GENETIC SUSCEPTIBILITY TO ADVERSE DRUG REACTIONS: A CLINICAL EXPRESSION OF DEFICIENT DRUG OXIDATION PHENOTYPE

Thesis submitted by

RASHMIKANT RASIKLAL SHAH

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Department of Pharmacology,
St Mary's Hospital Medical School,
Paddington,
LONDON W2 1PG

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DEDICATION

To

My Parents
With love and gratitude

and

My Son, Devron
who has brought me
great joy and happiness

ABSTRACT

1. Apart from their morbid and mortal consequences, adverse drug reactions(ADR's) have serious socio-economic impacts. A watch-repairer with perhexiline-induced neuropathy illustrates the magnitude of tragedy.

2. Most ADR's have pharmacokinetic basis. Drug metabolism, the major pharmacokinetic determinant of drug effect, is under marked genetic control. Acetylation polymorphism exemplifies the vital role of genetic factors in drug response.

3. However, the major route for drug metabolism is oxidation. Recently, human studies with debrisoquine have uncovered genetic polymorphism in drug oxidation. Oxidative 4-hydroxylation of debrisoquine is controlled by two alleles, D^H and D^L, at a single gene locus. The autosomal recessive inheritance of the variant (D^L) allele, responsible for impairment in oxidation, yields two population phenotypes: Extensive (EM's , about 91%) and poor (PM's , about 9%) metabolisers. Available evidence suggests that the same alleles probably control oxidations of other drugs.

4. Phenformin oxidation was studied in a previously debrisoquine-phenotyped panel. The results strongly suggested its genetic control with wide variation in inter-individual capacity. Population and family studies confirmed this. Poor metabolisers attained higher plasma phenformin and rise in blood lactate levels. This, together with impaired debrisoquine

oxidation in patients surviving phenformin-induced lactic acidosis, confirmed previous suspicions of an individual genetic diathesis and suggested a higher prevalence of this toxicity than hitherto reported.

5. Maturity-onset diabetic patients, not normally at risk, were found to have enhanced drug oxidising capacity. This results in recommendations of standard phenformin dosage which further prejudices those already at risk. Consequently, guide-lines, based on non-genetic epidemiological factors, have failed to contain phenformin toxicity.

6. Retrospective studies further emphasized the role of impaired debrisoquine oxidation status in the diathesis to another ADR studied: perhexiline-induced neuropathy. Prospectively, debrisoquine oxidation status proved to be predictive in alerting to the presence of subclinical neuropathy due to perhexiline.

7. Prior phenotyping of the watch-repairer would have prevented his tragedy.

8. In conclusion, phenotyped panel approach can be usefully exploited to establish co-inheritance of drug oxidations and debrisoquine phenotyping test offers a means to safer use of these drugs.

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A STATEMENT ON THE SHARE OF AUTHOR'S WORK

I have been responsible for designing, initiating and executing three population studies to estimate the global heterogeneity of the poor metaboliser phenotype of debrisoquine. These studies included the populations from India, Bangladesh and South African Xhosa tribe. I have also been responsible for analysis of urine samples, and the data therefrom, from India and Bangladesh.

All the studies on phenformin oxidation were designed and executed by me. I have been responsible for ethical