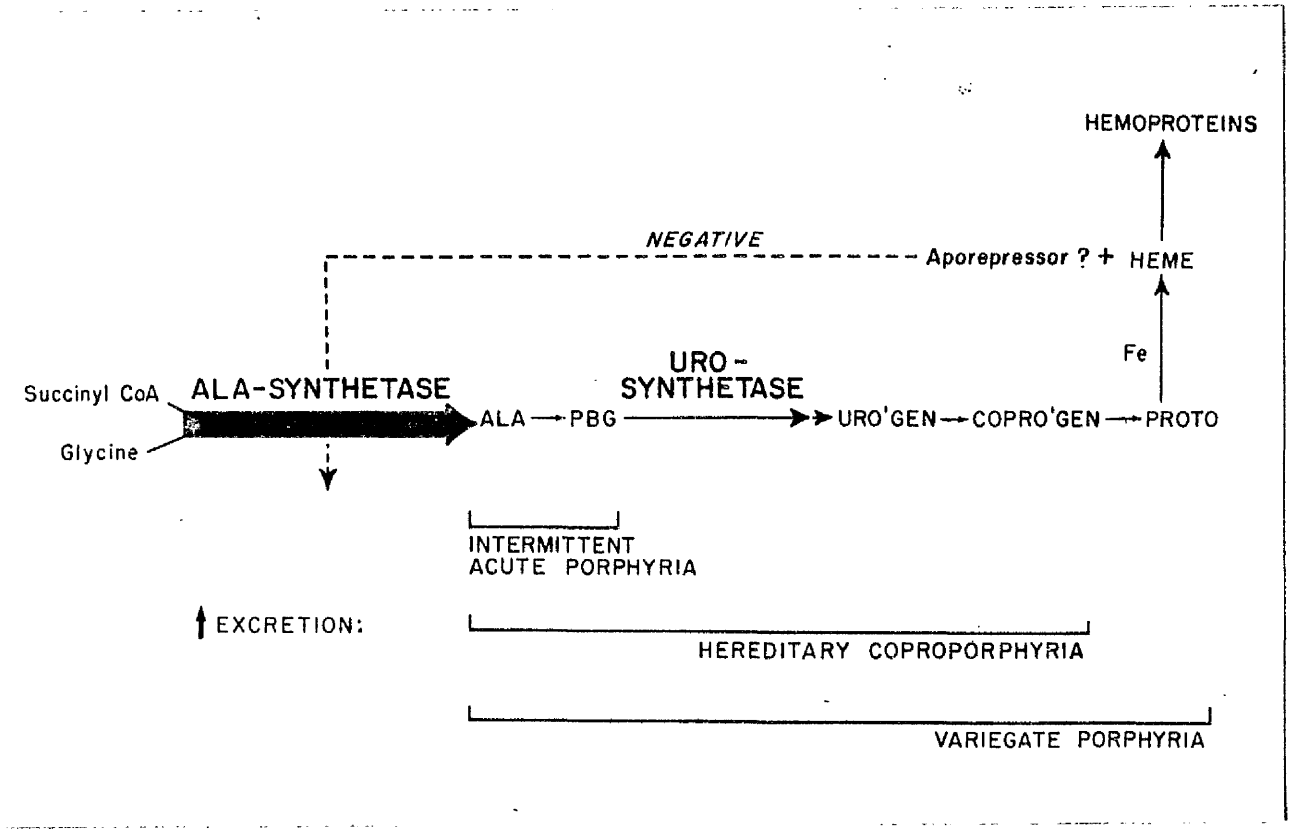
(b) Clinical Features and Enzyme Defects

The hepatic porphyrias are a group of disorders whose principal clinical manifestations are intermittent attacks of neuropsychiatric dysfunction and/or photosensitivity. They include intermittent acute porphyria (IAP), hereditary coproporphyria (HCP) variegate porphyria (VP) and porphyria cutanea tarda (PCT). All four are biochemically characterized by increased formation, accumulation and excretion of porphyrin precursors and/or porphyrins in the liver, reflecting dysfunction of hepatic heme biosynthesis (Meyer and Schmid, 1977).

IAP, HCP and VP constitute the three classical hereditary hepatic porphyrias. In these three disorders acute, often life-threatening attacks of an identical neuropsychiatric syndrome commonly are precipitated by normal therapeutic doses of a variety of drugs. These three forms of hepatic porphyria can be distinguished, however, on the basis of additional clinical manifestations (such as the presence or absence of skin photosensitivity) and a unique pattern of porphyrin precursor and/or porphyrin excretion.

In PCT, less clearly defined genetic and acquired abnormalities of hepatic heme biosynthesis interact in causing hepatic accumulation of porphyrins. In contrast to IAP, HCP and VP, the major clinical manifestations of PCT are limited to the skin and the disease frequently is associated with evidence of hepatic dysfunction. Acute attacks of neuropsychiatric disturbance and the associated massive excretion of porphyrin precursors do not occur in PCT; moreover, drugs that aggravate or precipitate symptoms in IAP, HCP and VP in general have no adverse effect in PCT. In regard to the sensitivity to drugs, PCT therefore clearly is different from the classical hereditary hepatic porphyrias.

Fig. 16



Schematic representation of haem biosynthesis and excretion patterns of porphyrin precursors and porphyrins in hereditary hepatic porphyrias. The heavy arrow for ALA-synthetase illustrates the increased activity for this enzyme. (Meyer et al, 1972).

In all the hepatic porphyrias, the liver appears to be the major site where the inborn enzymatic error manifests itself metabolically by accumulation and, consequently, by excretion of porphyrins and porphyrin precursors. This does not mean that the genetic defect is limited to the liver, as in IAP for example, characteristic enzymatic abnormalities have been demonstrated in other tissues studied (Meyer, 1973). It is now established that increased activity of hepatic δ - aminolevulinic acid synthetase (ALA-synthetase), the first and rate limiting enzyme in heme synthesis, is an enzymatic abnormality common to the three classical hereditary hepatic porphyrias (Elder et al., 1972; Meyer Schmid, 1973; Fig. 16). Furthermore, there is some evidence that during acute attacks the activity of this enzyme is enhanced still further (Taddeini and Watson, 1968; Strand et al., 1970; McIntyre et al., 1971) apparently contributing to the accumulation of porphyrin precursors characteristically seen at this time. However, although strikingly increased activity of hepatic ALA-synthetase is a constant feature IAP, HCP and VP, this is not seen in PCT, even in severe cases (Meyer and Schmid, 1977). Furthermore it has to be realized that increased activity of ALA-synthetase could not by itself account for the distinct patterns of porphyrin or porphyrin precursor excretion that typifies each of the hepatic porphyrias (Fig. 16).

Recent studies indicate that in IAP, reduced activity of uroporphyrinogen I synthetase (which catalyses the condensation of 4 moles of PBG to one mole of uroporphyrinogen) represents the primary genetic defect in heme synthesis (Meyer, et al., 1972). A partial block at this site conveniently explains both the specific pattern of porphyrin precursor excretion in IAP, and suggests that increased hepatic ALA-synthetase activity is a secondary phenomenon, possibly the result of a decreased negative feedback regulation by heme (Sassa and Granick, 1970; Strand et al., 1972). The demonstration of a primary partial defect in heme synthesis in IAP supports the concept that the other forms of hereditary hepatic porphyria may be explained by analogous enzymatic defects at more distant sites in the pathway of heme biosynthesis. (Elder et al, 1976).

i. Intermittent Acute Porphyria (IAP)

is characterised by acute attacks of neuropsychiatric manifestations including abdominal colic, hypertension, peripheral and central neuropathies and mental disturbance. Photosensitivity does not occur. Acute attacks are frequently precipitated by drugs as well as by hormonal, nutritional and other metabolic factors. Like the other hereditary hepatic porphyrias it is inherited as an autosomal dominant disorder. The primary genetic defect in IAP is a generalized deficiency or uroporphyrinogen I synthetase. In the liver this defect results in a secondary rise and apparently in enhanced "inducibility" of ALA-synthetase. As a consequence, urinary excretion of the porphyrin precursors δ-aminolevulinic acid (ALA) and porphobilinogen (PBG) is increased, without a proportional increase in porphyrin excretion (Fig. 16).

ii. Hereditary Coproporphyria (HCP)

resembles IAP both in its drug sensitivity and the occurrence of acute attacks with neuropsychiatric manifestations. In addition photosensitivity may occur. It is inherited as an autosomal dominant disorder with variable clinical expression. The suspected defect is a deficiency of coproporphyrinogen oxidase, which secondarily affects the regulation of hepatic ALA-synthetase. The characteristic chemical finding is the unremitting excretion of large amounts of coproporphyrin in the faeces and, to a lesser extent, in the urine. During acute attacks, urinary excretion of the porphyrin precursors ALA and PBG is increased.

Recent studies have shown that the activity of coproporphyrinogen oxidase in cultured skin fibroblasts from patients with hereditary coproporphyria is approximately half that in normal subjects or patients with other forms of porphyria (Elder et al, 1976).

iii. Variegate Porphyria (VP)

also is inherited as an autosomal dominant disorder; characterized by drug sensitivity, and may present with neuropsychiatric manifestations. However, in addition, chronic skin sensitivity to light and to mechanical trauma is a prominent feature. Biochemically VP is characterized by greatly enhanced excretion of faecal protoporphyrin and, to a lesser degree, of coproporphyrin. The suspected primary defect is a deficiency of an enzyme between protoporphyrinogen and heme. As in HCP, the urinary excretion of ALA and PBG is increased during acute attacks.

iv. Porphyria Cutanea Tarda (PCT)

Photosensitivity is the only noteworthy clinical manifestation of this form of hepatic porphyria. Biochemically it is characterized by excessive urinary excretion of uroporphyrins, together with smaller amounts of copro- and other porphyrins. Recent research suggests that, at least in some patients, PCT may be related to a dominantly inherited defect in uroporphyrinogen decarboxylase, but the metabolic expression of this genetic anomaly appears to require additional acquired factors such as hepatic iron overload, alcohol and oestrogens. In PCT, the underlying primary genetic defect (in contrast to the classical hereditary hepatic porphyrias) does not appear to result in significantly altered regulation of the heme pathway, as ALA-synthetase activity remains normal or only minimally increased, even in overt cases. This observation probably accounts for the critical clinical and biochemical difference between PCT and the classical hereditary hepatic porphyrias - namely the absence of acute neuropsychiatric attacks, normal urinary excretion of porphyrin precursors ALA and PBG and the lack of sensitivity to drugs such as barbiturates.

(c) Biochemical Basis for Clinical Features in the Hepatic Porphyrias

It has been difficult to establish a causal relationship between the metabolic defects in porphyrin and heme biosynthesis and the various clinical abnormalities in the hereditary hepatic porphyrias, because little is known of the pharmacological effects of porphyrins and porphyrin precursors. However, it is most likely that porphyrin accumulation in plasma or skin is responsible for photosensitivity in VP, HCP, and PCT (Rimington et al., 1967). The other noncutaneous features characteristic of an acute attack of hereditary hepatic porphyria can all be explained by disturbances of central or peripheral nervous system function (Ridley, 1969), but the pathogenetic link between the biochemical abnormality in heme synthesis and these clinical manifestations remains obscure. Numerous hypotheses have been advanced to explain the neuropsychiatric manifestations but none is supported by convincing experimental evidence. The neurological dysfunction cannot yet be defined in biochemical terms, but a partial enzymatic block in heme synthesis has a number of possible theoretical implications for the pathogenesis of neurotoxicity. If it is assumed that enzyme activity is also deficient in cells of the nervous system, this could lead to intraneuronal accumulation of porphyrin precursors, and possibly result in neurocytotoxicity. Alternatively, if heme synthesis in the nervous system were regulated in a different manner from that in the liver, the enzymatic defect could result in impaired availability of heme for hemoproteins which might in turn result in metabolic impairment. It is also conceivable that the neuropsychiatric manifestations might be caused by depletion of critical metabolites or by accumulation of neurotoxic substances which, while not directly related to heme synthesis, may be a consequence of the inherited defect (Meyer and Schmid, 1974). However, the most attractive explanation at present for the neuropsychiatric phenomena is that excess porphyrin precursors

(ALA and/or PBG) formed in the liver may gain access to the nervous system, and there exert a toxic effect. This hypothesis is supported by the observation that the neurological manifestations occur only in those hepatic porphyrias in which there is excessive hepatic accumulation and urinary excretion of porphyrin precursors (IAP, VP and HCP). Moreover, in patients with IAP, a rough correlation has been found between the concentration of ALA and PBG in serum, and the extent of neuropsychiatric manifestations (Dhar et al, 1975). Furthermore, the recently demonstrated effectiveness of haematin infusion in patients with acute attacks of hepatic porphyria (Watson, 1975) is also consistent with the concept that elevated plasma levels of ALA and/or PBG may be causally related to the pathogenesis of the neurological lesion. Because of the similarities between some clinical features of lead poisoning and acute porphyria (Dagg et al., 1965) ALA is a prime suspect as a mediator of the neurological lesion. Although various studies have shown ALA and PBG to affect nerve function in vitro, the significance of these effects for the pathogenesis of acute porphyria remains controversial. The neurotoxicity of the porphyrin precursors has not been conclusively demonstrated in vivo, but no studies have yet been carried out in the animal models of hepatic porphyria with generalized partial blocks in heme synthesis mimicking the genetic defect in man.

Table 14

ALCOHOL	Ethyl alcohol
ANTICONVULSANTS	Hydantoins; Succinimides
BARBITURATES	All varieties including thiopentone
CHEMOTHERAPEUTICS	Griseofulvin; Sulphonamides
HYPNOTICS	Dichloralphenazone; Glutethimide
HYPOGLYCAEMICS	Tolbutamide
HYPOTENSIVES	Methyldopa
TRANQUILLISERS	Meprobamate
STEROIDS	Oestrogens; Progestagens

Some drugs known or reported to precipitate acute attacks of porphyria.

(2) DRUGS AND PORPHYRIA

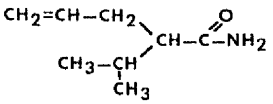
(a) Precipitation of Hereditary Hepatic Porphyrias by Drugs

In his classical monograph on intermittent acute porphyria published in 1937, Waldenstrom drew attention to the role of barbiturates in precipitating acute porphyria, an association which had first been reported some 30 years earlier by Dobrschansky (1906). Further evidence of a relationship between drug administration and acute attacks of hepatic porphyria has come from South Africa, where a large genetic reservoir of one form of inherited porphyria - variegate porphyria - has existed for over two centuries. Interestingly the clinical expression of this genetic disorder was virtually unrecognized until the therapeutic revolution of the past three to four decades, resulting in the widespread use of drugs such as the barbiturates. Since that time acute attacks of variegate porphyria have become a substantial problem in South Africa (Dean, 1963).

In all forms of the hereditary hepatic porphyrias both the intermittent nature of the clinical disorder, and the existence of latent cases (With, 1963; Meyer, 1973) provide evidence that the primary genetic defect is frequently not expressed, and have emphasized the importance of additional endogenous or environmental factors in initiating acute attacks. Imprudent drug administration appears to be particularly important in this respect, and has been implicated as a precipitating factor in between 50 and 75% of acute attacks (Stein and Tschudy, 1970; Eales, 1971). Moreover it has been claimed that drug related attacks are associated with a more severe neurological disturbance than spontaneous episodes of acute porphyria (Goldberg, 1959). Barbiturates remain the drugs most commonly associated with acute attacks of hepatic porphyria, and in one report featured in over 50% of acute attacks (Eales, 1971). A heterogeneous group of non-barbiturate sedatives and other structurally dissimilar drugs have also been implicated (Table 14) as

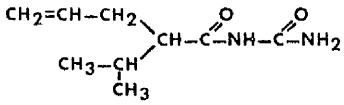
Fig. 17

I A)



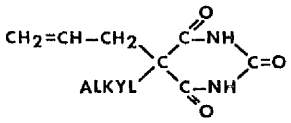
ALLYLISOPROPYL-ACETAMIDE (AIA)

B)



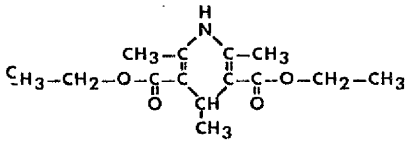
ALLYLISOPROPYLACETYL-UREA (SEDORMID)

C)



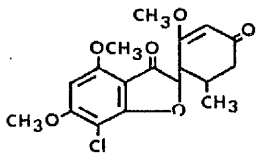
ALLYLALKYLBARBITURATE

II



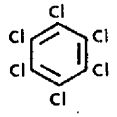
3,5-DICARBETHOXY-1, 4-DIHYDROCOLLIDINE (DDC)

III



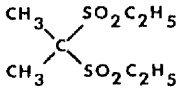
GRISEOFULVIN

IV



HEXACHLOROBENZENE

V



SULFONAL

Some compounds used to produce experimental porphyria.

possible precipitating factors of the acute attack. The association of drugs and acute attacks may have been underestimated as cases of deliberate suppresion of a history of prior drug ingestion have been recognised. The natural course of the disease is variable and unpredictable, and because of this the evidence associating any given drug with an acute attack is open to question. Many patients have been taking several drugs at the time of an acute attack, and under these circumstances it is difficult to implicate one drug rather than another. Furthermore, the primary indication for drug treatment (e.g. infection), rather than the drug itself (e.g. sulphonamide), may have been the major factor responsible for precipitating an acute attack (Stein and Tschudy, 1970; Eales, 1971). However, for the barbiturates at least, the evidence for a causal relationship between drug intake and an acute attack seems undeniable. There are many well documented reports of previously healthy individuals who developed acute attacks following thiopentone anaesthesia for minor extraabdominal surgery. On the other hand the susceptibility of porphyric subjects to barbiturates seems to be unpredictable, as there are may patients who have undergone drug administration or thiopentone anaesthesia without ill effect.

(b) Toxic and Experimental Porphyria

In contrast to the large number of common drugs which have been implicated in precipitating acute attacks in the hereditary hepatic porphyrias, a much smaller number of compounds is known to be capable of producing an experimental form of porphyria in laboratory rodents or cell culture which biochemically resembles the genetic variety (Tschudy & Bonowsky, 1972). (Fig. 17)

The concept of toxic porphyria originated from the report by Stokvis in 1889 of a patient who passed red urine, became paralysed and died after ingesting Sulphonal (diethylsulfondimethylmethane). This sedative proved to be highly toxic, and it was later estimated that between 7 and 10% of patients who took it developed symptoms of acute porphyria (With, 1971). This figure is greatly in excess of the prevalence of inherited hepatic porphyria (estimated at between 1:10,000 and 1:100,000 in European and North American populations) thus suggesting that Sulphonal was directly porphyrogenic and not simply responsible for precipitating acute attacks in genetically predisposed subjects. This and subsequent clinical observations provided the basis for the development of experimental models of porphyria in laboratory animals. Later studies, prompted by the clinical observation that Sedormid (allylisopropylcarbamide) resulted in the development of porphyria in a patient who had ingested large quantities of the sedative (Duesberg, 1932), demonstrated that this drug was also porphyrogenic in rodents (Schmid & Schwartz, 1952). Subsequent investigation of various other allyl substituted compounds revealed that allylisopropylacetamide (AIA), a more soluble and less sedative congener of Sedormid, was very active in producing hepatic porphyria in rodents (Goldberg & Rimington, 1955). This compound has been extensively investigated and is now widely used as an experimental porphyrinogen. A further example of an experimental porphyrinogen developed on the basis of earlier clinical observations is hexachlorobenzene. Accidental ingestion of this compound which was developed as a wheat fungicide resulted in thousands of cases of hepatic porphyria with cutaneous manifestations (Schmid, 1960). Later laboratory studies confirmed that hexachlorobenzene, too, could produce a chemical porphyria in rodents (Ockner & Schmid, 1961). Griseofulvin, a clinically useful fungicidal antibiotic with some structural similarity to hexachlorobenzene has also been shown to produce an experimental chemical porphyria in rodents (De Matteis and Rimington, 1963), as have other compounds related to hexachlorobenzene.

One of the most potent and widely used experimental porphyrinogenic compounds is 1,4-dihydro-3,5-dicarbethoxy-collidine (DDC), a trimethylpyridine derivative which is unrelated to any common drug (Solomon & Figge, 1959)

Experimental porphyrinogenic compounds have been shown to reproduce the biochemical features of an attack of hepatic porphyria by causing marked induction of hepatic ALA-synthetase with overproduction, accumulation and massive excretion or porphyrins and porphyrin precursors in laboratory rodents and cell culture (De Matteis, 1971; Tschudy & Bonkowsky, 1972).

For any given member of this small group of compounds (Fig. 17) structure does seem critical in determing activity. For example with AIA and its analogues, the porphyrinogenicity of the molecule appears to be associated with the allyl moiety. Similarly, modifications of griseofulvin (such as its hydroxy ether analogue, or iso-griseofulvin) are unable to produce the chemical porphyria seen with the parent compound (Tschudy & Bonkowsky, 1972). However the experimental porphyrinogens are chemically diverse, and the separate members of this group of compounds do not share any obvious structural features which might account for the activity. Despite the lack of structural parallels between these potent porphyrinogenic compounds, they do share the property of lipid solubility, and thus are potential inducers of hepatic microsomal enzymes (Conney, 1967). Indeed, in addition to their well recognised effects involving heme synthesis, these compounds initiate extensive morphological and biochemical changes characteristic of hepatic microsomal enzyme induction, including tubular hypertrophy of the SER and increase in hepatic size and portein content (Sweeney et al, 1971); Tschudy & Bonkowsky, 1972). Interestingly, the non-porphyrinogenic analogues of these compounds retain the capacity to produce these striking effects on liver structure and function.

A first clue to understanding the mechanism of action of the potent porphyrinogens came from the recognition that all these compounds share the crucial property of (initially) lowering the intracellular concentration of hepatic heme and hemoproteins (such as cytochrome P450 and catalase) (Schmid & Schwartz, 1952; Meyer & Marver, 1971; De Matteis, 1971, 1973). Griseofulvin apparently produces this effect by inhibiting the activity of ferrochelatase, the terminal enzyme in heme biosynthesis, while AIA and its analogues accelerate the degradation of heme and hemoproteins to as yet unidentified green pigments. DDC appears to have effects on both ferrochelatase and heme degradation (for review of these findings see Tschudy & Bonkowsky, 1972).

The activity of allyl substituted porphyrinogenic compounds depends on transformation of the allyl moiety to an active metabolite by the liver microsomes. Experimental studies have shown that prior treatment of an animal with phenobarbitone which stimulates microsomal drug metabolism, enhances the capacity of subsequently administered AIA to degrade heme, presumably by increasing the activity of the microsomal mixed function oxidase system responsible for converting the allyl group to its active metabolite (De Matteis, 1973). Paradoxically, however, such prior treatment with enzyme inducing drugs diminishes the porphyrinogenic capacity of AIA (Kaufman et al, 1970).

Whatever the primary mode of action of an individual porphyrinogenic compound in diminishing the intracellular hepatic heme concentration, the consequences are the same, namely depression of heme and cytochrome p450 concentrations. Because of the role of heme in the feedback regulation of the pathway this may explain the massive reciprocal increase in ALA-synthetase (Fig. 16).

As barbiturates are the most frequent offenders in precipitating clinical attacks of acute porphyria in genetically predisposed subjects, they are widely used as the prototype drug in experimental studies. Like the various porphyrinogenic compounds already discussed, barbiturates and other drugs implicated in precipitating acute attacks in patients with inherited porphyria are structurally diverse. However, this varied group of drugs again shares with the experimental porphyrinogenic compounds the property of lipid solubility. Indeed, many of these drugs are recognised as potent inducers of cytochrome P450 and the non-specific hepatic microsomal mixed function oxidase system (Conney, 1967). Normally induction of cytochrome P450 by such drugs in laboratory animals is preceded by a slight rise in hepatic ALA-synthetase activity. This response has been interpreted as providing the additional heme required for increased synthesis of hemoproteins, and contrasts strikingly with the divergent effect of porphyrinogenic compounds on hepatic ALA-synthetase activity and cytochrome P450 concentration already mentioned.

Barbiturates do not cause clinical attacks of porphyria in normal subjects nor do they increase porphyrin or porphyrin precursor excretion in laboratory rodents. These and other common drugs, which are innocuous in normal individuals, but which provoke acute attacks in subjects with inherited hepatic porphyria, are thought to do so by eliciting a sustained increase in ALA-synthetase activity (Elder et al, 1972). Why they should cause this idiosyncratic reaction in patients with a genetically determined abnormality in heme synthesis has until recently been unexplained. Studies from various laboratories have indicated that a similar basic regulatory mechanism may account for massive induction of hepatic ALA-synthetase in both experimental porphyria and the human pharacogenetic disorder.

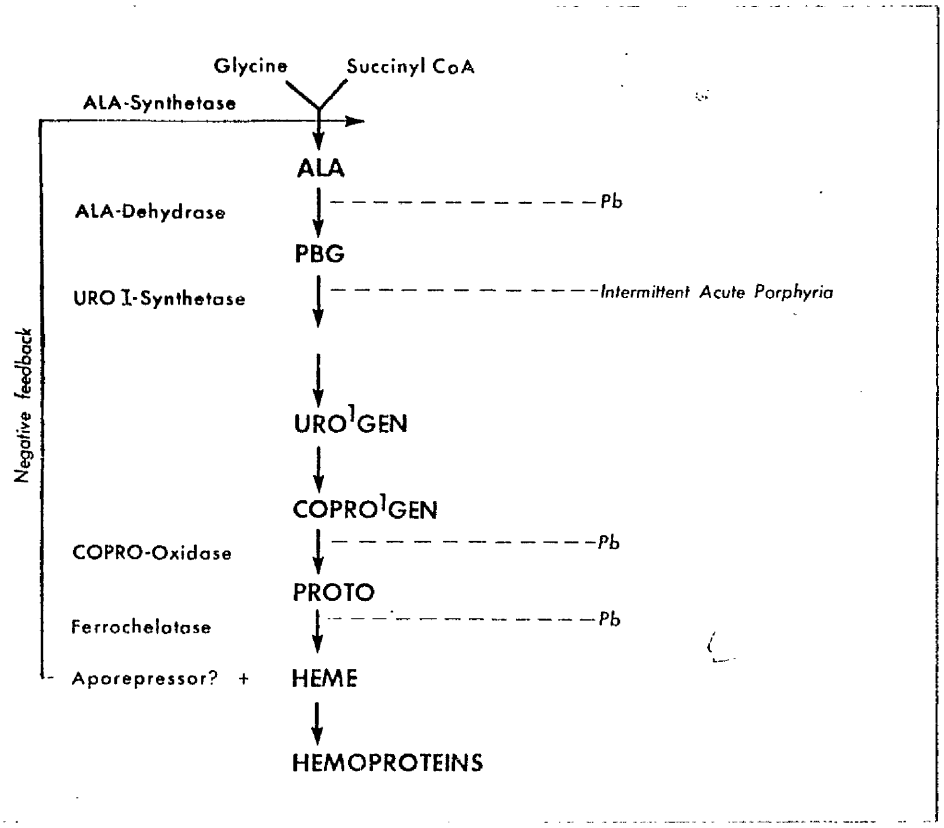
The subsequent sections of this chapter describe our laboratory studies on an experimental model for the exacerbation of hereditary hepatic porphyria by drugs. From these studies we have developed a hypothesis which may explain the idiosyncrasy to common drugs shown by susceptible patients and the development of experimental chemical porphyria in a variety of laboratory models.

(3) STUDIES WITH EXPERIMENTAL PARTIAL BLOCKS IN HEME SYNTHESIS

The commonest cause of acute porphyria in Europe and North America is intermittent acute porphyria (IAP) which is clinically characterised by intermittent attacks of neuro-psychiatric symptoms (Meyer & Schmid, 1977). As discussed earlier, these attacks are frequently precipitated by therapeutic doses of commonly used drugs and steroids, but the idiosyncrasy to these otherwise innocuous agents has remained unexplained. Acute attacks are associated with excessive urinary excretion of the porphyrin precursors δ-aminolaevulinic acid (ALA) and porphobilinogen (PBG), which appears to be a result of greatly increased activity of hepatic δ-aminolaevulinic acid synthetase (ALA-synthetase) (Tschudy et al, 1965; Strand et al, 1970; Sweeney et al, 1970) the initial and rate-controlling enzyme in heme biosynthesis (Fig. 16).

The present experimental studies were prompted by recent observations indicating that IAP is further characterised by a partial deficiency of uroporphyrinogen I synthetase (URO-synthetase) which catalyses the conversion of PBG to uroporphyrinogen (Fig. 16). Enzymatic studies in vitro (Strand et al, 1970; Miyagi et al, 1971; Meyer et al, 1972; Meyer, 1973; Sassa et al, 1974) and metabolic investigations in vivo (Meyer et al, 1972) suggest that this partial block of heme synthesis at the level of URO-synthetase represents the primary genetic defect which secondarily may lead to derepression of ALA-synthetase through negative feedback regulation.

Fig. 18



Schematic representation of haem biosynthesis showing proposed location of enzymatic defect in intermittent acute porphyria, and enzymatic steps inhibited by lead. The following abbreviations are used: ALA-synthetase (δ-amino laevulinic acid synthetase); ALA-dehydrase (δ-aminolaevulinic acid dehydrase): URO I-synthetase (uroporphyrinogen I-synthetase); COPRO-oxidase (coproporphyrinogen oxidase); ALA (δ-aminolaevulinic acid); PBG (porphobilinogen); URO GEN (uroporphyrinogen); COPRO GEN (coproporphyrinogen); PROTO (protoporphyrin); Pb (lead).

(a) Effect of lead on Hepatic δ-aminolaevulinic acid synthetase activity in the rat: A model for drug sensitivity in intermittent acute porphyria

In the present studies we tested the hypothesis that the unique sensitivity to many drugs, which is a characteristic feature of IAP (and the other hereditary hepatic porphyrias), may be a consequence of the primary defect in heme synthesis.

Partial inhibition of heme synthesis was produced experimentally in rats by lead, which inhibits several enzymes of the pathway (Fig. 18) including ALA-dehydratase, URO-synthetase and ferrochelatase (Lichtman & Feldman, 1963; Dresel & Falk, 1956; Kreimer-Birnbaum & Grinstein, 1965; Tephly et al, 1971; Goldberg, 1972; Piper & Tephly, 1974).

i. Methods: animals

Male Sprague-Dawley rats (130 - 180g) were fasted for 12 hours before, and during the course of the experiments. Lead (administered intravenously by tail vein injection and given as lead chloride, $PbCl_2$) and sodium phenobarbitone (administered intra-peritoneally) were dissolved in 0.9% sodium chloride and given in various doses as indicated in the lengends to Figs. 19 to 21. Cycloheximide was administered intraperitoneally and the appropriate dose to inhibit drug induced enzyme synthesis (2 mg/kg) was determined from preliminary survival experiments (Table 15) and from the inhibition of cytochrome P 450 synthesis after phenobarbitone (Fig. 23).

HEPATIC ENZYME STUDIES. At various times after treatment, as indicated in the legends to Figs 19-21 the animals were decapitated, the livers perfused in situ with cold isotonic saline, excised and homogenised in 0.25 M cold sucrose, and enzyme activities determined in homogenates and microsomes.

URINARY ALA EXCRETION. Fasted rats were kept for a further 24 hours in metabolic cages without food, but with water ad libitum. Urine was collected, and the total 24 hour urinary ALA excretion was determined (Fig. 25).

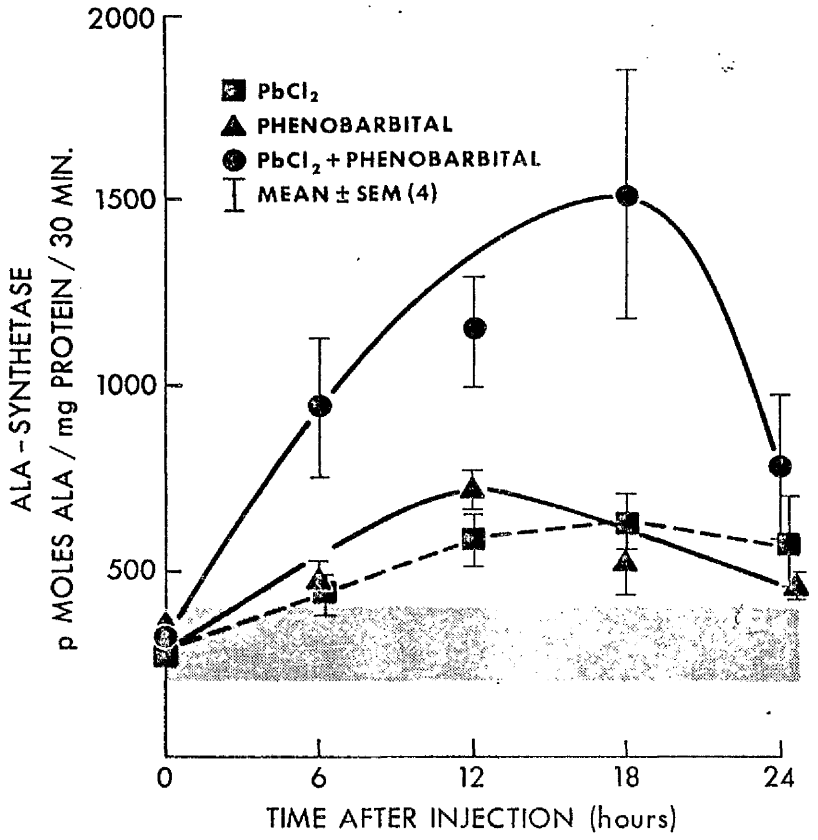
IN VIVO DRUG METABOLISM was assessed by Pentobarbital sleeping time (Borzecella & Manthei, 1957). Pentobarbital (20 mg/kg) was administered intra-peritoneally to various groups of control and treated rats as described in the legend to Table 16.

The time taken from injection of pentobarbital to return of the righting reflex was noted in two phases - "early", and "complete." The latter was taken as the end-point for determining sleeping time. The tests were all performed in a "blind" fashion with the observer unaware of the treatment category, or of the time of pentobarbital injection.

Analytical Methods

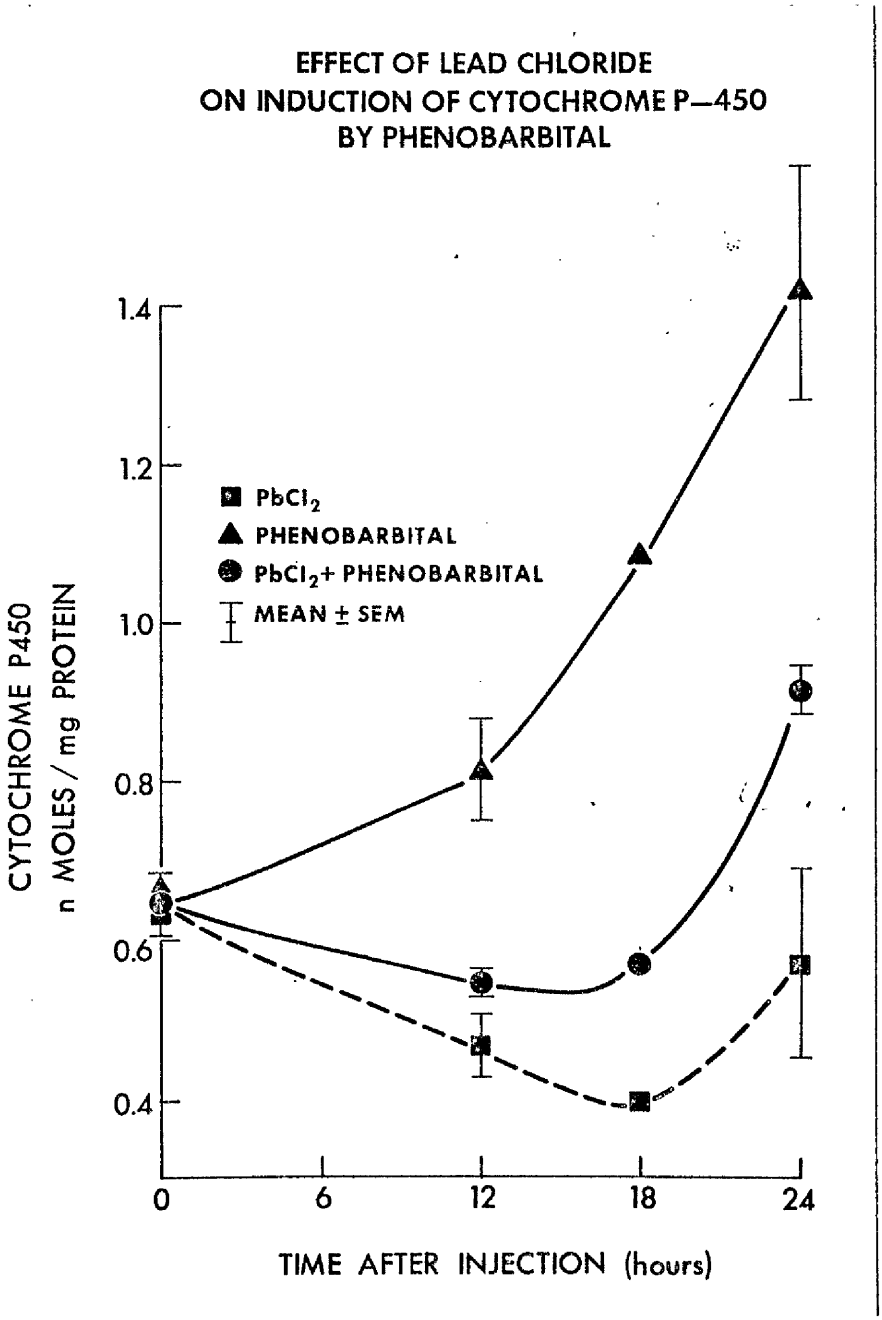
ALA-synthetase activity in liver homogenates was assayed by the incorporation of 1, 4 (14C) succinate into δ-aminolaevulinic acid, followed by isolation of the ALA-14C by three sequential chromatographic procedures (Strand et al, 1972A). Cytochrome P 450 content of liver microsomes was measured by the method of Omura & Sato (1964) using an Aminco DW-2 spectrophotometer. Paranitro anisole (pNA) - demethylase activity was determined in microsomes by the method of Netter & Seidel (1964). Protein concentration was measured by the method of Lowry et al (1951). Urinary ALA and PBG were estimated by the method of Davis & Andelman (1967) using disposable Bio-Rad Columns.

Fig. 19



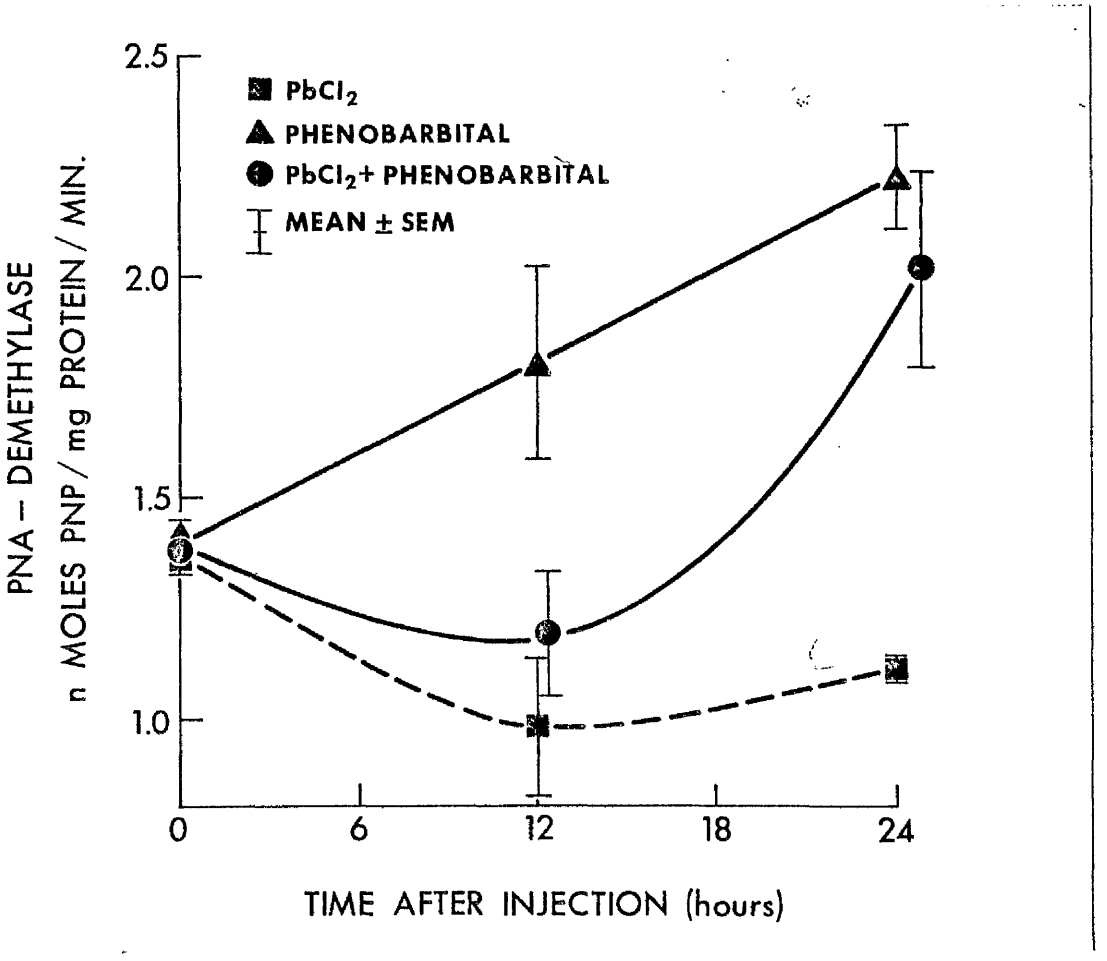
Hepatic ALA-synthetase activity up to 24 hours after a single injection of lead chloride (10 mg/kg intravenously), phenobarbitone (100 mg/kg intraperitoneally), and lead + phenobarbitone in combination. The shaded area indicates the control range for ALA-synthetase activity (mean ± SEM) in liver homogenates of rats injected with sodium chloride (154 mmol/L) and fasted between 12 and 36 hours.

Fig. 20



Microsomal cytochrome P450 concentration up to 24 hours after a single injection of lead chloride (10 mg/kg intravenously), phenobarbitone (100 mg/kg intraperitoneally), and lead + phenobarbitone in combination. Control values for microsomal cytochrome P 450 content (mean ± SEM) of rats injected with sodium chloride (154 mmol/L) are shown at zero time. No significant difference in cytochrome P450 content was found for rats fasted between 12 and 36 hours.

Fig. 21



P-nitroanisoledemethylase activity after a single injection of lead chloride (10 mg/kg intravenously), phenobarbitone (100 mg/kg intraperitoneally), and lead + phenobarbitone in combination. Control values for p-nitroanisole demethylase activity (mean ± SEM) of rats injected with sodium chloride (154 mmol/L) are shown at zero time. No significant difference in p-nitroanisole demethylase activity was found for rats fasted between 12 and 36 hours.

Table 15.

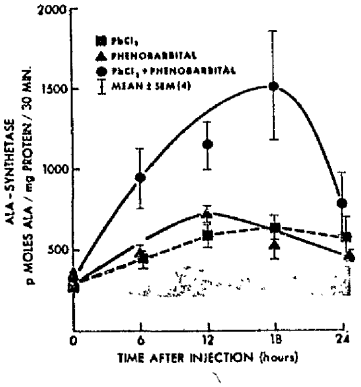
DOSE OF CYCLOHEXIMIDE (mg/kg)

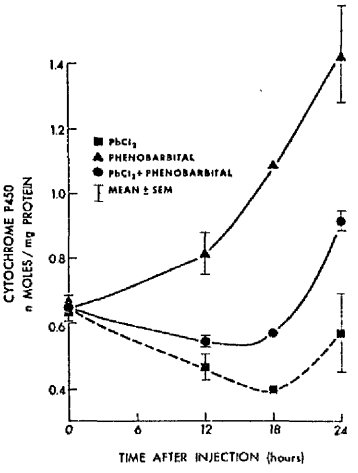
PROPORTION OF RATS SURVIVING (time in hours following injection)

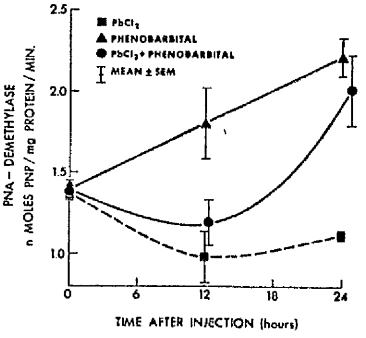
	50	10	5	2
0	Well 4/4	Well 4/4	Well 4/4	Well 4/4
3	Drowsy 4/4	Drowsy 4/4	Drowsy 4/4	Well 4/4
6	Drowsy/sick 3/4	Drowsy/sick 4/4	Drowsy 4/4	Well 4/4
9	0/4	1/4	Drowsy 3/4	Well 4/4
12	0/8	0/8	Sick 3/4	Well 4/4
24	0/8	0/8	0/4	Well 4/4

Survival of fasted male rats given phenobarbitone (100 mg/kg intraperitoneally) with cycloheximide in varying doses (2 to 50 mg/kg intraperitoneally) observed for up to 24 hours.

Fig. 22







Composite slide of Figs. 19 to 21. Phenobarbitone and lead chloride were injected at zero time to male rats fasted for 24 hours. Groups of rats were killed at the times indicated, and enzyme activity was determined in liver homogenates and microsomes. Results at each time point are expressed mean + SEM from at least 4 experiments each utilising pooled livers from 2 to 4 rats.

ii. Results

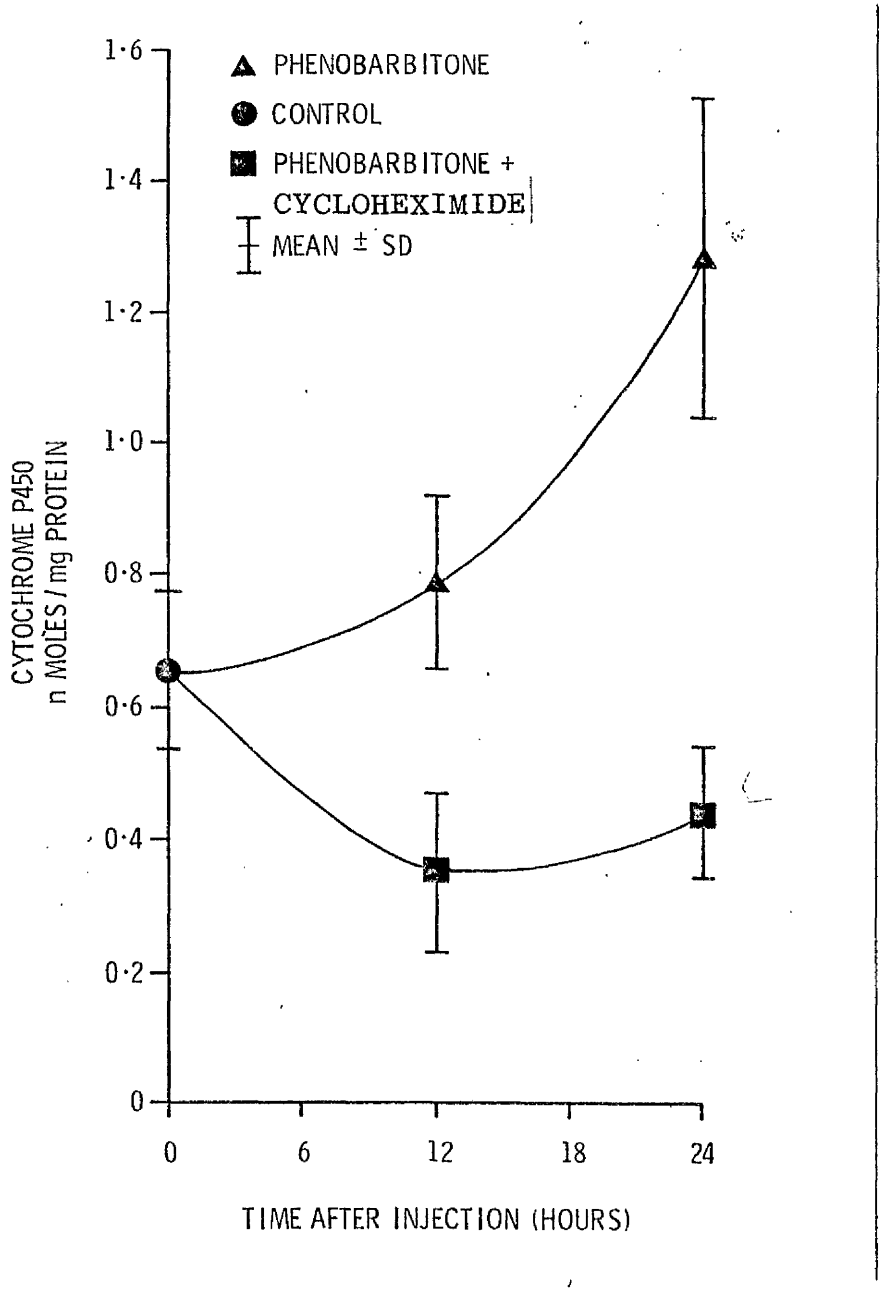
EFFECT of LEAD and PHENOBARBITONE on HEPATIC ALA-SYNTHETASE ACTIVITY

The effect of a single injection of lead or of phenobarbitone or of a combination of the two, on the activity of ALA-synthetase, the concentration of cytochrome P 450 and the pNA demethylase activity, are shown in Figs. 19 to 21 At these doses ($PbCl_2$ 10 mg/kg intravenously, phenobarbitone 100 mg/kg intraperitoneally) both lead and phenobarbitone caused a rise in ALA-synthetase activity which was maximal 12 - 18 hours after injection (121% and 82% above control for lead and phenobarbitone respectively). However, when phenobarbitone was given in combination with lead, induction of ALA-synthetase was greatly enhanced (430% above control). Cycloheximide (2 mg/kg), intraperitoneally administered 30 minutes before the injection of either lead, phenobarbitone, or the combination of lead and phenobarbitone, completed abolished the rise in ALA-synthetase activity (Fig 24).

Additional studies revealed that the potentiation of the effect of lead on drug mediated induction of ALA-synthetase activity was related both to the dose of lead and also to the dose of the inducing agent, phenobarbitone. Dose response studies for both phenobarbitone (5 to 100 mg/kg intraperitoneally), with and without lead (10 mg/kg intravenously), and lead (2.5 to 20 mg/kg intravenously), with and without phenobarbitone (100 mg/kg intraperitoneally) were performed.

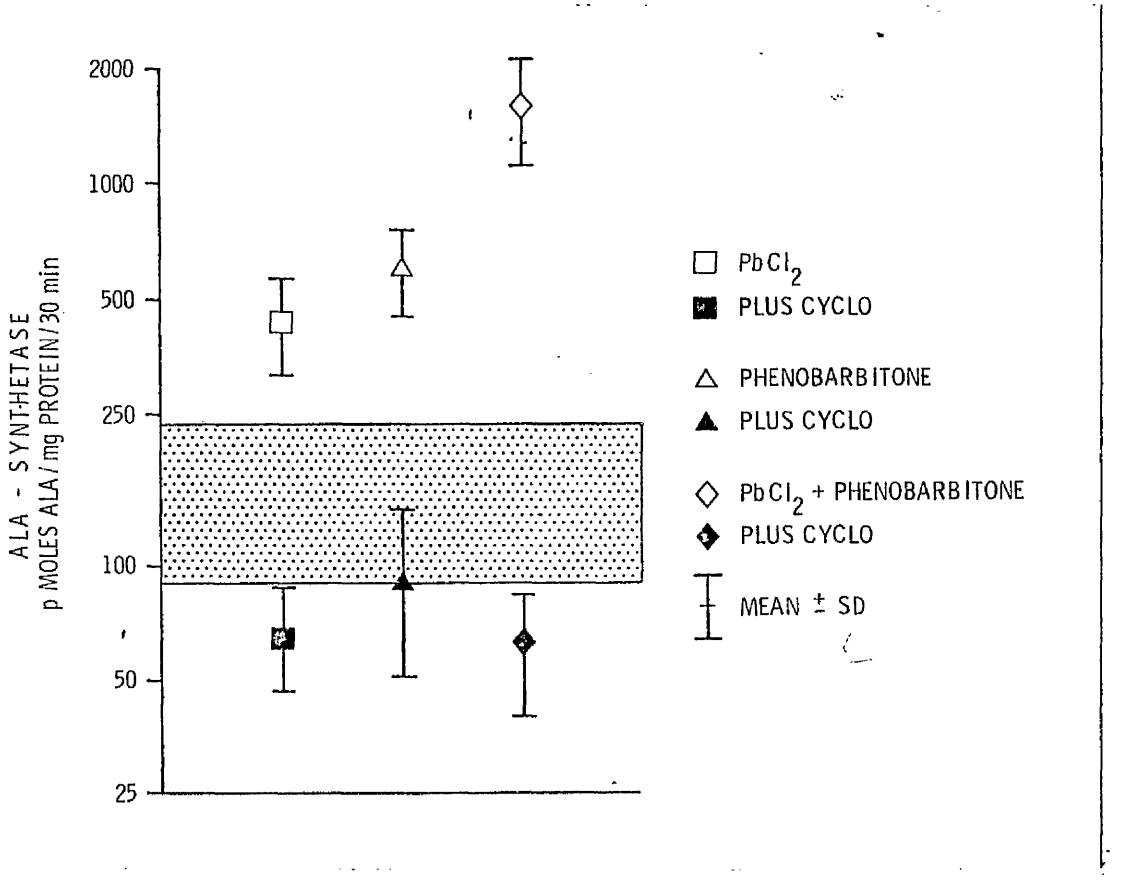
From the earlier time-response study of the effect of lead (10 mg/kg intravenously) and phenobarbitone (100 mg/kg intraperitoneally) on ALA-synthetase activity (Fig. 19) it was observed that maximum enzyme activity occurred approximately 12 hours after drug administration. This time was thus selected for the dose-response study illustrated in Fig. 26. , . although the assumption that maximal activity of ALA-synthetase occurred at this time for all doses of the drugs administered was not tested.

Fig. 23.



Microsomal cytochrome P450 concentration up to 24 hours after a single injection of phenobarbitone (100 mg/kg intraperitoneally ▲) alone, or in combination with cycloheximide (2 mg/kg intraperitoneally ■). Control range for microsomal cytochrome P450 concentration shown at zero time. ● All values mean ± SD.

Fig. 24.



Hepatic ALA-synthetase activity measured 12 hours after administration of lead chloride □ (10mg/kg intravenously), phenobarbitone △ (100 mg/kg intraperitoneally), and the combination of lead + phenobarbitone ◇, with and without cycloheximide (2 mg/kg intraperitoneally given 30 min before the other agents). Hatched area denotes control range (mean ± SD).

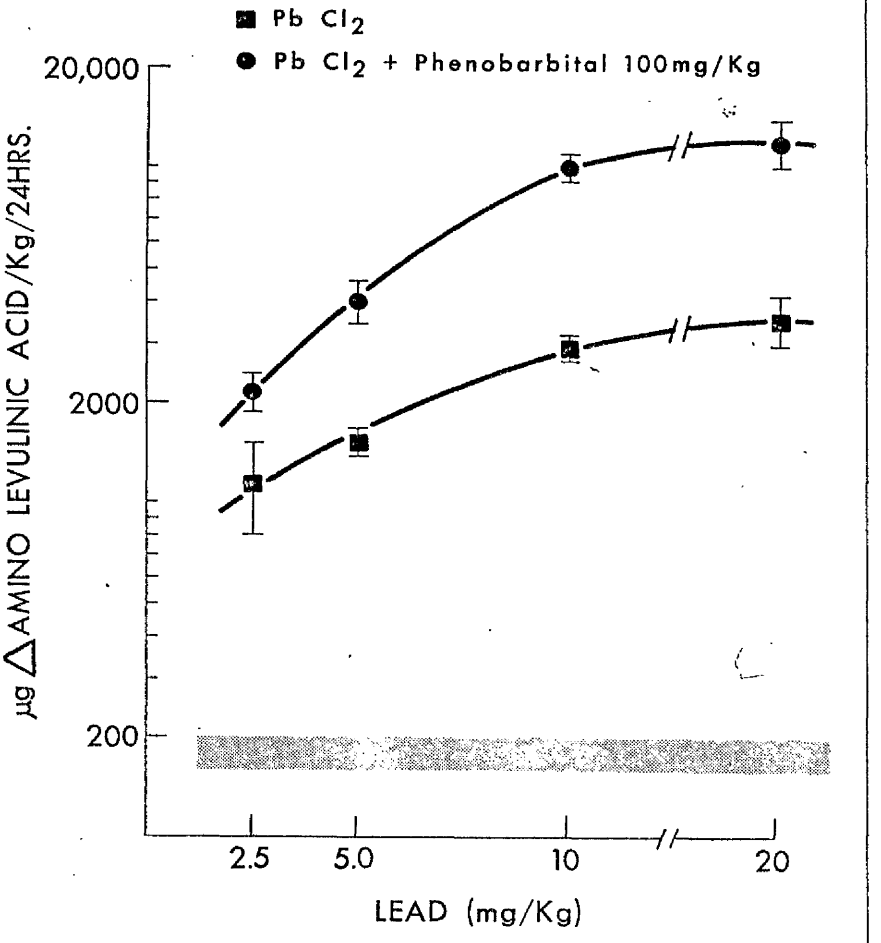
As is shown in Fig. 26 phenobarbitone in a dose of 5 mg/kg had no effect on the activity of ALA-synthetase either alone or in combination with lead. In doses of 10 and 25 mg/kg again there was no effect with phenobarbitone alone, but in rats in which heme synthesis was partially blocked by lead, these same doses caused marked induction of ALA-synthetase activity.

A similar synergistic effect of lead was observed with other barbiturates (aprobarbital, barbital, butabarbital, hexobarbital, pentobarbital, secobarbital and thiopental) all given in equimolar doses (100 umol/kg); antipyrine 25 mg/kg; chloral hydrate 100 mg/kg, and small doses of the porphyrogenic compound 2-allyl-2-isopropylacetamide (AIA). ALA-synthetase activity 12 hours after administration of AIA (100 mg/kg intraperitoneally) was 52% above the control value, while a 220% increase was seen when AIA was administered with lead.

EFFECT of LEAD and PHENOBARBITONE on URINARY ALA EXCRETION

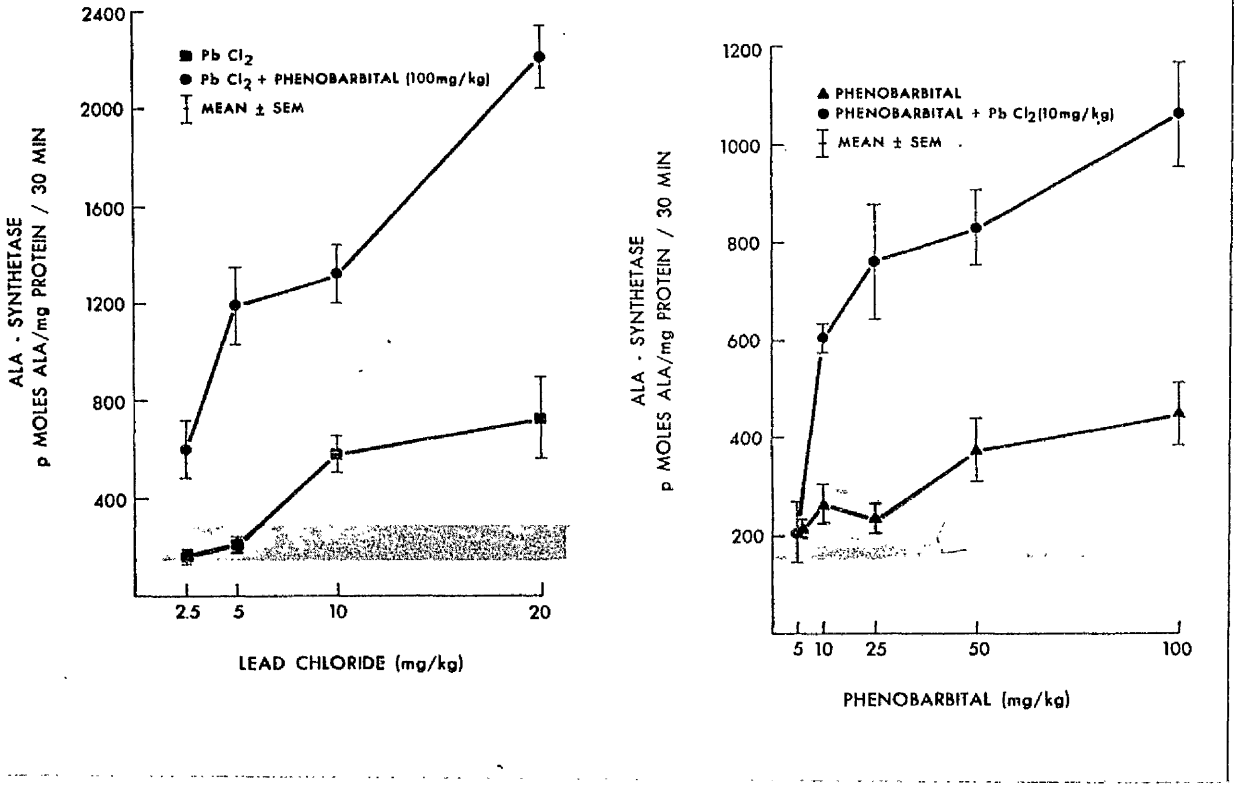
The effects of lead and phenobarbitone on hepatic ALA-synthetase activity were paralleled by similar changes in the urinary excretion of the porphyrin precursor ALA (Fig. 25). lead chloride in a single dose (10 mg/kg) intravenously caused a significant increase in the urinary excretion of ALA over the subsequent 24 hours, from the control value of 181 ± 25 mg/kg to 2925 ± 170 mg/kg/24 h (mean $\pm$ SEM). Phenobarbitone (100 mg/kg) intrperitoneally given alone had no effect on urinary ALA excretion, but when the drug was administered in combination with lead, ALA excretion was strikingly enhanced to 10230 ± 790 ug/kg/24 h. These effects on urinary ALA excretion (like those on hepatic ALA-synthetase activity) were dose related. PBG excretion was not detectably increased in treated animals.

Fig. 25



Dose-response study of the effect of lead (2.5 to 20 mg/kg intravenously) with and without phenobarbitone (100 mg/kg intraperitoneally) on the urinary excretion of δ-aminolaevulinic acid (ALA). Male rats were fasted for 24 hours before injection, and kept for a further 24 hours without food but with water ad libitum, in metabolic cages. Total ALA excretion in urine was measured and results expressed as mean ± SEM from at least 4 experiments. The shaded area indicates the normal range for urinary ALA excretion.

Fig. 26



Dose-response study of the effect of lead and phenobarbitone on hepatic ALA-synthetase activity. Dose-response for phenobarbitone (5 to 100 mg/kg intraperitoneally) in the presence and absence of lead (100 mg/kg intravenously) on the right; and for lead chloride (2.5 to 20 mg/kg intravenously), in the presence and absence of phenobarbitone (100 mg/kg intraperitoneally) on the left; Fasted male rats were injected and killed 12 hours later, and enzyme activity determined in liver homogenates. Results are expressed as means ± SEM from at least 4 experiments each utilising pooled livers from 2 to 4 rats. The shaded area indicates the normal range for ALA-synthetase activity in rat liver homogenates.

EFFECT of LEAD and PHENOBARBITONE on MICROSOMAL CYTOCHROME P 450 CONCENTRATION and ON DRUG METABOLISM *in vitro* and *in vivo*

Lead administration resulted in a significant initial fall in hepatic cytochrome P 450 concentration, and the combination of lead and phenobarbitone delayed and impaired the well-known adaptive rise in cytochrome P 450 concentration normally observed after phenobarbitone treatment. Microsomal pNA-demethylase activity paralleled cytochrome P 450 concentration under these three experimental conditions (Fig. 21). Pentobarbital sleeping time of rats given phenobarbitone and lead was significantly greater than that of control rats or rats given phenobarbitone alone (Table 16).

iii. Discussion

These findings suggest that while partial impairment of heme biosynthesis by small doses of lead has only a minor effect on ALA-synthetase activity it greatly enhances the sensitivity of ALA-synthetase to induction by drugs which when given alone have little or no inducing effect on the enzyme.

The massive induction of ALA-synthetase caused by lead and phenobarbitone in combination is reminiscent of the effect of potent porphyrogenic chemicals such as allylisopropylacetamide (AIA), 3,5-diethoxycarbonyl-1, 4-dihydro-2,4,6-trimethylpyridine (DDC) and griseofulvin. Their effect may be related in part to the decrease in the intracellular concentration of heme and cytochrome P 450 observed after administration of these chemicals (Wada et al, 1968; Waterfield et al, 1969; De Matteis, 1970; Meyer & Marver, 1971; Sweeney et al, 1972; Satyanarayana et al, 1972; De Matteis, 1973B; De Matteis & Gibbs, 1975).

Drug metabolism is further compromised by the failure of induction of cytochrome P 450 and drug metabolising enzymes normally seen in response to inducing drugs such as phenobarbitone (Fig. 20).

Table 16

SLEEPING TIME (min)

	Mean	SD	
CONTROL (n = 8)	53.7	19.2	
PHENOBARBITONE (n = 7)	60.6	12.2	N.S.
LEAD + PHENO (n = 6)	86.8	24.7	$p < 0.005$

Effect of phenobarbitone (100 mg/kg intraperitoneally) alone, and lead (10 mg/kg intravenously) + phenobarbitone (100 mg/kg intraperitoneally) on pentobarbitol sleeping time. Rats were fasted for 12 hours before injection, and after a further 12 hours pentobarbitol (40 mg/kg intraperitoneally) was administered, and sleeping time (complete return of righting reflex) determined in a "blind" fashion. Control rats received intravenous and/or intraperitoneal saline injection.

One consequence of impaired metabolism of an inducing agent in this setting is that its stimulatory effect on ALA-synthetase will be enhanced, as enzyme induction is a dose dependent phenomenon (Conney, 1967) and is influenced by the rate of biotransformation of the inducer in the target organ (Remmer, 1970). Inducers of experimental porphyria are detoxified by drug metabolising enzymes of the hepatic endoplasmic reticulum, and augmentation of this transformation reduces the bioavailability of the inducer and thereby its effectiveness (Kaufman et al, 1970). Conversely, slowing the metabolism of an inducing drug would increase its effectiveness as an inducer and thus may contribute to massive induction of ALA-synthetase. In the present studies (confirming the earlier reports of Alvares et al, 1972) and Scoppa et al (1973) administration of lead to rats impaired drug metabolism in vitro, and also in vivo as assessed by pentobarbitol sleeping time.

The idiosyncratic reaction to many common drugs in patients with the hereditary hepatic porphyrias has naturally inhibited clinical studies of drug metabolism in this group of disorders. However it is of interest that two recent clinical studies demonstrated impaired hydroxylation of salicylamide and antipyrine in patients with overt IAP, while glucuronidation (phase II drug metabolism) was unaffected. (Song et al, 1974; Anderson et al, 1976).

(4) COMMON BASIS FOR HUMAN AND EXPERIMENTAL PORPHYRIA

The experimental studies described above have shown that while partial impairman of heme biosynthesis in laboratory rodents has only minor effects on ALA-synthetase activity, it greatly enhances the sensitivity of ALA-synthetase to induction by a variety of drugs and steroids which when given alone, have little or no inducing effect on the enzyme. Curiously however such drugs are by themselves capable of causing striking induction of ALA-synthetase and porphyrin accumulation in cultured chick embryo liver cells (Granick, 1966). The chick embryo liver system thus appears to be a model for human inherited hepatic porphyria and has been used to screen for drugs potentially dangerous to porphyria subjects (Rifkind et al, 1973). In the chick embryo liver, the stimulatory effect on ALA-synthetase activity appears to be related to the lipid solubility of the test drug (De Matteis, 1971).

(a) Dual Requirements for Massive Induction of Hepatic δ-Aminolaevulinic Acid Synthetase

A wide variety of drugs have been implicated in precipitating acute attacks in patients with hereditary hepatic porphyrias (Table 14 ; Goldberg, 1959; Taddeini & Watson, 1968; Stein & Tschudy, 1970; Eales, 1971). Although of diverse chemical structure, they have in common the general property of lipid solubility. Many of these compounds induce the microsomal hemoprotein, cytochrome P450, an effect which is preceded by a modest increase in ALA-synthetase activity, presumably to provide additional heme for hemoprotein synthesis. However, even at high concentration, administration of these drugs to animals is not accompanied by porphyrin accumulation nor do they result in a porphyria-like syndrome in normal subjects.

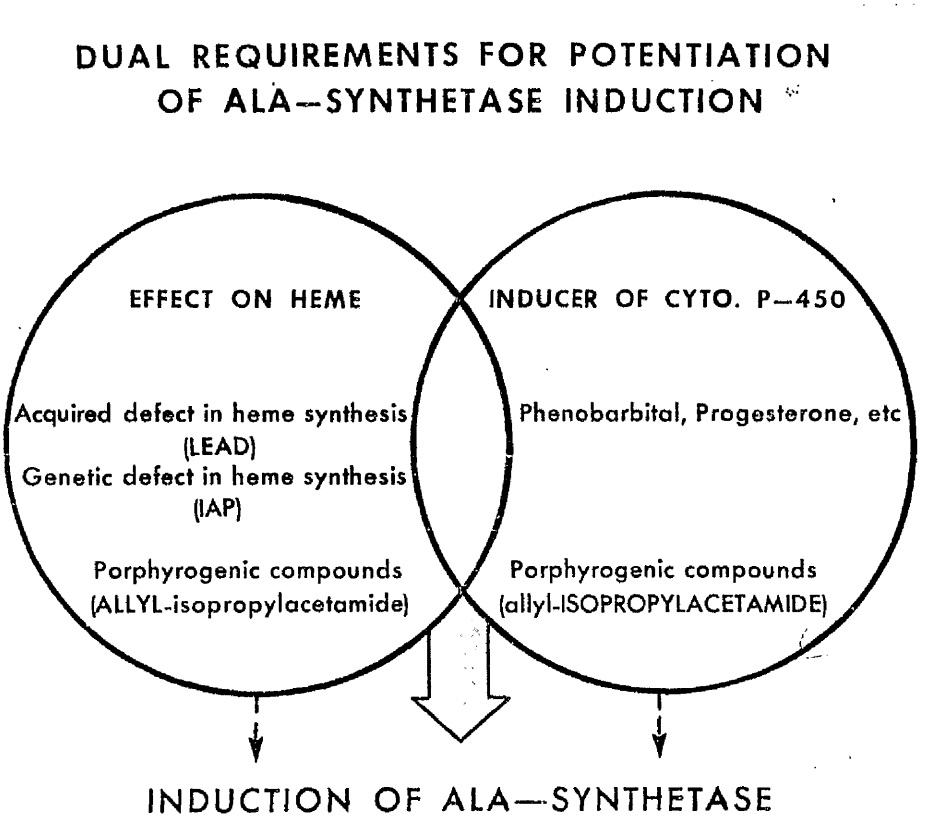
In marked contrast to this large number of commonly used drugs is the small group of compounds, including AIA, DDC, and griseofulvin, which cause massive induction of ALA-synthetase and produce a biochemical situation in rodents mimicking human hepatic porphyria. (Fig. 17).

Interestingly, these lipid soluble compounds, although apparently structurally unrelated, all interfere with the intracellular concentration of heme, either by inhibiting enzymatic steps in heme biosynthesis (for example, DDC; griseofulvin) or by accelerating breakdown of heme and hemoproteins (for example, AIA) resulting in initial falls in cytochrome P450 concentration. By decreasing intracellular heme, these chemicals may affect ALA-synthetase activity, at least in part, by interfering with the feedback regulation of this enzyme. (Sassa & Granick, 1970; Strand et al, 1972B; Sinclair & Granick, 1975).

A unifying explanation both for the drug idiosyncrasy seen in the hereditary hepatic porphyrias, and for the mode of action of the potent porphyrinogenic agents in laboratory animals, is suggested by the studies presented above. Thus, the mechanism of action of the potent porphyrinogenic compounds may be analogous to the combination of lead and phenobarbitone, in that they all possess the dual properties of affecting heme (by inhibiting its synthesis or increasing its breakdown) and having the lipid solubility characteristics of inducers of ALA-synthetase and of microsomal enzymes. Indeed a similar potentiation of ALA-synthetase activity has recently been observed when phenobarbitone or phenylbutazone were administered together with small doses of DDC or AIA (De Matteis & Gibbs, 1972; Bock et al, 1973; Padmanaban et al, 1973).

Increased activity of hepatic ALA-synthetase with consequent overproduction of porphyrins and/or porphyrin precursors (features common to both the acute attacks of hereditary hepatic porphyria and experimental chemical porphyria) appears to result from the coexistence of two separate requirements, each of which alone may have only moderate or no effect on the enzyme. These are a) an effect on heme synthesis or degradation, and b) exposure to lipid soluble inducers of hemoprotein synthesis, such as certain drugs, and possibly endogenous substances (Fig 27). Thus experimental reduction of a certain pool of intracellular heme, for example when heme synthesis is partially blocked by lead, may moderately induce ALA synthetase.

Fig. 27.



Dual requirements for potentiation of the induction of hepatic ALA-synthetase.

Likewise phenobarbitone and other inducers of hemoprotein cytochrome P450 may also moderately stimulate the activity of this enzymes, but without affecting the porphyrin or porphyrin precursor excretion.

However, when both factors are present together, a synergistic effect is seen with massive induction of ALA-synthetase (depicted by the broad arrow in Fig.27), which is accompanied by greatly enhanced excretion of precursors (Maxwell and Meyer, 1974,1976).

The analogy with IAP is clear, as in this clinical setting, defective adaption of heme synthesis is a consequence of the intrinsic genetic disorder. It has been proposed that the partial defect in heme synthesis in IAP may result in derepression of ALA-synthetase through negative feedback regulation, and that because of the kinetic properties of the enzymatic steps following ALA-synthetase, the increase in PBG production may be sufficient to partially overcome the defect in heme synthesis (Meyer and Schmid, 1973). Thus, despite the partial block, the "effective" heme concentration and heme available for basal hemoprotein synthesis may approach normal levels, provided ALA-synthetase, and, therefore, heme synthesis remains induced. Such a "compensated" block may reflect the situation in a patient with "latent" prophyria where clinical symptoms are absent and precursor excretion in the urine may be negligible. However, this precarious equilibrium is readily disturbed by any of a large number of drugs (and possibly naturally occurring endogenous inducers) which have little or no effect of ALA-synthetase in the normal hepatocyte but may precipitate "acute porphyria" in individuals with a genetic defect in heme synthesis.

The exact mechanism of increased sensitivity of ALA-synthetase to induction in the presence of partial inhibition of heme synthesis remains to be determined but it appears to be related to the negative feedback regulation of heme synthesis. Thus, the inability of an hepatocyte with compromised heme synthesis to respond appropriately to a sudden demand for increased synthesis of heme (such as is created by inducers of hemoprotein cytochrome P450) would result in persistence of the regulatory feedback signal and hence exaggerated stimulation of ALA-synthetase activity. Recent studies (Correia and Meyer, 1975) have shown that partial enzymatic blocks in heme synthesis disturb the

normally closely coordinated synthesis of mitochondrial heme and microsomal apoprotein during induction of cytochrome P450, and result in a relative excess of free apocytochrome P450 in the liver. Further studies have suggested that increased synthesis of apocytochrome P450 is the primary event in drug-mediated induction of holocytochrome P450 (Correia and Meyer, 1975; Rajamanickam et al., 1975). This has provided support to the speculation that free apocytochrome P450 may quantitatively reflect the regulatory signal for induction of ALA-synthetase by drugs. Massive induction of ALA-synthetase by inducers of cytochrome P450 in the presence of partial blocks in heme synthesis may represent an attempt to overcome the block and provide the heme required for combination with free apocytochrome P450. Whether the regulatory signal for synthesis of ALA-synthetase is mediated directly via free apoprotein concentration ("positive feedback") (Padmanaban et al., 1973) or in response to the prevailing deficiency of a certain heme pool ("negative feedback") (De Matteis and Gibbs, 1972) or by some other mechanism, remains to be determined.

The recent clinical studies indicating impaired hepatic biotransformation of salicylamide and antipyrine in patients with IAP (Song et al., 1974; Anderson et al., 1976) raises the possibility that impaired hepatic biotransformation may contribute to the drug idiosyncrasy in hepatic porphyria. Our experimental studies were consistent with these observations as the in vitro studies in lead treated rats showed impaired demethylation of paranitroanisole (Maxwell & Meyer, 1976), and pentobarbital sleeping times were prolonged. One consequence of impaired metabolism of an inducing agent is that its stimulatory effect on ALA-synthetase activity will be enhanced as enzyme induction is a dose-dependent phenomenon and may be influenced by the rate of biotransformation of the inducer in the target organ. Slowing the metabolism of an inducing drug would increase its effectiveness as an inducer, and thus may contribute to the massive induction of ALA-synthetase.

The scheme depicted in Fig 27 may also be relevant to an understanding of the mechanism of action of potent experimental porphyrinogenic compounds such as AIA, DDC and griseofulvin. These compounds may functionally be analogous to the experimental combination of lead and phenobarbital in

that each possesses in a single molecule the dual properties for massive induction of ALA-synthetase. This duality is most readily apparent with allyl substituted compounds where the allyl group is responsible for the effect on heme, while the remainder of the lipid soluble molecule may provide the inducing moiety.

The paradoxical effect of prior exposure of an experimental animal to enzyme inducing drugs in enhancing heme degradation, but at the same time diminishing the porphyrinogenic effect of subsequently administered AIA (Kaufman et al., 1970; De Matteis, 1973a; Schmid, 1973) can now also be understood on a rational basis. Thus, while microsomal enzyme induction will enhance the effect of AIA on heme degradation by accelerating the biotransformation of the allyl moiety to the active metabolite responsible for this effect (De Matteis, 1971b) it also accelerates the metabolism of the remainder of the molecule (the inducing moiety) (Kaufman et al., 1970) reducing its availability and thus its inducing potential. This latter effect apparently negates the former, and could account for the net reduction in ALA-synthetase activity observed.

Finally, the concept of dual requirements for massive induction of ALA-synthetase and consequent porphyrin and/or precursor excretion may also explain why the chick embryo liver provides a model for the drug sensitivity of hereditary hepatic porphyria. Recent observations indicate that there may be relative defects in heme synthesis in chick embryo liver cells in culture (Doss, 1971; De Matteis, 1973c). Such defects in heme synthesis (analogous to the genetic defect in IAP or that experimentally produced in rats by lead) could explain the ready induction of ALA-synthetase and porphyrin accumulation in response to barbiturates and other common drugs in the chick embryo liver. Interestingly in this system the effect of drugs appears to be most strongly correlated with their lipid solubility (De Matteis, 1971)

(b) Role of Enzyme Inducing Agents in Acute Human Porphyria

The laboratory studies described in the earlier section of this chapter are of some practical clinical relevance. Many of the previously unexplained features of the hereditary hepatic porphyrias can now be understood on a mechanistic basis. In particular the "idiosyncratic" reaction to many drugs which is such a characteristic feature of the hereditary hepatic porphyrias can be seen now to be a direct consequence of the primary genetic defect in porphyrin and heme synthesis. Clearly, drugs with enzyme-inducing properties (Table 14) pose a major threat to these patients and should be avoided. Thus acute attacks of porphyria can be regarded as another adverse effect of enzyme induction (in genetically predisposed individuals).

A necessary corollary to this definition of the hereditary hepatic porphyrias as a group of pharmacogenetic disorders of heme synthesis characterised by a unique sensitivity to enzyme inducing agents is the recognition that those drugs which may be safely used in this group of patients are relatively weak or ineffective inducers of microsomal haemoproteins (Table 17). (Maxwell, 1976). A further consequence of the dual requirements underlying massive induction of hepatic ALA-synthetase is the recognition that it is inappropriate to screen drugs as safe for use in patients with inherited hepatic porphyria simply on the basis of their failure to affect ALA-synthetase activity in laboratory animals with an intact heme pathway, as for example in the report of Beattie et al., (1973).

While these recent studies have given some insight into the basis of these pharmacogenetic disorders of heme synthesis, the concept of dual requirements for massive induction of ALA-synthetase in IAP, HCP and VP is necessarily an oversimplification. In an individual with the underlying genetic disorder, response to drug administration is unpredictable, and it is likely that a number of factors will determine whether or not an acute attack will be precipitated.

Table 17

ANALGESICS	Aspirin; morphine and related opiates; Paracetamol
ANTIBIOTICS	Penicillin
ANTIHISTAMINES	Diphenhydramine; Mepyramine
CARDIOVASCULAR	Digoxin; Propranolol
HYPOTENSIVES	Bethanidine; Guanethidine; Propranolol
MISCELLANEOUS	Atropine; Neostigmine/Prostigmine; Vitamins B, C, E.

Some drugs considered to be safe (or probably safe) in the hereditary hepatic porphyrias.

These include the genetically determined activity of the defective enzyme, dose of drug absorbed and its inducing potential. A variety of other factors such as the hormonal environment and nutritional state ("glucose effect" Bonkowsky et al., 1973) have been shown to influence the inducibility of mitochondrial ALA-synthetase and there is considerable evidence that the inducibility of the microsomal P450 system is to a large extent genetically determined (Vesell, 1975). Moreover, the apparent insensitivity of patients with PCT to the effects of enzyme inducing drugs is puzzling. However, the explanation for this discrepancy, as suggested earlier, seems to lie in the fact that in this form of hereditary hepatic porphyria, the partial enzymatic defect is insufficient to affect the feedback-regulation of ALA-synthetase by heme. Hence ALA-synthetase activity and its inducibility remain unaltered. Finally, our explanation for the pathogenesis of this group of pharmacogenetic disorders requires that the development of the neurological syndrome is directly related to massive induction of ALA-synthetase and consequent accumulation of porphyrin precursors, for which there is as yet only indirect evidence (Meyer and Schmid, 1974).

REFERENCES

AARTS, E. M. (1965) Evidence for the function of D-glucaric acid as an indicator for drug induced enhanced metabolism through the glucuronic acid pathway in man.
Biochemical Pharmacology 14, 359

AGGELER, P. M., O'REILLY, R. A., LEONG, L., KOWEY, P. E. (1967) Potentiation of the anticoagulant effect of warfarin by phenylbutazone.
New England Journal of Medicine 276, 496

AGGELER, P. M., O'REILLY, R. A. (1969) Effect of heptabarbital on the response to bishydroxycoumarin in man.
Journal of Laboratory and Clinical Medicine 74, 229

ALEXANDERSON, B., EVANS, D. A. P., SJOQUIST, F. (1969) Steady state plasma levels of nortriptyline in twins: influence of genetic factors and drug therapy.
British Medical Journal 4, 764

ALVARES, A. P., SCHILLING, G., LEVIN, W., KUNTZMAN, R., (1967) Studies on the induction of Co-binding pigments in liver microsomes by phenobarbital and 3-methyl cholanthrene.
Biochemical and Biophysical Research Communications 29, 521

ALVARES, A. P., SCHILLING, G., LEVIN, W., KUNTZMAN, R., BRAND, L., MARK, L., (1969) Cytochromes P 450 and b5 in human liver microsomes
Clinical Pharmacology and Therapeutics 10, 655

ALVARES, A. P., LEIGH, S., COHN, J., KAPPAS, A., (1972) Lead and methyl mercury: effects of acute exposure on cytochrome P 450 and the mixed function oxidase system in the liver.
Journal of Experimental Medicine 135, 1406

AMERICAN ACADEMY OF PEDIATRICS (1963) Committee on nutrition: the prophylactic requirements and toxicity of vitamin D.
Pediatrics 31, 512

AMOROSO, E. C., PORTER, D. G. (1970) Endocrine Functions of the Placenta
In "Scientific Foundations of Obstetrics and Gynaecology" Edited by E. E. Philipp, J. Barnes and M. Newton. William Heinemann Medical Books, Ltd., London. p 556

ANDERS, M. W., MAINWERING, G. J. (1966) Inhibition of Drug Metabolism.
Molecular Pharmacology 2, 319

ANDERSON, K. E., ALVARES, A. P., SASSA, S., KAPPAS, A. (1976) Studies in porphyria. V. Drug oxidation rates in hereditary hepatic porphyria.
Clin. Pharm. and Therap. 19, 47

APONTE, C. J., PETRELLI, M. P. (1973) Anticonvulsants and vitamin D metabolism.
Journal of American Medical Association 225, 1248

APPLEGARTH, D. A., DUNN, H. G. (1972) Failure of Enzymatic Induction.
New England Journal of Medicine 287, 148

ARIAS, I. M. (1961) Ethereal and N-linked glucuronide formation by normal and Gunn rats in vitro and in vivo.
Biochemical and Biophysical Research Communications 6, 81

ARIAS, I. M., JOHNSON, L., WOLFSON, S. (1961) Biliary excretion of injected conjugated and unconjugated bilirubin by normal and Gunn rats
American Journal of Physiology 200, 1091

ARIAS, I. M., DOYLE, D., SCHIMKE, R. T. (1969) Studies on the synthesis and degradation of proteins of the endoplasmic reticulum of rat liver
Journal of Biological Chemistry 244, 12 and 3303

ARIAS, I. M., GARTNER, L. M., COHEN, M., BEN-EZZER, J., LEVI, A. J. (1969) Chronic non-hemolytic unconjugated hyperbilirubinemia with glucuronyl transferase deficiency. Clinical biochemical, pharmacologic and genetic evidence for heterogeneity
American Journal of Medicine 47, 395

AVANT, G. R., KLOTZ, U., ATKINSON, G. R., HOYUMPA, A., SCHENKER, S., (1974) Altered disposition of diazepam in liver disease.
Journal of Clinical Investigation 53, 2A

BARKA, T., POPPER, H. (1967) Liver enlargement and drug toxicity.
Medicine (Baltimore) 46, 103

BARRY, J. E., DANKS, D. M., (1974) Anticonvulsants and congenital abnormalities.
Lancet 2, 48

BAUER, S., DE VINO, T. (1969)
Technicon International Congress 3, 31

BAUGH, C. M., KRUMDIECK, C. L. (1969) Effects of phenytoin on folic acid conjugase in man
Lancet 2, 519

BAYLIS, G. N., CROWLEY, J. M., PREECE, J. M., SYLVESTER, P. E., MARKS, V. (1971) Influence of folic acid on blood phenytoin levels. Lancet 1, 62

BEATTIE, A. D., MOORE, M. R., GOLDBERG, A., WARD, R. L. (1973) Acute intermittent porphyria: Response of tachycardia and hypertension to propranolol
British Medical Journal 3, 257

BENN, A., SWAN, C. H. J., COOKE, W. T., BLAIR, J. A., MATTY, A. J., SMITH, M. E., (1971) Effect of intraluminal pH on the absorption of pteroyl monoglutamic acid.
British Medical Journal 1, 148

BERMAN, R. E., FISHER, D. E. (1970) Phenobarbital for neonatal jaundice
Journal of Pediatrics 76, 945

BERK, P. D., MARTIN, J. F., BLASCHKE, T. F., SCHARSCHMIDT, B. F., PLOTZ, P. H. (1975) Unconjugated hyperbilirubinaemia: physiologic evaluation and experimental approaches to therapy.
Annals of Internal Medicine 82, 552

BERNHEIM, F. (1941) The reduction of neoprontosil by tissues in vitro.
Journal of Pharmacology and Experimental Therapeutics 71, 344

BERNSTEIN, L. H., CUTSTEIN, S., WEINER, C. V. (1970) γ-glutamyl carboxypeptidase (conjugase), the folic acid releasing enzyme of intestinal mucosa
American Journal of Clinical Nutrition 23, 919

BERTHELOT, P., ERLINGER, S., DHUMEAUX, D., PREAUX, A. M. (1970) Mechanisms of phenobarbital-induced hypercholeresis in the rat
American Journal of Physiology 219, 809

BERTINO, J. R., BOOTH, B. A., BIEBER, A. L., BASHORE, A., SARTORELLI, A. C., (1964) Studies on the inhibition of dihydrofolate antagonists
Journal of Biological Chemistry 239, 479

BILLING, B. H., BLACK, M. (1971) The action of drugs on bilirubin metabolism in man
Annals of the New York Academy of Sciences 179, 403

BLACK, M., SHERLOCK, S. (1970) Treatment of Gilbert's Syndrome with phenobarbitone
Lancet 1, 1359

BLASCHKE, T. F., BERK, P. D. (1972) Augmentation of bilirubin UDP-glucuronyl transferase activity in rat liver homogenates by glutethimide.
Proceedings of the Society for Experimental Biology and Medicine 140, 1315

BLASCHKE, T. F., BERK, P. D., RODKEY, F. L., SCHARSCHMIDT, B. F., COLLISON, H. A., WAGGONER, J. G., (1974) Drugs and the Liver I. Effects of glutethimide and phenobarbital on hepatic bilirubin clearance, plasma bilirubin turnover and carbon monoxide production in man.
Biochemical Pharmacology 23, 2795

BOCK, K. W., WEINER, R., FROHLING, W. (1973) Regulation of δ-aminolaevulinate synthetase by steroids and drugs in isolated perfused rat liver.
Enzyme 16, 295

BOLT, H. M., KAPPUS, H., BOLT, M. (1974) Rifampicin and oral contraception.
Lancet 1, 1280

BONICKE, R., LISBOA, B. P. (1957) Uber die erbbedingtheit der intraindividuellen konstanz der Isoniazid ausscheidung beim menschen.
Natur wissenschaften 44, 314

BONKOWSKY, H. L., COLLINS, A., DOHERTY, J. M., TSCHUDY, D. P. (1973) The glucose effect in rat liver.
Biochimica et Biophysica Acta 320, 561

BORZECELLA, J. F., MANTHEI, R. W. (1957) Factors influencing pentobarbital sleeping time in mice.
Archives Internationales de Pharmacodynamie et de Therapie III, 296

BOYER, J. L. (1971) Phenobarbital enhancement of bile flow.
Gastroenterology 60, 614

BOYER, J. L., BLOOMER, J. R., MADDREY, W. C. (1973) Estimates of canalicular bile secretion and its determinants in man utilising the biliary clearance of ^{14}C mannitol.
Journal of Clinical Investigation 52, 11A

BOYLE, I. T., MIRAVET, L., GRAY, R. W., HOLICK, M. F., DE LUCA, H. F. (1972) The response of intestinal calcium transport to 25-hydroxy and 1,25-dihydroxy vitamin D in nephrectomised rats.
Endocrinology 90, 605

BRANCH, R. A., HERBERT, C. M., READ, A. E. (1973) Determinants of serum antipyrine half-lives in patients with liver disease.
Gut 14, 569

BRANCH, R. A., HIES, A. S., SWAND, D. G. (1973) The disposition of propranolol VIII. General implications of the effects of liver blood flow on elimination from the perfused rat liver.
Drug Metabolism and Disposition 1, 687

BRANCH, R. A., JAMES, J. A., READ, A. E. (1974) The influence of chronic liver disease on the elimination of d-propranolol, antipyrine and indocyanine green.
Gut 15, 837

BRAUND, D. G., LANGLOIS, B. E., CONNER, D. J., MOORE, E. E. (1971) Feeding phenobarbital and activated carbon to accelerate dieldrin residue removal in a contaminated dairy herd.
Journal of Dairy Science 54, 435

BRECKENRIDGE, A., ORME, M. (1971) Clinical implications of enzyme induction.
Annals of the New York Academy of Sciences 179, 421

BRECKENRIDGE, A., ORME, M. L. E., THORGEIRSSON, S., DAVIES, D. S., BROOKS, R. V. (1971) Drug interactions with warfarin; Studies with Dichloralphenazone, Chloral hydrate and phenazone.
Clinical Science 40, 351

BRECKENRIDGE, A., ORME, M. L. E., DAVIES, L., THORGEIRSSON, S. S., DAVIES, D. S. (1973) Dose dependent enzyme induction.
Clinical Pharmacology and Therapeutics 14, 514

BRODIE, B. B., AXELROD, J. (1950) The fate of antipyrine in man.
Journal of Pharmacology and Experimental Therapeutics 98, 97

BRODIE, B. B., AXELROD, J., COOPER, J. R., GAUDETTE, L., LA DU, B. N., MITOMA, C., OREINFRIEND, S. (1955) Detoxification of drugs and other foreign compounds by liver microsomes.
Science 121, 603

BRODIE, B. B., GILETTE, J. R., LA DU, B. N., (1958) Enzymatic metabolism of drugs and other foreign compounds.
Annual Review of Biochemistry 27, 427

BRODIE, B. B., BURNS, J. J., WEINER, M. (1959) Metabolism of drugs in subjects with Laennec's Cirrhosis.
Medicina Experimentalis 1, 290

BRODIE, B. B. (1964) Distribution and fate of drug: Therapeutic implications.
In Absorption and Distribution of Drugs. ed. T. B. Binns, pp 199 - 255, Williams and Wilkins, Baltimore, Md.

BRODIE, B. B., [illegible], A. K., [illegible], G., [illegible], W. D., (1971) Drug metabolism in man: past, present and future.
Annals of the New York Academy of Sciences 179, 11

BROOKS, S. M., [illegible], E. E., [illegible], G. J., SULLIVAN, I., [illegible], K. (1972) Adverse effects of phenobarbital on corticosteroid metabolism in patients with bronchial asthma.
New England Journal of Medicine 286, 1125

BROWN, A. T., [illegible], W. W. (1958) Studies on the neonatal development of the glucuronide conjugating system.
Journal of Clinical Investigation 37, 332

BROWN, R. R., MILLER, J. A., MILLER, E. C. (1954) The metabolism of methylated amino azo dyes.
Journal of Biological Chemistry 209, 211

BURNS, J. J. (1959) Biosynthesis of L-ascorbic acid: basic defect in scurvy.
American Journal of Medicine 26, 740

BURNS, J. J., [illegible], A. H. (1965) Enzyme stimulation and inhibition in the metabolism of drugs.
Proceedings of the Royal Society of Medicine 58, 955

BURNS, J. J., [illegible], S. [illegible], [illegible], B., [illegible], A. H. (1965) Application of drug metabolism to drug toxicity studies.
Annals of the New York Academy of Sciences 123, 273

BURNS, J. J. (1969) Variation of drug metabolism in animals and the prediction of drug action in man.
Annals of the New York Academy of Sciences, 151, 959

BURSTEIN, S., [illegible], E. L. (1965) Phenobarbital induced increase in 6 β -hydroxycortisol excretion: clue to its significance in human urine.
Journal of Clinical Endocrinology and Metabolism 25, 293

BUTLER, T. C. (1955) The metabolic hydroxylation of phenobarbital.
Journal of Pharmacology and Experimental Therapeutics 116, 326

CAMAPA-WILSON, R., ROWE, D. J. F. (1970) Electrophoretic separation of tissue-specific serum alkaline phosphatase.
Journal of Clinical Pathology, 23, 499

CARSWELL, F., KERR, M. M., [illegible], I. R. (1972) Sequential trial of effect of phenobarbitone on serum bilirubin of preterm infants.
Archives of Disease in Childhood 47, 621

CARTEI, G., PANZETTA, O., MACCARATO, R. (1971) BSP excretion in Gilbert's syndrome before and after phenobarbital. Abstract 62. Proc. 6th Meeting European Association for Study of the Liver, London.

CARTER, D. E., GOLDMAN, J. M., BRESSLER, R., HUXTABLE, R. J., CHRISTIAN, C. D., HEINE, M. W. (1974) Effect of oral contraceptives on drug metabolism. Clinical Pharmacology and Therapeutics 15, 22.

CATALANO, P. M., CULLEN, S. I. (1966) Warfarin antagonism by griseofulvin. Clinical Research 14, 266

CATZ, C., YAFFE, S. J. (1962) Pharmacological modification of bilirubin conjugation in the newborn. American Journal of Diseases of Children 104, 516.

CATZ, C., YAFFE, S. J. (196) Barbiturate enhancement of bilirubin conjugation in young and adult animals Pediatric Research 2, 361

CHANARIN, I., ANDERSON, B. B., MOLLIN, D. L. (1958) The absorption of Folic Acid. British Journal of Haematology 4, 156

CHANARIN, I. (1969) in "The Megaloblastic Anaemias" p 834, Oxford, Blackwell.

CHANARIN, I. (1970) The Anaemias, in "Biochemical Disorders in Human Disease" p 188, Eds R. H. J. Thompson and I. D. P. Wooton, London, Churchill.

CHATTERJEE, I. B., KAR, N. C., GHOSH, N. C., GUHA, B. C. (1961) Aspects of Ascorbic acid biosynthesis in animals. Annals of the New York Academy of Sciences 92, 36

CHRISTIANSEN, C., KRISTENSEN, M., RODBRO, P., (1972) Latent osteomalacia in epileptic patients on anticonvulsants. British Medical Journal 3, 738

CHRISTIANSEN, C., RODBRO, P., LUND, M. (1273) Effect of vitamin D on bone mineral mass in normal subjects and in epileptic patients on anticonvulsants: a controlled therapeutic trial. British Medic l Journal 2, 208

CLARK, R., THOMPSON, R. P. H., BORIRAKCHANYAVAT, V., WIDDOP, B., DAVIDSON, A. R., GOULDING, R., WILLIAMS, R. (1973) Hepatic damage and death from overdose of paracetamol. Lancet 1, 66.

[illegible] (1967) History of the liver. Hospital Medicine 1, 494.

CLEMMESEN, J., FUGLSANG-FREDERIKSEN, V., PLUM, C. M. (1974) Are anticonvulsants oncogenic?
Lancet 1, 705

COCCIA, P. F., WESTERFELD, W. W. (1967) The metabolism of chlorpromazine by liver microsomal enzyme systems.
Journal of Pharmacology and experimental Therapeutics 157, 446

COHN, M. O. (1973) in "The Liver: Quantitative Aspects of Structure and Function." p 240, Karger, Basel.

CONNEY, A. H., MILLER, E. C., MILLER, J. A. (1958) The metabolism of methylated aminoazo dyes: Evidence for induction of enzyme synthesis in the rat by 3-methylcholanthrene.
Cancer Research 16, 450

CONNEY, A. H., BURNS, J. J. (1959) Stimulatory effect of foreign compounds on ascorbic acid biosynthesis and on drug metabolising enzymes.
Nature 184, 363

CONNEY, A. H., DAVISON, C., GASTEL, R., BURNS, J. J. (1960) Adaptive increases in drug metabolism induced by phenobarbital and other drugs.
Journal of Pharmacology and experimental Therapeutics, 130, 1.

CONNEY, A. H., BRAY, G. A., EVANS, C., BURNS, J. J. (1961) Metabolic interactions between L-ascorbic acid and drugs.
Annals of the New York Academy of Sciences 92, 115

CONNEY, A. H. (1967) Pharmacological implications of microsomal enzyme induction.
Pharmacol Rev 19, 317

CONNEY, A. H. (1969) Drug metabolism and therapeutics.
New England Journal of Medicine 280, 653

COOPER, D. Y., LEVIN, S., NARASIMHULU, S., ROSENTHAL, O., ESTABROOK, R. W. (1965) Photochemical action spectrum of the terminal oxidase of mixed function oxidase systems.
Science 147, 400

CORCINO, J. J., WAXMAN, S., HERBERT, V. (1971) Uptake of tritiated folates by human bone marrow cells in vitro.
British Journal of Haematology 20, 503

CORREIA, M. A., MEYER, U. A. (1975) Apocytochrome P 450: Reconstitution of functional cytochrome with hemin in vitro.
Proceedings of the National Academy of Sciences USA 72, 400.

CORROCHER, R., HOFFBRAND, A. V. (1972) Subcellular localisation of folate and effect of methotrexate on the incorporation of radioactive folic acid into guinea pig liver folate.
Clinical Science 43, 815

COYNE, M. J., BONORRIS, G. G., CHUNG, A., GOLDSTEIN, L. I., LAHANA, D., SCHOENFIELD, L. J., (1975) Treatment of gallstones with Chenodeoxycholic Acid and Phenobarbital. New England Journal of Medicine 292, 604.

CREAN, G. P., RUMSEY, R. D. E. (1971) Hyperplasia of the gastric mucosa during pregnancy and lactation in the rat. Journal of Physiology 215, 181.

CREAVEN, P. J. DAVIES, W. H., WILLIAMS, R. T. (1966) The effect of butylated hydroxytoluene, butylated hydroxyanisole and acetyl gallate upon liver weight and biphenyl 4-hydroxylase activity in the rat. Journal of Pharmacy and Pharmacology 18, 485.

CRIGLER, J. F., GOLD, N. I. (1966) Sodium phenobarbital induced decrease in serum bilirubin in an infant with congenital non-hemolytic jaundice and kernicterus. Journal of Clinical Investigation 45, 998.

CRIGLER, J. F., GOLD, N. I. (1967) Effect of sodium phenobarbital on the metabolism of bilirubin -^{3}H and ^{14}C in an infant with congenital nonhemolytic jaundice and kernicterus. Journal of Clinical Investigation 46, 1047.

CUCINELL, S. A., CONNEY, A. H., SANSUR, M., BURNS, J. J. (1965) Drug interactions in man I. Lowering effect of phenobarbital on plasma levels of bishydroxycoumarin and diphenylhydantoin. Clinical Pharmacology and Therapeutics 6, 420.

CUCINELL, S. A., ODESSKY, L., WEISS, M., DAYTON, P. G. (1966) The effect of chloral hydrate on bishydroxycoumarin metabolism. Journal of the American Medical Association 197, 366.

CURRY, S. H. (1968) Method for the estimation of nanogram quantities of chlorpromazine and some of its relatively non-polar metabolites in plasma, using gas-chromatography, with an electron capture detector. Analytical Chemistry 40, 1251.

CURRY, S. H., DERR, J. E., MALING, H. M. (1970) The physiological disposition of chlorpromazine in the rat and dog. Proceedings of the Society for Experimental Biology and Medicine 134, 314.

DAGG, J. H., GOLDBERG, A., LOCHHEAD, A., SMITH, J. A. (1965) The relationship of lead poisoning to acute intermittent porphyria. Quarterly Journal of Medicine 34, 163.

DAVIES, D. P., [illegible] (1967) Malabsorption of folic acid due to diphenylhydantoin.
Blood 30, 341.

DAVIES, G., SIEKEVITZ, P., PALADE, G. E. (1966) Biogenesis of endoplasmic reticulum membranes.
Journal of Cell Biology 30, 73 and 97.

DAVIES, D. S., GIGON, P. L., GILLETTE, J. R. (1969) Species and sex differences in electron transport systems in liver microsomes and their relationship to ethylmorphine demethylation.
Life Sciences 8, 85.

DAVIES, D. S., THORGEIRSSON, S. S., BRODIE-BRIDGE, A., ORME, M. (1973) Interindividual differences in rates of drug oxidation in man.
Drug metabolism and Disposition 1, 411.

DAVIES, J. P., WILKINSON, J. P., GARTY, G. H., BARNETT, A. (1969) Effect of anticonvulsant drugs on dicophane (DDT) residues in man.
Lancet 1, 7.

DAVIS, J. R., ALTEMAN, S. L. (1967) Urinary delta-amino-laevulinic acid (ALA) levels in lead poisoning. I A modified method for the rapid determination of urinary delta-amino-laevulinic acid using disposable ion exchange chromatography columns.
Archives of Environmental Health 15, 53.

DAVIS, M., [illegible], C. J., [illegible], E., MAXWELL, J. D., [illegible], R. (1973) Induction of hepatic enzymes during normal pregnancy.
Journal of Obstetrics and Gynaecology of the British Commonwealth 80, 690.

DEAN, G. (1963) The prevalence of the porphyrias.
South African Journal of Laboratory and Clinical Medicine 9, 145.

DE LUCA, H. F. (1969) Recent advances in the metabolism and function of vitamin D.
Federation Proceedings 28, 1678.

DE MATTEIS, F., RIMINGTON, C. (1963) Disturbance of porphyrin metabolism caused by griseofulvin in mice.
British Journal of Dermatology 75, 91.

DE MATTEIS, F., (1970) Rapid loss of cytochrome P 450 and haem caused in the liver microsomes by the porphyrogenic agent 2-allyl-2-isopropylacetamide.
FEBS-letters 6, 343.

DE MATTEIS, F. (1971A) Drugs and porphyria.
South African Medical Journal 45, 126.

DE MATTEIS, F. (1971B) Loss of haem in rat liver caused by the porphyrogenic agent 2-allyl-2-isopropylacetamide.
Biochemical Journal 124, 767.

DE MATTEIS, F. (1972) The effects of drugs on the activities of δ -aminolaevulinate synthetase and other enzymes in the pathway of heme biosynthesis.
Biochemical Journal 130, 52P.

DE MATTEIS, F., GIBBS, A. H. (1972) Stimulation of liver δ -aminolaevulinate synthetase by drugs and its relevance to drug induced accumulation of cytochrome P 450.
Biochem. J. 126, 1149.

DE MATTEIS, F. (1973A) Drug induced destruction of cytochrome P 450.
Drug Metabolism and Disposition 1, 267.

DE MATTEIS, F. (1973B) Drug interactions in experimental hepatic porphyria: a model for the exacerbation by drugs of human variegate porphyria.
Enzyme 16,266.

DE MATTEIS, F. (1973C) Effect of drugs on δ -aminolaevulinate synthetase. Differential response between rat and chick liver cells.
Hoppe Seyler Zeit fur Physiol Chemie 354, 856.

DE MATTEIS, F., GIBBS, A. H. (1975) Stimulation of the pathway of porphyrin synthesis in the liver of rats and mice by griseofulvin 3, 5-diethoxycarbonyl-1, 4-dihydrocollidine and related drugs: Evidence for 2 basically different mechanisms.
Biochem. J. 146, 285.

DENT, C. E. (1962) Some problems of hyperparathyroidism.
British Medical Journal 2, 1419.

DENT, C. E., RICHENS, A., ROWE, D. J. F., STAMP, T. C. B. (1970) Osteomalacia with long term anticonvulsant therapy in epilepsy.
British Medical Journal 4, 69.

DENT, C. E., SMITH, R. (1969) Nutritional osteomalacia.
Quarterly Journal of Medicine 32, 195.

DHAR, G. J., ROSENTHALER, I., PERRY, E. J., CARDINAL, R. AND WATSON, C. J. (1975) Effects of hematin in hepatic porphyria. Further studies. Ann, Intern. Med. 83, 20.

DOBRSCHANSKY, M. (1906) Einiges über Malonal.
Weiner Medizinische Presse 47, 2145.

DOE, W. F., HOFFBRAND, A. V., REED, P. I., SCOTT, I. M. (1971)
Jejunal pH and folic acid.
British Medical Journal 1, 699.

DOLLERY, C. T. (1972) Enzyme induction.
British Journal of Anaesthesia 44, 961.

DOSS, M., KALTEPOTH, B. (1971) Porphyrin biosynthesis in liver cell cultures.
South African Medical Journal 45, 73.

DRESEL, E. I. B., FALK, J. E. (1956) Studies on the biosynthesis of blood pigments. 2 . Haem and porphyrin formation in intact chicken erythrocytes.
Biochemical Journal 63, 72.

DUESBERG, R. (1932) Toxische Porphyrie.
Munchener Medizinische Wochenschrift 79, 1821.

DUNDEE, J. W. (1952) Thiopentone narcosis in the presence of hepatic dysfunction.
British Journal of Anaesthesia 24, 81.

DUTTON, G. J. (1966) in "Glucuronic acid, free and combined.
Chemistry, Biochemistry, Pharmacology and Medicine."
Academic Press, New York.

EALES, L. (1971) Acute porphyria: The precipitating and aggravating factors.
South African Medical Journal 45, 120.

EICHNER, E. R., PIERCE, H. I., HILLMAN, R. S. (1971) Folate balance in dietary induced megaloblastic anaemia.
New England Journal of Medicine 284, 933.

EICHNER, E. R., HILLMAN, R. S. (1973) Effect of alcohol on serum folate level.
Journal of Clinical Investigation 52, 585.

ELDER, G. H., GRAY, C. H., NICHOLSON, D. C. (1972) The porphyrias: a review.
Journal of Clinical Pathology 25, 1013.

ELDER, G. H., EVANS, J. O., THOMAS, N., COX, R., BRODIE, M. J., MOORE, M. R., GOLDBERG, A., NICHOLSON, D. C. (1976) The primary enzyme defect in hereditary coproporphyria.
Lancet ii, 1217.

ELSBORG, L. (1972) Binding of folic acid to human plasma proteins.
Acta Haematologica 48, 207.

EHRLICH, L., LASKY, M. (1935) The origin of L-xylose (urine pentose).
Journal of Biological Chemistry 110, 443.

ERLINGER, S., DUMONT, M. (1972) Influence of canalicular bile flow on sulfobromophthalein transport maximum in bile in the dog.
Digestion 7, 355.

ERNSTER, L., ORRENIUS, S. (1965) Substrate induced synthesis of the hydroxylating enzyme system of liver microsomes.
Federation Proceedings 24, 1190.

ESTABROOK, R. W., COOPER, D. Y., ROSENTHAL, O. (1963) The light reversible monoxide inhibition of the steroid C21 hydroxylase system of the adrenal cortex.
Biochemische Zeitschrift 338, 741.

EVANS, D. A. P. (1960) Genetic control of isoniazid metabolism in man.
British Medical Journal 2, 485.

EVANS, D. A. P. (1971) Genetic Study of drug metabolism.
Chemico-Biological Interactions. 3, 262.

EVANS, A. R., FORRESTER, R. M., DIGGORE, C. (1970) Neonatal haemorrhage following maternal anticonvulsant therapy.
Lancet 1, 517.

FAHIM, M. S., HALL, D. G., FAHIM, Z. (1969) Urinary D-glucaric acid. An index of microsomal enzyme activity in human females.
American Journal of Obstetrics and Gynecology 105, 124.

FEHLING, C., JAGERSTAD, M., LUNDSTRAND, K., WESTESSON A-K (1973) The effect anticonvulsant therapy upon the absorption of folates.
Clinical Science 44, 595

FERGUSON, H. C. (1966) Effect of red cedar chip bedding on hexobarbital and pentobarbital sleep time.
Journal of Pharmaceutical Science 55, 1142.

FOOD, COSMETICS AND TOXICOLOGY (1970). Join the microsomal bandwagon 8, 579.

FORREST, J. A. H., FINLAYSON, N. D. C., ADJEPON-YAMOAH, K. K., PRESCOTT, L. F. (1975) Antipyrine, lignocaine and paracetamol metabolism in chronic liver disease.
Gut 16, 828.

FOUTS, J. R., ROGERS, L. A. (1965) Morphological changes in the liver accompanying stimulation of microsomal drug metabolising enzyme activity by phenobarbital, chlordane, benzpyrene or methylcholanthrene in rats.
Journal of Pharmacology and Experimental Therapeutics 147, 112.

FOUTS, J. R. (1973) Discussion of "induction of the unspecific microsomal hydroxylase in the human liver."
Drug Metabolism and Disposition 1, 229.

FRANKLIN, J. L., ROSENBERG, I. H. (1971) Folate absorption in patients with inflammatory bowel disease: effects of Azulfidine.
Clinical Research 19, 657.

FRASER, R., MACINTYRE, I. (1970) Disorders of Bone and Calcium Metabolism p751.
In Biochemical Disorders in Human Disease,
ed. R. H. S. Thompson and I. D. P. Wootton, London, Churchill.

GARROD, A. E. (1902) The incidence of alkaptonuria: a study in chemical individuality.
Lancet 2, 1616.

GERSON, C. D., HEPNER, G. W., BROWN, N., COHEN, N., HERBERT, V., JANOWITZ, H. D. (1972) Inhibition by diphenylhydantoin of folic acid absorption in man.
Gastroenterology 63, 246.

GIKALOV, I., KUPFER, A., PLATZER, R., PIRCHER, J., PREISIG, R. (1975) Breath analysis of hepatic microsomal function in man.
Digestion 12, 254A.

GILLETTE, J. R., KAMM, J. J. (1960) The enzymatic formation of sulphoxides: The oxidation of chlorpromazine and 4, 4'-diamino-diphenylsulphide by guinea pig liver microsomes.
Journal of Pharmacology and Experimental Therapeutics 130, 262.

GILLETTE, J. R. (1963) Metabolism of drugs and other foreign compounds by enzymatic mechanisms.
Progress in Drug Research 6, 13.

GILLETTE, J. R. (1966) Biochemistry of drug oxidation and reduction by enzymes in hepatic endoplasmic reticulum.
Advances in Pharmacology 4, 219.

GILLETTE, J. R. (1971) Factors affecting drug metabolism.
Annals of the New York Academy of Sciences 179, 43.

GIRDWOOD, R. W., LEWIS, J. A. R. (1956) Megaloblastic anaemia occurring during primidone therapy.
British Medical Journal 1, 146.

GOLDBERG, A., RIMINGTON, C. (1955) Experimentally produced porphyria in animals.
Proceedings of the Royal Society of London: C1 B: Biological Sciences 143, 257.

GOLDBERG, A. (1959) Acute intermittent porphyria.
Quarterly Journal of Medicine 28, 183.

GOLDBERG, A. (1972) Lead poisoning and haem biosynthesis.
British Journal of Haematology 23, 521.

GORESKY, N. A., KLOTZ, S. A. (1969) The relation between bile flow and transport maximum for bilirubin in the dog.
Gastroenterology 56, 398.

GRANICK, S. (1966) The induction in vitro of the synthesis of δ -aminolaevulinic acid synthetase in chemical porphyria, a response to certain drugs, sex hormones and foreign chemicals.
Journal of Biological Chemistry 241, 1359.

GRUN, A., [illegible], F. (1970) Stabilization of heme during the elevation of microsomal cytochromes P-450 and b-5 by phenobarbital in otherwise [illegible].
Scandinavian Journal of Clinical and Laboratory Investigation 25, 115.

GRUNDY, S., [illegible], S. A. (1970) Hepatic biliary excretion of sulfobromophthalein sodium in sheep.
American Journal of Digestive Diseases 15, 37.

HASTINGS, A. B. (1964) Porphyrin carriers in enzymatic hydroxylation of steroids.
Federation Proceedings 23, 490.

[illegible], M. J., [illegible], C. J., [illegible], S. P., [illegible], L. V. (1972) Phenobarbital alteration in vitamin D metabolism.
Journal of Clinical Investigation 51, 741.

[illegible], S. J., [illegible], P. A., [illegible], S. P., HARPER, J. G. (1972) Effect of chronic phenobarbital and dilantin on serum 25 hydroxycholecalciferol levels in adults.
New England Journal of Medicine 287, 900.

[illegible], M. J. (1973) Anticonvulsant therapy and vitamin D.
[illegible] of Internal Medicine 78, 308.

[illegible], S. J., [illegible], A. A., [illegible], S. P., [illegible], W. C., [illegible], J. G. (1973) Serum 25-OHD and bone mass in children given anticonvulsant drugs.
New England Journal of Medicine 288, 550.

[illegible] (1971) The effect of progesterone on hepatic metabolism in animals and humans.
Journal of Reproductive Medicine 6, 116.

HALPIN, T. C., JONES, A. R., BISHOP, H. L., LERNER, S. (1972) Prophylaxis of neonatal hyperbilirubinaemia with phenobarbital.
Obstetrics and Gynecology 40, 85.

[illegible] (1965) Inhibition Studies on folic acid metabolism with drugs suspected to act on the myeloproliferative system.
Clinica Chimica Acta 12, 144.

HANNA, F. M., FAHIM, M., HALL, D. G. (1970) D-glucaric acid excretion: Age and sex related differences.
Federation Proceedings 29, 277.

HARBISON, R. D., BECKER, B. A. (1969) Relation of dosage and time of administration of diphenylhydantoin to its teratogenic effect in mice.
Teratology 2, 305.

HARGREAVES, T., HOLTON, J. B. (1962) Jaundice of the newborn due to novobiocin.
Lancet 1, 839.

HARRISON, Y. E., WEST, W. L. (1971) Stimulatory effect of charcoal broiled ground beef on hydroxylation of 3, 4 benzpyrene by enzymes in rat liver and placenta.
Biochemical Pharmacology 20, 2105.

HART, L. G., SHULTICE, R. W., FOUTS, J. R. (1963) Stimulatory effect of chlordane on hepatic microsomal drug metabolism in the rat.
Toxicology and Applied Pharmacology 5, 371.

HAWKES, C. H., [illegible], PRYOR, H., TOWNSEND, H. R. A. (1970) The value of E.E.G. frequency analysis in hepatic encephalopathy.
Journal of the Royal College of Surgeons of Edinburgh 15, 151.

HAWKINS, C. F., MEYNELL, M. J. (1958) Macrocytosis and Macrocytic Anaemia caused by Anticonvulsant Drugs.
Quarterly Journal of Medicine 27, 45.

[illegible] (1969) The absorption of pteroyl glutamic (folic) acid in [illegible].
British Journal of Haematology 16, 241.

HEPNER, G. W., VESELL, E. S. (1974) Aminopyrine metabolism in man: Effects of Phenobarbital, Disulfiram and Portal Cirrhosis. New England Journal of Medicine 291, 1384.

HIGGINS, G., YAO, F. (1959) Influence of hormones on liver I. Effects of Steroids and thyroxine on pyridine nucleotide linked dehydrogenases.
Journal of experimental Medicine 110, 899.

HILL, B. T. (1972) The increased anti-tumour activity of chlorambucil after pretreatment with phenobarbitone.
Biochemical Journal 129, 44p.

HOFFBRAND, A. V., BEVERLEY, F. A., NEWCOMBE, B. F. A., MOLLIN, D. L. (1966) Method of assay of red cell folate activity and the value of the assay as a test for folate deficiency.
Journal of Clinical Pathology 19, 17.

HOFFBRAND, A. V., NECHELES, T. F. (1968) Mechanism of folate deficiency in patients receiving phenytoin.
Lancet 2, 528.

HOFFMAN, D. G., WORTH, H. M., EMMERSON, J. L., ANDERSON, R. C. (1970) Stimulation of Hepatic Drug-metabolising enzymes by DDT; the relationship to liver enlargement and hepatotoxicity in the rat.
Toxicology and Applied Pharmacology (1970) 16, 171.

HOLTZMAN, J. L., GILLETTE, J. R. (1968) The effect of phenobarbital on the turnover of microsomal phospholipid in male and female rats.
Journal of Biological Chemistry 243, 3020.

HOOPER, W. D., BOCHNER, F., EADIE, M. J., TYRER, J. H. (1974) Plasma protein binding of diphenylhydantoin. Effects of sex hormones renal and hepatic disease.
Clinical Pharmacology and Therapeutics 15, 276.

HOULIHAN, C. M., SCOTT, J. M., BOYLE, P. H., WEIR, D. G., (1972) The effect of phenytoin on the absorption of synthetic folic acid polyglutamates.
Gut 13, 189.

HSIA, D. Y. Y., RIABOV, S., DOWBEN, R. M. (1963) Inhibition of glucuronyl transferase by steroid hormones.
Archives of Biochemistry 103, 181.

HUNTER, J., MAXWELL, J. D., CARRELLA, M., STEWART, D. A., WILLIAMS, R. (1971A) Urinary D-glucaric acid excretion as a test for hepatic enzyme induction in man.
Lancet 1, 572.

HUNTER, J., MAXWELL, J. D., WILLIAMS, R. (1971B) D-glucaric acid excretion as a test for hepatic enzyme induction.
Lancet 2, 47.

HUNTER, J., MAXWELL, J. D., STEWART, D. A., PARSONS, V., WILLIAMS, R. (1971C) Altered calcium metabolism in epileptic children on anticonvulsants.
British Medical Journal 4, 202.

HUNTER, J., THOMPSON, R. P. H., RAKE, M. O., WILLIAMS, R. (1971D) Controlled trial of phetharbital, a non-hypnotic barbiturate, in unconjugated hyperbilirubinaemia.
British Medical Journal, 2, 497.

HUNTER, J., MAXWELL, J. D., STEWART, D. A., WILLIAMS, R., ROBINSON, J., RICHARDSON, A. (1972) Increased hepatic microsomal enzyme activity from occupational exposure to certain organochlorine pesticides.
Nature 237, 399.

HUNTER, J., MAXWELL, J. D., WILLIAMS, R. (1972) Enzyme induction by environmental agents.
In Eighth Symposium on Advanced Medicine, p 243.
Ed. G. Neale, Pitman Medical, London.

HUNTER, J. O., THOMPSON, R. P. H., DUNN, P. M., WILLIAMS, R. (1973) Inheritance of type 2 Crigler-Najjar hyperbilirubinaemia.
Gut 14, 46.

HUNTER, J., MAXWELL, J. D., STEWART, D. A., WILLIAMS, R. (1973) Urinary D-glucaric acid excretion and total liver content of cytochrome P-450 in guinea pigs: Relationship during enzyme induction and following inhibition of protein synthesis.
Biochemical Pharmacology 22, 743.

HUNTER, J. (1976) Enzyme Induction and Inhibition in Human Pregnancy. p 131 in Anticonvulsant Drugs and Enzyme Induction.
Eds. A. Richens and F. Peter Woodford, Associated Scientific Publishers.

HVIDBERG, E. F., ANDREASEN, P. B., RANEK, L. (1974) Plasma half-life of phenylbutazone in patients with impaired liver function.
Clinical Pharmacology and Therapeutics 15, 171.

IKEDA, M., ULLRICH, V., STAUDINGER, H. (1968) Metabolism in vitro of coumarin by enzymic and non-enzymic systems.
Biochemical Pharmacology 17, 1663.

IMAI, Y., SATO, R. (1966) Studies on the substrate interactions with P-450 in drug hydroxylations by liver microsomes.
Journal of Biochemistry, 62, 239.

JAGER, K. W. (1970) Aldrin, dieldrin, endrin and telodrin: An epidemiological and toxicological study of long term exposure.
Elsevier Pub. Co., Amsterdam and New York.

KAMADA, T., YAMADA, H., [illegible], Y., [illegible], Y. (1961) Effect of some drugs on lactonase activity of rat liver.
Journal of Biochemistry (Tokyo) 50, 74.

KEMENY,, J., TARJAN, R. (1966) Investigations on the effects of chronically administered small amounts of DDT in mice.
Experientia 22, 748.

KHANNA, N. N., HARPUR, E. R., STERN, L. (1969) In vitro effect of sodium phenobarbital and diethyl-nicotinamide (coramine) on the protein binding of bilirubin.
Clinical Biochemistry 2, 349.

KINOSHITA, F. K., FRAWLEY, J. P., DUBOIS, K. P. (1966) Quantitative measurement of induction of hepatic microsomal enzymes by various dietary levels of DDT and toxaphene in rats.
Toxicology and Applied Pharmacology 9, 505.

KLAASSEN, C. D., PLAA, G. L. (1968) Studies on the mechanism of phenobarbital enhanced sulfobromophthalein disappearance.
Journal of Pharmacology and Experimental Therapeutics 161, 361.

KLAASSEN, C. D. (1969) Biliary flow after microsomal enzyme induction.
Journal of Pharmacology and Experimental Therapeutics 168, 218.

KLINGENBERG, M. (1958) Pigments of rat liver microsomes.
Archives of Biochemistry 75, 376.

KLIPSTEIN, F. A. (1964) Subnormal serum folate and macrocytosis associated with anticonvulsant drug therapy.
Blood 23, 68.

KOCH-WESER, J., SELLERS, E. M. (1971) Drug interactions with coumarin anticoagulants.
New England Journal of Medicine 285, 487 and 493.

KODICEK, E., LAWSON, D. E., WILSON, P. W. (1970) Biological activity of a polar metabolite of vitamin D.
Nature (Lond) 228, 763.

KODICEK, E. (1974) The story of Vitamin D: From vitamin to hormone.
Lancet 1, 325.

KOLMODIN, B., AZARNOFF, D. L., SJOQVIST, F. (1969) Effect of environmental factors on drug metabolism. Decreased plasma half-life of antipyrine in workers exposed to chlorinated hydrocarbon insecticides.
Clinical Pharmacology and Therapeutics 10, 638.

[illegible], (1973)
Assessment of the 14 C-Glycocholic acid breath test.
[illegible] Journal 3, [illegible].

JEZEQUEL, A. M., ORLANDI, F., TENCONI, L. T. (1971)
Changes of the smooth endoplasmic reticulum induced by
rifampicin in human and guinea pig hepatocytes.
Gut 12, [illegible].

JEZEQUEL, A. M., KOCH, M., ORLANDI, F. (1974) A morphometric
study of the endoplasmic reticulum in human hepatocytes:
correlation between morphological and biochemical data in
subjects under treatment with certain drugs.
Gut 15, 737.

JICK, H., SHUSTER, L. (1966) The turnover of microsomal
NADPH cytochrome c reductase in the livers of mice treated
with phenobarbital.
Journal of Biological Chemistry 241, 5366.

JOLLOW, D. J., MITCHELL, J. R., POTTER, W. Z., DAVIS, D. C.,
GILLETTE, J. R., BRODIE, B. B. (1973). Acetaminophen induced
hepatic necrosis. II. Role of covalent binding in vitro.
Journal of Pharmacology and Experimental Therapeutics 187, 195.

JUDAH, J. D., McLEAN, A. E. M., McLEAN, E. K. (1970)
Biochemical mechanisms of liver injury.
American Journal of Medicine 49, 609.

KADAR, D., INABA, T., ENDRENYI, L., JOHNSON, G. E.,
KALOW, W. (1973) Comparative drug elimination capacity in
man. Glutethimide, amobarbital, antipyrine and sulfinpyrazone.
Clinical Pharmacology and Therapeutics 14, 552.

KAHN, S. B., FEIN, S. A., BRODSKY, I. (1968) Effects of
trimethoprim on folate metabolism in man.
Clinical Pharmacology and Therapeutics 9, 550.

KAPPAS, A., SONG, C. S., LEVERE, R. D., SACHSON, R. A.,
GRANICK, S. (1968) The induction of δ-aminolevulinic
acid synthetase in vivo in chick embryo liver by natural
steroids.
Proceedings of the National Academy of Sciences USA 61, 509.

KATO, R., CHIESARA, E., VASSANELLI, P. (1962)
Factors influencing induction of hepatic microsomal drug
metabolising enzymes.
Biochemical Pharmacology 11, 211.

KATO, R., [illegible], Y., CHIESARA, E. (1963) Enhanced
phenobarbital induction of hepatic microsomal drug metabolising
enzymes.
Biochemical Pharmacology 12, 365.

KAUFMAN, L., SWANSON, A. L., MARVER, H. S., (1970) Chemically
induced porphyria: Prevention by prior treatment with pheno-
barbital.
Science 170, 320.

KRASNER, N., DOW, J., MOORE, M. R., GOLDBERG, A., (1974)
Ascorbic acid saturation and ethanol metabolism.
Lancet 2, 693.

KREEK, M. J., GARFIELD, J. W., GUTJAHR, C. L., GIUSTI, L. M.
(1976) Rifampin-induced methadone withdrawal.
New England Journal of Medicine 294, 1104.

KREIMER-BIRNBAUM, M., GRINSTEIN, M. (1965) Prophyrin
biosynthesis III. Porphyrin metabolism in experimental lead
poisoning.
Biochimica et Biophsica Acta III, 110

KROGER, M. (1972). Insecticide residues in human milk.
Journal of Pediatrics 80, 401.

KRUSE, R. (1968) Osteopathien bei antiepileptischer
langzeittherapie.
Monatsschrift fur Kinderheilkunde 116, 378.

KUNIN, C. M., GLAZKO, A. J., FINLAND, M. (1959) Persistence
of antibiotics in blood of patients with acute renal failure. II
Chloramphenicol and its metabolic products in the blood of
patients with severe renal disease or hepatic cirrhosis.
Journal of Clinical Investigation 38, 1498.

KUNTZMAN, R., JACOBSON, M., CONNEY, A. H. (1966) Effect of
phenylbutazone on cortisol metabolism in man.
Pharmacologist 8, 195.

KUNTZMAN, R., JACOBSON, M., LEVIN, W., CONNEY, A. H., (1968)
Stimulatory effect of N-phenylbarbital on cortisol hydroxylation
in man.
Biochemical Pharmacology 17, 565.

KUNTZMAN, R., (1969) Drugs and enzyme induction.
Annual Review of Pharmacology 9, 21.

KURIYAMA, Y., OMURA, T., SIEKEVITZ, P., PALADE, G. E. (1969)
Effects of phenobarbital on the synthesis and degradation of the
protein components of rat liver microsomal proteins.
Journal of Biological Chemistry 244, 2017.

KUTT, H., WINTERS, W., SCHERMAN, R., McDOWELL, F. (1964)
Diphenylhydantoin and Phenobarbital toxicity: the role of
liver disease.
Archives of Neurology 11, 649.

KUTT, H., WINTERS, W., McDOWELL, F. H. (1966) Depression of
parahydroxylation of diphenylhydantoin by antituberculosis
chemotherapy.
Neurology 16, 594.

LAIDLAW, J., READ, A. E., SHERLOCK, S. (1961) Morphine tolerance in hepatic cirrhosis.
Gastroenterology 40, 389.

LAIDLAW, J., [illegible] (1966) Numerical indices derived from the analysis of E. E. G. records.
Electroencephalography and Clinical Neurophysiology 21, 202.

LAUTERBURG, B., BIRCHER, J. (1973) Hepatic microsomal drug metabolising capacity measured in vivo by breath analysis.
Gastroenterology 65 , A32/556.

LATHAM, A. N., MILLBANK, L., RICHENS, A., ROWE, D. J. F. (1973) Liver enzyme induction by anticonvulsant drugs, and its relationship to disturbed calcium and folic acid metabolism.
Journal of Clinical Pharmacology 13, 337.

LEHOUX, J. G., FAVREAU, [illegible], LEBLANC, [illegible], CHAPDELAINE, A., SANDOR, T. (1972) The effect of folic acid and aminopterin on the steroid 11β and 21-hydroxylation by domestic duck adrenocortical tissue preparations.
Journal of Steroid Biochemistry 3, 773.

LEVI, A. J., SHERLOCK, S., WALKER, D. (1968) Phenylbutazone and isoniazid metabolism in patients with liver disease in relation to previous drug therapy.
Lancet 1, 1275.

LEVI, A. J., GATMAITAN, Z., ARIAS, I. M. (1969) Two hepatic cytoplasmic protein fractions, Y and Z, and their possible role in the hepatic uptake of bilirubin, sulfobromophthalein and other anions.
Journal of Clinical Investigation 48, 2156.

LEWIS, R. J., TRAGER, W. F., CHAN, K. K., BRECKENRIDGE, A., ORME, M., ROWLAND, M., SCHARY, W. (1974) Warfarin: stereochemical aspects of its metabolism and the interaction with phenylbutazone.
Journal of Clinical Investigation 53, 1607.

LIEBERMAN, F. L. (1963) The effect of liver disease on the rate of ethanol metabolism in man.
Gastroenterology 44, 261.

LICHTMAN, H. C., FELDMAN, F. (1963) In vitro pyrrole and porphyrin synthesis in lead poisoning and iron deficiency.
Journal of Clinical Investigation 42, 830.

LIVINGSTON, S., BERMAN, W., PAULI, L. L. (1973) Anticonvulsant drugs and vitamin D metabolism.
Journal of the American Medical Association 224, 1634.

LLOYD, T., MORI, T., KAUFMAN, S. (1971) 6-methyl tetrahydropterin. Isolation and identification as the highly active hydroxylase cofactor from tetrahydrofolate.
Biochemistry 10, 2330.

LONGENECKER, H. E., FRICKE, H. H., KING, C. G. (1940) The effect of organic compounds upon vitamin C synthesis in the rat.
Journal of Biological Chemistry 135, 497.

LOSITO, R., OWEN, C. A., FLOCK, E. V. (1968) Metabolic studies of vitamin K1-14C and menadione-14C in the normal and hepatectomised rats.
Thrombosis et Diathesis Haemorrhagica 12, 383

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., RANDALL, R. J. (1951) Protein measurement with the folin phenol reagent.
Journal of Biological Chemistry 193, 265.

LU, A. Y. H., STROBEL, H. W., COON, M. J. (1969) Hydroxylation of benzphetamine and other drugs by a solubilised form of cytochrome P 450 from liver microsomes.
Biochemical and Biophysical Research Communications 36, 545.

LU, A. Y., WEST, S. B., RYAN, D., LEVIN, W. (1973) Characterisation of partially purified cytochromes P-450 and P-448 from rat liver microsomes.
Drug metabolism and Disposition. 1, 29.

LUMB, G. A., MAWER, E. B., STANBURY, S. W. (1971) The apparent vitamin D resistance of chronic renal failure.
American Journal of Medicine 50, 421.

MACDONALD, M. G., ROBINSON, D. S., SYLWESTER, D., JAFFE, J. J. (1969) The effects of phenobarbital, chloral betaine, and glutethimide administration on warfarin plasma levels and hypoprothrombinemic responses in man.
Clinical Pharmacology and Therapeutics 10, 80.

McINTYRE, N., PEARSON, A. J. G., ALLAN, D. J., CRASKE, S., WEST, G. M. L., MOORE, M. R., PAXTON, J., BEATTIE, A. D., GOLDBERG, A. (1971) Hepatic δ-aminolaevulinic acid synthetase in an attack of hereditary coproporphyria and during remission.
Lancet 1, 560.

McLEAN, A. E. M., MAGEE, P. N. (1970) Increased renal carcinogenesis by dimethylnitrosamine in protein deficient rats.
British Journal of Experimental Pathology 51, 587.

McLUEN, E. F., FOUTS, J. R. (1961) The effects of obstructive jaundice on drug metabolism in rabbits.
Journal of Pharmacology and Experimental Therapeutics 131, 7.

McM[illegible], G. P., [illegible], [illegible] (1970)
Phenobarbitone in rhesus haemolytic disease: a controlled trial.
Lancet 2, [illegible].

[illegible], T., [illegible], J. C. (1971) Effect of inducers of drug metabolising enzymes on diethylnitrosamine metabolism and toxicity.
Biochemical Journal 123, 8P.

MALPAS, A., [illegible], A. J., JOYCE, C. R. B., SCOTT, D. F. (1970) Persistent behavioural and electroencephalographic changes after single doses of nitrazepam and amylobarbitone sodium.
British Medical Journal 1, 762.

MANNERING, C. J. (1971) Microsomal enzyme systems which catalyse drug metabolism. In "Fundamentals of Drug Metabolism and Drug Disposition" p 206. Eds La Du, Mandel and Way. Williams and Wilkins.

[illegible], E., [illegible], F., [illegible], E. H., VETTER, H. (1952) Die haematologischen Komplikationen der Epilepsiebehandlung mit Hydantoinkörpern.
Medizinische Klinik 47, 1397.

MAR[illegible], [illegible], [illegible] (1964) The metabolism of tritiated digoxin in cirrhotic patients.
Gastroenterology 47, 517.

MARGOLIS, J. I. (1929) Chronic pentosuria and migraine.
American Journal of Medical Science 177, 348.

[illegible], T., [illegible], [illegible], [illegible], S., [illegible], P. (1972) New carrier protein(s) of folic acid in human serum.
Acta Haematologica [illegible], 145.

[illegible], [illegible], [illegible], J. (1960) Inhibition of mammalian glucose-6-phosphate dehydrogenase by steroids.
Proceedings of the National Academy of Sciences USA 46, 447.

[illegible] (1963) Metabolism of D-glucuronolactone in mammalian systems. [illegible] identification of D-glucaric acid as a normal constituent of urine.
Biochemical Journal [illegible], 77.

[illegible] (19[illegible]) The effect of cirrhosis of the liver on microsomal detoxification and cytochrome P-450.
British Journal of Experimental Pathology [illegible], [illegible].

[illegible], [illegible] (1971) Role of steroid hormones in microsomal enzyme induction.
Biochemical Pharmacology 20, 1723.

[illegible], [illegible], [illegible] (1968) Biotransformation in the liver: implications for human disease.
Gastroenterology 55, 232.

[illegible] (1957) Mechanisms of oxygen metabolism.
Science 125, 1105.

[illegible] (1965) Oxidases. Annual Review of Biochemistry 34, 595.

[illegible], [illegible], [illegible] (1945) The influence of the liver and kidney on the duration of anaesthesia produced by barbiturates.
Anaesthesiology 6, 483.

[illegible], [illegible], [illegible], [illegible] (1972) Metabolism of sulphobromophthalein in patients with chronic liver disease.
British Journal of Pharmacology 44, 542.

[illegible], [illegible], [illegible], [illegible], [illegible], [illegible], [illegible] (1972) Plasma disappearance and cerebral effects of chlormethiazole in cirrhosis.
Clinical Science 43, 143.

[illegible], [illegible], [illegible], [illegible], [illegible], [illegible], [illegible], [illegible] (1972) Folate deficiency after anticonvulsant drugs: an effect of hepatic enzyme induction?
British Medical Journal 1, 227.

[illegible], [illegible], [illegible], [illegible], [illegible], [illegible], [illegible] Effect of phenobarbitone on bile flow and bilirubin metabolism in man and the rat.
Digestion 9, 138.

[illegible], [illegible], [illegible] (1974) Drug sensitivity in hepatic porphyria: relationship to defect in heme synthesis.
Journal of Clinical Investigation 53, 514.

[illegible], [illegible] (1976) Enzyme Induction and Drug Sensitivity in Hereditary Hepatic Porphyria.
p 127 in Anticonvulsant Drugs and Enzyme Induction.
Eds. A. Richens & F. Peter Woodford.
Associated Scientific Publishers.

[illegible], [illegible] (1976) Discussion of paper by Clemmesen, J.
p 127 in Anticonvulsant Drugs and Enzyme Induction.
Eds. A. Richens & F. Peter Woodford.
Associated Scientific Publishers.

MAXWELL, J. D., MEYER, U. A. (1976) Effect of lead on hepatic δ - aminolaevulinic acid synthetase activity in the rat: A model for drug sensitivity in intermittent acute porphyria.
European Journal of Clinical Investigation 6, 373

MAXWELL, J. D., MEYER, U. A. (1977) Pharmacogenetics in the field of heme metabolism: Drug sensitivity in hereditary hepatic porphyria. In 'Haem and Haemoproteins'. Handbook of experimental Pharmacology.
Springer-Verlag. Berlin-Heidelberg-New York. In press.

MELONI, T., CAGNAZZO, G., DORE, A., CUCILLO, S. (1973) Phenobarbital for prevention of hyperbilirubinemia in glucose - 6 - phosphate dehydrogenase-deficient infants.
Journal of Pediatrics 82, 1048.

MEYER, U. A., MARVER, H. S. (1971) Chemically induced porphyria: increased microsomal heme turnover after treatment with allyl isopropylacetamide.
Science 171, 64.

MEYER, U. A., MARVER, H. S. (1971) Enhancement of the fractional catabolic rate of microsomal heme in chemically induced porphyria.
South African Medical Journal 45, 175.

MEYER, U. A., STRAND, L. J., DOSS, M., REES, A. C., MARVER, H. S. (1972) Intermittent acute porphyria: demonstration of a genetic defect in porphobilinogen metabolism.
New England Journal of Medicine 286, 1277.

MEYER, U. A. (1973) Intermittent acute porphyria. Clinical and Biochemical studies of disordered heme biosynthesis.
Enzyme 16, 334.

MEYER, U. A., SCHMID, R. (1973) Hereditary hepatic porphyrias.
Federation Proceedings 32, 1649.

MEYER, U. A., SCHMID, R. (1974) Intermittent acute porphyria. The enzymatic defect. Research Publications of the Association for Research in Nervous and Mental Disease 53, 211.

MEYER, U. A., SCHMID, R. (1977) The porphyrias. In: The Metabolic Basis of Inherited Disease, edited by J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, 4th edition, New York, McGraw Hill.

MEYNELL, M. J. (1966) Haemoblastic anaemia in anticonvulsant therapy.
Lancet 1, 487.

MITCHELL, J. R., JOLLOW, D. J., POTTER, W. Z., DAVIS, D. C., GILLETTE, J. R., BRODIE, B. B. (1973) Acetaminophen induced hepatic necrosis I. Role of drug metabolism.
Journal of Pharmacology and experimental Therapeutics 187, 185.

MITCHELL, J. R., JOLLOW, D. J., GILLETTE, J. R., BRODIE, B. B. (1973A) Drug metabolism as a cause of toxicity. Drug Metabolism and Disposition 1, 418.

MITOMA, C., SORICH, T. J., NEUBAUER, S. E. (1968) The effect of caffeine on drug metabolism. Life Sciences 7, 145.

MIYAGI, K., CARDINAL, R., BOSSENMAIER, I., WATSON, C. J. (1971) The serum porphobilinogen and hepatic porphobilinogen deaminase in normal and porphyric individuals. Journal of Laboratory and Clinical Medicine 78, 683.

MOK, H. Y. I., PERRY, P. M., DOWLING, R. H. (1974) The control of bile acid pool size: Effect of jejunal resection and phenobarbitone on bile acid metabolism in the rat. Gut 15, 247.

MONSON, R. R., ROSENBERG, L., HARTZ, S. C., SHAPIRO, S., HEINONEN, O. P., SLONE, D. (1973) Diphenylhydantoin and selected congenital malformations. New England Journal of Medicine 289, 1049.

MOSES, S. W., LEVIN, S., CHAYOTH, R., STEINITZ, K. (1966) Enzyme induction in a case of glycogen storage disease. Pediatrics 38, 111.

MOTULSKY, A. G. (1969) Drugs and genes. Annals of Internal Medicine 70, 1264.

MOUNTAIN, K. R., HIRSH, J., GALLUS, A. S. (1970) Neonatal coagulation defect due to anticonvulsant drug treatment in pregnancy. Lancet 1, 265.

MOWAT, A. P., (1968) Developmental effects on liver D-glucuronolactone dehydrogenase levels and on D-glucaric acid excretion in urine: hormonal effects on D-glucaric acid excretion in urine. Journal of Endocrinology 42, 585.

MUELLER, G. C., MILLER, J. A. (1949) The reductive cleavage of 4-dimethylaminoazobenzene by rat liver: the intracellular distribution of the enzyme system and its requirement for triphosphopyridine nucleotide. Journal of Biological Chemistry 180, 1125.

MUMFORD, J. P. (1974) Drugs affecting oral contraceptives. British Medical Journal 2, 333.

[illegible], L. (1960) Ganglionblocker.
Fortschritte der Arzneimittelforschung 2, 297.

[illegible], L. S., [illegible], P. V. (1969) The effects of pregnancy on the hydroxylation and reduction of drugs and cytochrome P 450 content of liver microsomes.
Biochemical Journal 113, 129

[illegible], C. (1964) Rate of metabolism of tolbutamide in test subjects with liver disease or with impaired renal function.
American Journal of the Medical Sciences 243, 657.

[illegible] R, H. J., [illegible], C. (1964) An adaptively stimulated O-demethylating system in rat liver microsomes and its kinetic properties.
Journal of Pharmacology and Experimental Therapeutics 146, 61.

[illegible]-[illegible], L., [illegible], W., [illegible], D. (1973) Wirkung von Rifampicin auf den [illegible].
Menstruationszyklus und die Ostrogen ausscheidung bei einnahme oraler kontrazeptiva.
Deutsche Medizinische Wochenschrift 98, 1521.

[illegible], [illegible], [illegible], [illegible] (1961) Acquired porphyria in man and [illegible].
Nature 192, 139.

[illegible], [illegible], [illegible], [illegible], [illegible], [illegible], [illegible], [illegible] (1971) Changes in liver blood flow during [illegible].
Biochemical Pharmacology 20, 2561.

[illegible], O. [illegible], [illegible], C. H. (1970) The influence of folic acid on phenytoin metabolism and the 24 hours fluctuation in urinary output of HPPH.
Acta Pharmacologica et Toxicologica
(Kobenhavn) 28, 265.

[illegible], [illegible], [illegible], [illegible], [illegible], [illegible] (1966) Factors determining the basal rate of organic anion secretion by the liver and further evidence on the hepatic site of action of the hormone secretin.
Journal of Physiology 186, 134.

[illegible], [illegible], [illegible], [illegible], [illegible], [illegible], [illegible] (1971) Effect of age and sex on human drug metabolism.
British Medical Journal 3, 607.

[illegible], [illegible], [illegible], [illegible], [illegible], [illegible] (1972) Impairment of human drug metabolism by oral contraceptive steroids.
Clinical Pharmacology and Therapeutics 13, 552.

[illegible], [illegible], [illegible], [illegible] (1964) [illegible] carbon monoxide binding pigment of liver microsomes.
Journal of Biological Chemistry 239, 2370.

OMURA, T., SATO, R., COOPER, D. Y., ROSENTHAL, O., ESTABROOK, R. W. (1965) Function of cytochrome P-450 of microsomes.
Federation Proceedings 24, 1181.

O'REILLY, R. A., AGGELER, P. M., HOAG, M. S., LEONG, L. (1962) Studies on the coumarin anticoagulant drugs.
Thrombosis et Diathesis Haemorrhagica 8, 82.

OSBORNE, R. H., DE GEORGE, F. V. (1959) Genetic basis of morphological variation, an evaluation and application of the twin study method.
Harvard University Press, Cambridge, Mass.

OSTROWER, V. G., COHN, P., [illegible], [illegible] (1973) The effect of phenobarbital (Pb) on ileal absorption of cholic acid (CA).
(Abst.) Gastroenterology 64, 781.

PADMANABAN, G., RAO, M. R. S., MALATHI, K. (1973) A model for the regulation of δ-aminolaevulinate synthetase induction in rat liver.
Biochemical Journal 134, 847.

PARKE, D. V., RAHMAN, H. (1969) The effects of some terpenoids and other dietary anutrients on hepatic drug-metabolising enzymes.
Biochemical Journal 113, 12P.

PARKE, D. V., RAHMAN, H. (1969) Induction of a new hepatic microsomal haemoprotein by safrole and isosafrole.
Biochemical Journal 123, 9P.

PARKES, J. D., MURRAY-LYON, I. M., WILLIAMS, R. (1970) Neuropsychiatric and electroencephalographic changes after transplantation of the liver.
Quarterly Journal of Medicine 39, 515.

PECK, C. C., SHEINER, L. B., MARTIN, C. M., COMBS, D. T., MELMON, K. L. (1973) Computer-assisted digoxin therapy.
New England Journal of Medicine 289, 441.

PELKONEN, O., KALTIALA, E. H., LARMI, T. K. I., KARKI, N. T., (1973) Comparison of activities of drug metabolising enzymes in human fetal and adult livers.
Clinical Pharmacology and Therapeutics 14, 840.

PEREZ, V., GORODISCH, S., CASAVILLA, F., MARUFFO, C. (1971) Ultrastructure of human liver at the end of normal pregnancy.
American Journal of Obstetrics and Gynecology 112, 428.

PERRY, J., CHANARIN, I. (1972) Observations on folate absorption with particular reference to folate polyglutamate and possible inhibitors to its absorption.
Gut 13, 544.

PESCH, L. A., SEGAL, S., TOPPER, Y. J. (1960) Progesterone effects on galactose metabolism in prepubertal patients with congenital galactosaemia and in rats maintained on high galactose diets.
Journal of Clinical Investigation 39, 178.

PILCHER, C. W. T., THOMPSON, R. P. H., WILLIAMS, R. (1972) Effect of phenobarbitone on hepatic microsomal enzymes of the male rat.
Biochemical Pharmacology 21, 129.

PIPER, W. N., TEPHLY, T. R. (1974) Differential inhibition of erythrocytic and hepatic uroporphyrinogen I synthetase activity by lead.
Federation Proceedings 33, 588.

PLATT, D. S., COCKRILL, B. L. (1969) Biochemical changes in rat liver in response to treatment with drugs and other agents.
Biochemical Pharmacology 18, 429 - 445 - 459.

POLAND, A., SMITH, D., KUNTZMAN, R., JACOBSON, M., CONNEY, A. H. (1970) Effect of intensive occupational exposure to DDT on phenylbutazone and cortisol metabolism in human subjects.
Clinical Pharmacology and Therapeutics 11, 724.

PONCHON, G., DE LUCA, H. F. (1969) The role of the liver in the metabolism of vitamin D.
Journal of Clinical Investigation 48, 1273.

PONCHON, G., KENNAN, A. L., DE LUCA, H. F. (1969) "Activation" of vitamin D by the liver.
Journal of Clinical Investigation 48, 2032.

PORTER, C. A., MOWAT, A. P., COOK, P. J. L., HAYNES, D. W. T., SHILKIN, K. B., WILLIAMS, R. (1972) α_1 Antitrypsin deficiency and neonatal hepatitis.
British Medical Journal 3, 435.

POSNER, H. S., MITOMA, C., ROTHBERG, S., UDENFRIEND, S. (1961) Enzymic hydroxylation of aromatic compounds.
Archives of Biochemistry and Biophysics 94, 230.

POTREPKA, R. F., SPRATT, J. L. (1971) Effect of phenobarbital and 3-methylcholanthrene pretreatment on guinea pig hepatic microsomal bilirubin glucuronyl transferase activity.
Biochemical Pharmacology 20, 861.

PREISIG, R., WILLIAMS, R., SWEETING, J., BRADLEY, S. E., (1966) Changes in sulfobromophthalein transport and storage by the liver during viral hepatitis in man.
American Journal of Medicine 40, 170.

PRESCOTT, L. F. (1969) Pharmacokinetic drug interactions.
Lancet 2, 1239.

QUINN, G. P., AXELROD, J., BRODIE, B. B. (1958) Species, strain and sex differences in metabolism of hexobarbitone, amidopyrine, antipyrine and aniline.
Biochemical Pharmacology 1, 152.

RADZIALOWSKI, F. M., BOUSQUET, W. F. (1968) Daily rhythmic variation in hepatic drug metabolism in the rat and mouse.
Journal of Pharmacology and Experimental Therapeutics 163, 229.

RAJAMANICKAM, C., RAO, M. R. S., PADMANABAN, G. (1975) On the sequence of reactions leading to cytochrome P 450 synthesis - Effect of drugs.
Journal of Biological Chemistry 250, 2305.

RAMBOER, C., THOMPSON, R. P. H., WILLIAMS, R. (1969) Controlled trials of phenobarbitone therapy in neonatal jaundice.
Lancet 1, 966.

RAWLINS, M. D. (1974) Variability of response to drugs in man.
British Journal of Hospital Medicine 12, 803.

READ, A. E., LAIDLAW, J., McCARTHY, C. F. (1969) Effects of chlorpromazine in patients with hepatic disease.
British Medical Journal 2, 497.

REDINGER, R. N., SMALL, D. M. (1973) The effect of phenobarbital upon bile salt synthesis and pool size, biliary lipid secretion, and bile composition.
Journal of Clinical Investigation 52, 161.

REMMER, H., MERKER, H. J. (1963) Drug induced changes in the liver endoplasmic reticulum.
Science 142, 1657.

REMMER, H., MERKER, H. J. (1965) Effect of drugs on the formation of smooth endoplasmic reticulum and drug metabolising enzymes.
Annals of the New York Academy of Sciences 123, 79.

REMMER, H., SCHENKMAN, J., ESTABROOK, R. W., SASAME, H., GILLETTE, J., NARASIMHULU, S., COOPER, D. Y., ROSENTHAL, O. (1966) Drug interaction with hepatic microsomal cytochrome.
Molecular Pharmacology 2, 187.

REMMER, H. (1970) The role of the liver in drug metabolism.
American Journal of Medicine 49, 617.

REMMER, H., SCHOENE, B., FLEISCHMANN, R., [illegible] (1973) Induction of the unspecific microsomal hydroxylase in the human liver. p 152 in "The liver: quantitative aspects of structure and function." Karger, Basel.

REMMER, H., SCHOENE, B., FLEISCHMANN, R. A. (1973b) Induction of the unspecific microsomal hydroxylase in the human liver. Drug Metabolism and Disposition 1, 224.

REYES, H., LEVI, A. J., GATMAITAN, Z., ARIAS, I.M. (1969) Organic anion-binding protein in rat liver: drug induction and its physiologic consequence. Proceedings of the National Academy of Sciences USA, 64, 168.

REYNOLDS, E. H. (1968) Mental effects of anticonvulsants and folic acid metabolism. Brain 91, 197.

REYNOLDS, E. H., [illegible], J., CHANARIN, I. (1969) Folic acid and anticonvulsants. Lancet 1, 1264.

RICHENS, A., ROWE, D. J. F. (1970) Disturbance of calcium metabolism by anticonvulsant drugs. British Medical Journal 4, 73.

RICHENS, A., WATERS, A. H. (1971) Acute effect of phenytoin on serum folate concentration. British Journal of Pharmacology 41, 414P.

RIDLEY, A. (1969) The neuropathology of acute intermittent porphyria. Quarterly Journal of Medicine 38, 307.

RIEGELMAN, S., LOO, J. C. K., ROWLAND, M. (1968) Shortcomings in pharmacokinetic analysis by conceiving the body to exhibit properties of a single compartment. Journal of Pharmaceutical Sciences 57, 117.

RIFKIND, A. B., GILLETTE, P. N., SONG, C. S., KAPPAS, A. (1973) Drug stimulation of δ-aminolevulinic acid synthetase and cytochrome P 450 in vivo in chick embryo liver. Journal of Pharmacology and Experimental Therapeutics 185, 214.

RIGGS, D. S. (1963) The Mathematical Approach to Physiological Problems. Williams and Wilkins, Baltimore.

RIMINGTON, C., MAGNUS, I. A., RYAN, E. A., CRIPPS, D. J. (1967) Porphyria and photosensitivity. Quarterly Journal of Medicine 36, 29.

ROBERTS, R. J., PLAA, G. L. (1967) Effect of phenobarbital on the excretion of an exogenous bilirubin load. Biochemical Pharmacology 16, 827.

ROBINSON, D. S., MACDONALD, M. G. (1966) The effect of phenobarbital administration on the control of coagulation achieved during warfarin therapy in man.
Journal of Pharmacology and Experimental Therapeutics 153, 250.

RODBRO, P., CHRISTIANSEN, C. (1975) Prophylactic vitamin D supplement to epileptic children on anticonvulsant drugs.
New England Journal of Medicine 293, 306.

ROSALKI, S. B., TARLOW, D., RAU, D. (1971) Plasma gamma glutamyl transpeptidase elevation in patients receiving enzyme inducing drugs.
Lancet 2, 376.

ROSENBERG, I. H., STREIFF, R. R., GODWIN, H. A., CASTLE, W. B. (1968) Impairment of intestinal deconjugation of dietary folate.
Lancet 2, 530.

ROSENBERG, I. H., GODWIN, H. A. (1971) The digestion and absorption of dietary folate.
Gastroenterology 60, 445.

ROWAN, P. A., HUNTER, J., MAXWELL, J. D. (1975) Hepatic microsomal enzyme induction during normal human pregnancy.
Digestion 12, 345 (Abstract).

ROWLAND, M. (1972) Drug administration and regimens, in "Clinical Pharmacology, Basic Principles in Therapeutics" Eds. K. Melmon and H. F. Morelli, The Macmillan Company, New York.

RUBIN, E., LIEBER, C. S. (1971) Alcoholism, alcohol and drugs.
Science 172, 1097.

SASSA, S., GRANICK, S. (1970) Induction of δ-aminolaevulinic acid synthetase in chick embryo liver cells in culture.
Proceedings of the National Academy of Sciences USA 67, 517.

SASSA S., GRANICK, S., BICKERS, D. R., BRADLOW, H. L., KAPPAS, A., (1974) A microassay for uroporphyrinogen I-synthetase, one of three abnormal enzyme activities in acute intermittent porphyria and its application to the study of the genetics of this disease.
Proceedings of the National Academy of Sciences U.S.A. 71, 732.

SATYANARAYANA RAO, M. R., MALATHI, K., PADMANABAN, G. (1972) The relationship between δ-aminolevulinate synthetase induction and the concentration of cytochrome P 450 and catalase in liver.
Biochemical Journal 127, 553.

SCHAEFER, K., [illegible], VON HERRATH, D., KRAFT, D., [illegible] (1972) Vitamin D Stoffwechsel und Antiepileptika.
Schweizerische Medizinische Wochenschrift 102, 785.

SCHAFFNER, F., POPPER, H. (1969) Cholestasis is the result of hypoactive hypertrophic smooth endoplasmic reticulum in the hepatocyte.
Lancet 2, 355.

SCHELINE, R. R. (1973) Metabolism of foreign compounds by gastrointestinal microorganisms.
Pharmacological Reviews 25, 451.

SCHENKMAN, J. B., BALL, J. A., ESTABROOK, R. W. (1967) On the use of nicotinamide in assays for microsomal mixed function oxidase activity.
Biochemical Pharmacology 16, 1071.

SCHENKMAN, J. B., REMMER, H., ESTABROOK, R. W. (1967A) Spectral studies on drug interactions with hepatic microsomal cytochrome.
Molecular Pharmacology 3, 113.

SCHIMKE, R. T., SWEENEY, E. W., BERLIN, C. M. (1965) The role of synthesis and degradation in the control of rat liver tryptophan pyrrolase.
Journal of Biological Chemistry 240, 322.

SCHMID, F. (1967) Osteopathien bei antiepileptischer Dauerbehandlung.
Fortschritte der Medizin 85, 381.

SCHMID, R., SCHWARTZ, S. (1952) Experimental porphyria III. Hepatic type produced by Sedormid.
Proceedings of the Society for Experimental Biology and Medicine 81, 685.

SCHMID, R. (1960) Cutaneous porphyria in Turkey.
New England Journal of Medicine 263, 397.

SCHMID, R. (1973) Discussion of paper by De Matteis, F.
Drug Metabolism and Disposition 1, 273.

SCHMID, R. (1976) An expanded model of bilirubin kinetics: effect of feeding, fasting and phenobarbital in Gilbert's syndrome.
Ciba Foundation Discussion Meeting.

SCHOENE, B., FLEISCHMANN, R. A., REMMER, H., v. OLDERSHAUSEN, H. F. (1972) Determination of drug metabolising enzymes in needle biopsies of human liver.
European Journal of Clinical Pharmacology 4, 65.

SCOPPA, P., ROUMENGOUS, M., PENNING, W. (1973) Hepatic drug metabolising activity in lead poisoned rats.
Experientia 22, 970.

SESSIONS, J. T., MINKEL, H. P., BULLARD, J. C., INGELFINGER, F. J. (1954) The effect of barbiturates in patients with liver disease.
Journal of Clinical Investigation 33, 1116.

SHAHIDI, N. T. (1968) Acetophenetidin induced methaemoglobinaemia.
Annals of the New York Academy of Sciences 151, 822.

[illegible] (1974) Effect of phenobarbital on liver functions in patients with Dubin-Johnson syndrome.
Gastroenterology 67, 303.

[illegible] (1972) Effect of phenobarbital on hyperbilirubinaemia, bile acid metabolism, and microsomal enzyme activity in chronic cholestasis of childhood.
Journal of Pediatrics 81, 115.

[illegible] (1971) Drug enzyme induction and drug interactions: literature tabulation.
Toxicology and Applied Pharmacology 18, 730.

[illegible] (1968) Diseases of the Liver and Biliary System.
Blackwell Scientific Publications, Oxford.

[illegible] (1966) Hepatic glucuronyl transferase activity and bilirubin Tm in pregnancy in the rat.
American Journal of Physiology 211, 967.

[illegible] ([illegible]) The role of the liver in the detoxication of thiopental by man.
Anaesthesiology 10, 421.

[illegible] (1969) Induction of drug metabolism III.
Molecular Pharmacology 5, 412.

[illegible] (1971) The effect of oral contraceptives on folate metabolism.
American Journal of Obstetrics and Gynecology 111, 782.

[illegible] (1974) Effect of phenobarbitone treatment on vitamin D metabolism in mammals.
Clinical Science and Molecular Medicine 46, 433.

[illegible] (1975) Vitamin D therapy for children on anticonvulsants.
New England Journal of Medicine 293, 1126.

[illegible] (1975) Heme control of the synthesis of δ-aminolevulinic acid synthetase in cultured chick embryo liver cells.
Annals of the New York Academy of Sciences 244, 509.

[illegible] (1971) [illegible]
and neonatal jaundice.
Archives of Disease in Childhood 46, 710.

[illegible] (1971) Phenobarbitone and neonatal jaundice.
The need for more knowledge.
Clinical Pediatrics 10, 683.

[illegible] (1973) Interindividual differences
in drug oxidation: clinical importance.
Drug Metabolism and Disposition 1, 469.

[illegible] (1966) Implications of the
conjugation of drugs and other exogenous compounds.
II "Glucuronic Acid, Free and Combined" Ed. G. J. Dutton,
p 457, Academic Press, New York.

[illegible] (1972) Prediction of the rate of human drug metabolism.
Abstract 1302. Fifth International Congress on Pharmacology,
San Francisco.

[illegible] (1949) The use of antipyrine in
the measurement of total body water in man.
Journal of Biological Chemistry 179, 31.

[illegible] (1959) Disturbance in
porphyrin metabolism caused by feeding diethyl 1, 4 - dihydro - 2, 4,
6 - trimethylpyridine - 3, 5, - dicarboxylate.
Proceedings of the Society for Experimental Biology and Medicine
100, 593.

SOLOMON, H. M., SCHROGIE, J. J. (1967) The effect of various
drugs on the binding of warfarin - 14C to human albumin.
Biochemical Pharmacology 16, 1219.

[illegible] (1971) Observer error and sampling
variability tested in evaluation of hepatitis and cirrhosis by
liver biopsy.
American Journal of Digestive Diseases 16, 1002.

[illegible] (1968) The influence of estrogens,
progestins and pregnancy on the liver.
Vitamins and Hormones 26, 147.

[illegible] (1974)
Salicylamide metabolism in acute intermittent porphyria.
Clinical Pharmacology and Therapeutics 15, 431.

SOTANIEMI, E. A., HAKKARAINEN, H. A., PURANEN, J. A., LAHTI, R. O. (1972) Radiologic bone changes and hypocalcemia with anticonvulsant therapy in epilepsy.
Annals of Internal Medicine 77, 389.

SOUTHREN, A. L., TOCHIMOTO, S., STROM, L., RATUSCHNI, A., ROSS, H., GORDON, G. (1966) Remission in Cushing's syndrome with o, p-DDD.
Journal of Clinical Endocrinology and Metabolism, 36, 268.

SOYKA, L. F. (1969) Determinants of hepatic aminopyrine demethylase activity.
Biochemical Pharmacology 18, 1029.

SPEIDEL, B. D., MEADOW, S. R. (1972) Maternal epilepsy and abnormalities of the foetus and newborn.
Lancet 2, 839.

STAMP, T. C. B., ROUND, J. M., ROWE, D. J. F., HADDAD, J. H. (1972) Plasma levels and therapeutic effect of 25 hydroxycalciferol in epileptic patients taking anticonvulsant drugs.
British Medical Journal 4, 9.

STAMP, T. C. B. (1973) Vitamin D metabolism: recent advances.
Archives Disease in Childhood 48, 2.

STEIN, J. A., TSCHUDY, D. P. (1970) Acute intermitten porphyria: A clinical and biochemical study of 46 patients.
Medicine (Baltimore) 49, 1.

STEIN, Y. & STEIN, O. (1969) Effect of phenobarbital on rat liver acyl-phosphohydrolases.
Israel Journal of Medical Science 5, 985.

STEINER, J. W., JEZEQUEL, A. M., PHILLIPS, M. J., MIYAI, K., ARAKAWA, K. (1965) Some aspects of the ultrastructural pathology of the liver.
Progress in Liver Disease, Vol II, pp 303 - 372.
(Ed. by H. Popper and F. Schaffner)
William Heinemann, London.

STENGER, R. J. (1970) Organelle pathology of the liver.
Gastroenterology 58, 554.

STEVENSON, I. H., BROWNING, M., CROOKS, J., O'MALLEY, K. (1972) Changes in human drug metabolism after long term exposure to hypnotics.
British Medical Journal 4, 322.

STIEHL, A., THALER, M. M., ADMIRAND, W. H. (1972) The effects of phenobarbital on bile salts and bilirubin in patients with intrahepatic and extrahepatic cholestasis.
New England Journal of Medicine 286, 858.

[illegible], R. E., [illegible], [illegible] (1968)
Inhibition of hexobarbital metabolism in the isolated
perfused liver of the rat.
Molecular Pharmacology 4, 15.

STOKVIS, B. J. (1889) Over twee zeldzame kleurstoffen in
urine van zieken.
Nederlands Tijdschrift voor Geneeskunde.
2nd Series 25 (2), 409.

STRAND, J. L., FELSHER, B. F., REDEKER, A. G., MARVER, H. S.
(1970) Heme biosynthesis in intermittent acute porphyria:
decreased hepatic conversion of porphobilinogen to porphyrins
and increased δ-aminolevulinic acid synthetase activity.
Proceedings of the National Academy of Sciences USA 67, 1315.

STRAND, L. J., [illegible], MANNING, J., BRANCH, S.,
MARVER, H. S. (1972A) Radiochemical microassay of δ-amino-
levulinic acid synthetase in hepatic and erythroid tissues.
Analytical Biochemistry 47, 457.

STRAND, L. J., MANNING, J., MARVER, H. S. (1972B) The
induction of δ-aminolevulinic acid synthetase in cultured
liver cells.
Journal of Biological Chemistry 247, 2820.

STREIFF, R. R. (1970) Folate deficiency and oral contraceptives.
Journal of the American Medical Association 214, 105.

[illegible], J. R., [illegible], H. J. (1971)
Intestinal folate absorption 1. 5-methyl tetrahydrofolic acid.
Journal of Clinical Investigation 50, 1910.

[illegible] (1971)
The experimental porphyrias: a group of distinctive metabolic
lesions.
South African Medical Journal 45, 68.

[illegible] (1970) Acute
intermittent porphyria. Increased ALA-synthetase activity
during an acute attack.
[illegible]

[illegible], L., [illegible] (1968) The clinical porphyrias.
[illegible] 5, [illegible]

[illegible] Inhibition of drug
[illegible]
[illegible]

[illegible] Effect of
[illegible]
[illegible], 200.

[illegible] (1970) The effect of pregnancy on the sensitivity of the rabbit liver to diethylnitrosamine.
Australian and New Zealand Journal of Obstetrics and Gynaecology 10, 175.

THALER, M. M., DALLMAN, P. R., GOODMAN, J. (1972) Phenobarbital-induced changes in NADPH-cytochrome c-reductase and smooth endoplasmic reticulum in human liver.
Journal of Pediatrics 80, 302.

THOMSON, P. D., ROWLAND, M., MELMON, K. L. (1971) The influence of heart failure, liver disease and renal failure on the disposition of lidocaine in man.
American Heart Journal 82, 417.

THOMSON, P. D., MELMON, K. L., RICHARDSON, J. A., COHN, K., STEINBRUNN, W., CUDIHEE, R., ROWLAND, M. (1973) Lidocaine pharmacokinetics in advanced heart failure, liver disease, and renal failure in humans.
Annals of Internal Medicine 78, 499.

THOMPSON, R. M., GERBER, N., SEIBERT, R. A., DESIDERIO, D. M. (1972) Identification of 2-methyl 1-1, 4-naphthohydroquinone monoglucuronide as a metabolite of 2-methyl 1-1, 4-naphthoquinone (menadione) in rat bile.
Research Communications in Chemical Pathology and Pharmacology 4, 543.

THOMPSON, R. P. H. (1969A) Modification of Michaelsson's method for the measurement of plasma total bilirubin.
Journal of Clinical Pathology 22, 439.

THOMPSON, R. P. H. (1969B) Hepatic Enzyme Induction and Jaundice: Clinical and Experimental Studies.
D. M. Thesis, University of Oxford.

THOMPSON, R. P. H., STATHERS, G. M., PILCHER, C. W. T., McLEAN, A. E. M., ROBINSON, J., WILLIAMS, R. (1969)
Treatment of unconjugated jaundice with dicophane.
Lancet 2, 4.

THOMPSON, R. P. H., EDDLESTON, A. L. W. F., WILLIAMS, R.
(1969) Low plasma bilirubin in epileptics on phenobarbitone.
Lancet 1, 21.

THOMPSON, R. P. H., WILLIAMS, R. (1970) Phenobarbitone in intrahepatic biliary atresia.
Lancet 2, 466.

THORPE, E., WALKER, A. I. T. (1973) The toxicology of dieldrin (HEOD) II. Comparative long-term oral toxicity studies in mice with dieldrin, DDT, phenobarbitone β-BHC and γ-BHC.
Food and Cosmetics Toxicology 11, 433.

THRASHER, K., WERK, E. E., CHOI, Y., SHOLITON, L. J., MEYER, W., OLINGER, C. (1969) The measurement, excretion and source of urinary 6-hydroxycortisol in humans.
Steroids 14, 455.

TIETZ, A., LINDBERG, M., KENNEDY, E. P. (1964) A new pteridine requiring enzyme system for the oxidation of glyceryl ethers.
Journal of Biological Chemistry 239, 4081.

TROLLE, D. (1968A) Phenobarbitone and neonatal icterus.
Lancet 1, 251.

TROLLE, D. (1968B) Decrease of total serum bilirubin concentration in newborn infants after phenobarbitone treatment.
Lancet 2, 705.

TSCHUDY, D. P., PERLROTH, M. G., MARVER, H. S., COLLINS, A., HUNTER, G., RECHCIGL, M. (1965) Acute intermittent porphyria: the first "overproduction disease" localised to a specific enzyme.
Proceedings of the National Academy of Sciences USA 53, 841.

TSCHUDY, D. P., BONKOWSKY, H. L. (1972) Experimental porphyria.
Federation Proceedings 31, 147.

TURTON, C. W. G., STANLEY, P., STAMP, T. C. B., MAXWELL, J. D., (1977) Altered vitamin D metabolism in pregnancy.
Lancet 1

UEDA, H., SAKURAI, T., OTA, M., NAKAJIMA, A., KAMII, K., MAEZAWA, H. (1963) Disappearance rate of tolbutamide in normal subjects and in diabetes mellitus, liver cirrhosis, and renal disease.
Diabetes 12, 414.

UPSON, D. W., GRONWALL, R. R., CORNELIUS, C. E. (1970) Maximal hepatic excretion of bilirubin in sheep.
Proceedings of the Society for Experimental Biology and Medicine 134, 9.

VALDES, O. S., MAURER, H. M., SHUMWAY, C. N., DRAPER, D. A., HOSSAINI, A. (1971) Controlled clinical trial of phenobarbital and/or light in reducing neonatal hyperbilirubinemia in a predominantly Negro population.
Journal of Pediatrics 79, 1015

VAN ROY, F. P., HEIRWEGH, K. P. M. (1968) Determination of bilirubin glucuronide and assay of glucuronyl transferase with bilirubin as acceptor.
Biochemical Journal 107, 507.

VESELL, E. S., PAGE, J. G. (1969) Genetic control of phenobarbital induced shortening of plasma antipyrine half-lives in man.
Journal of Clinical Investigation 48, 2202.

VESELL, E. S. (1972) Pharmacogenetics.
New England Journal of Medicine 287, 904.

VESELL, E. S. (1975) Pharmacogenetics.
Biochemical Pharmacology 24, 445.

VEST, M., SIGNER, E., WEISSER, K., OLAFSSON, A. (1970)
A double blind study of the effect of phenobarbital on neonatal hyperbilirubinemia and frequency of exchange transfusion.
Acta Paediatrica Scandinavica 59, 681.

VISSER, G. H. A., HUISJES, H. J., ELSHOVE, J. (1976)
Anticonvulsants and Fetal Malformations.
Lancet 1, 970.

VIUKARI, N. M. A. (1968) Folic acid and anticonvulsants.
Lancet 1, 980.

VOGEL, F. (1959) Moderne probleme der Humangenetik.
Ergebnisse der inneren Medizin und Kinderheilkunde 12, 52.

WADA, O., YANO, Y., URATA, G., NAKAO, K. (1968) Behaviour of hepatic microsomal cytochromes after treatment of mice with drugs known to disturb porphyrin metabolism in liver.
Biochemical Pharmacology 17, 595.

WALDENSTRÖM, J. (1937) Studien über Porphyrie.
Acta Medica Scandinavica. Supplement 82.

WALKER, A. I. T., THORPE, E., STEVENSON, D. E. (1973) The toxicology of dieldrin (HEOD) I. Long term studies in mice.
Food and Cosmetics Toxicology 11, 415.

WATERFIELD, M. D., DEL FAVERO, A., GRAY, C. H. (1969)
Effect of 1, 4 - dihydro-3, 5 dicarbethoxy collidine on hepatic microsomal haem, cytochrome b5 and cytochrome P 450 in rabbits and mice.
Biochimica et Biophysica Acta 184, 470.

WATSON, C. J. (1975) Hematin and porphyria.
New England Journal of Medicine 293, 605.

WATTENBERG, L. W., PAGE, M. A., LEONG, J. L. (1968) Induction of increased benzpyrene hydroxylase activity by flavones and related compounds.
Cancer Research 28, 934.

WATTENBERG, L. W. (1972) Dietary modification of intestinal and pulmonary aryl hydrocarbon hydroxylase activity.
Toxicology and Applied Pharmacology 23, 741.

WEIBEL, E. R., STÄUBLI, W., GNÄGI, H. R., HESS, F. A. (1969)
Correlated morphometric and biochemical studies on the liver cell.
I Morphometric model, stereologic methods and normal morphometric data for rat liver.
Journal of Cell Biology 42, 68.

[illegible], [illegible], [illegible], [illegible], [illegible], J. J. (1954) Observations on the metabolic transformation and effects of phenylbutazone in subjects with hepatic disease.
American Journal of the Medical Sciences, [illegible], 56.

[illegible], [illegible], HARRISON, [illegible], [illegible], J. J. (1967) Implications of enzyme induction in drug toxicity studies.
Toxicology and Applied Pharmacology 10, 340.

[illegible], [illegible], [illegible], [illegible], [illegible], [illegible], [illegible], [illegible] (19[illegible]) Stimulatory effect of cigarette smoking on the hydroxylation of 3, 4-benzpyrene and the N-demethylation of 3-methyl 4-monomethyl aminoazobenzene by enzymes in human placenta.
Clinical Pharmacology and Therapeutics 10, 100.

[illegible], [illegible], [illegible], [illegible], [illegible] (1964) Effect of diphenylhydantoin on cortisol metabolism in man.
Journal of Clinical Investigation 43, 1324.

[illegible], [illegible], [illegible], [illegible], [illegible], [illegible], [illegible] (1971) Cortisol production in epileptic patients treated with diphenylhydantoin.
Clinical Pharmacology and Therapeutics 12, 628.

[illegible], [illegible], [illegible], [illegible], [illegible] (19[illegible]) Hepatic storage and excretion of sulfobromophthalein sodium in the dog.
Journal of Clinical Investigation 3[illegible], 236.

[illegible], [illegible], [illegible], J. L., BRADLEY, S. E. (19[illegible]) Biliary transport and hepatic storage of sulfobromophthalein sodium in the unanesthetized dog, in normal man and in patients with hepatic disease.
Journal of Clinical Investigation 3[illegible], 1131.

[illegible], [illegible], [illegible], [illegible] (1972) Thymocytotoxic [illegible] activity in patients with active chronic hepatitis and primary biliary cirrhosis [illegible] treated with azathiprine.
Gut 13, [illegible].

[illegible], [illegible], [illegible], [illegible], [illegible] (1913) Tests for hepatic function and disease under experimental conditions. Phenoltetrachlorphthalein.
Bulletin of the Johns Hopkins Hospital 24, 343.

[illegible], [illegible], [illegible], [illegible], [illegible], [illegible] (1973) Changes in plasma γ glutamyl transpeptidase activity associated with alterations in drug metabolism in man.
British Medical Journal 1, [illegible].

[illegible], [illegible], [illegible], [illegible], J. L. (1971) The effect of hepatic blood flow on the hepatic removal rate of oxyphenbutazone in the dog.
Journal of Pharmacology and Experimental Therapeutics 177, 246.

[illegible], J. [illegible], [illegible], P. [illegible]. (1970) Genetic control of phenylbutazone metabolism in man.
British Medical Journal 4, 303.

[illegible], P. G. (1971) Species variations in drug biotransformation, in "Fundamentals of Drug Metabolism and Disposition".
W. Williams and Wilkins Co., Baltimore.

[illegible], R. T. (1971) The metabolism of certain drugs and food chemicals in man.
Annals of the New York Academy of Science 179, 141.

[illegible], R. T., (1972) Hepatic metabolism of drugs.
Gut 13, 579.

[illegible], J. T. (1971) Caution with phenobarbital.
Clinical Pediatrics 10, 684

[illegible], K., [illegible], S., [illegible], T. (1969) The hepatic metabolism of ethanol in patients with cirrhosis of the liver.
Scandinavian Journal of Clinical and Laboratory Investigation 23, 59.

[illegible], . . (1971) Variable effect of phenobarbital treatment of mice on hepatic UDP glucuronyl transferase activity when judged by slightly different enzyme assay techniques.
Biochemical Pharmacology 20, 1253.

WITT, G. K. (1963) Acute intermittent porphyria: Family studies on the excretion of PBG and delta-ALA with ion exchange chromatography.
Klin Chem Klin Biochem
1, 134.

[illegible], G. K. (1971) Toxic porphyria after treatment with sulphonal and trional.
South African Medical Journal 45, 133.

WOOLF, I. L., [illegible], B. M. (1972) Vitamin K and warfarin.
American Journal of Medicine 53, 261.

[illegible], J. A. (1965) Primidone in the treatment of major epilepsy.
Epilepsia 6, 67.

[illegible], C. J., [illegible], C., [illegible], T., [illegible], T. (1966) Enhancement of glucuronide - conjugating capacity in a hyperbilirubinemic infant due to apparent enzyme induction by phenobarbital.
New England Journal of Medicine 275, 1461

YEUNG, C. Y., TAM, L. S., CHAN, A., LEE, K. H. (1971) Phenobarbitone prophylaxis for neonatal hyperbilirubinaemia.
Pediatrics 48, 372.

YOSHIMURA, H. (1969) Metabolism of drugs LXII. Isolation and identification of morphine glucuronides in urine and bile of rabbits.
Biochemical Pharmacology 18, 279.

ZAIMIS, E. J. (1953) Motor end plate differences as a determining factor in the mode of action of neuromuscular blocking substances.
Journal of Physiology 122, 238.

ZEIDENBERG, P., ORRENIUS, S., ERNSTER, L. (1967) Increased levels of glucuronylating enzymes and associated rises in activities of mitochondrial oxidative enzymes upon phenobarbital administration in the rat.
Journal of Cell Biology 32, 528.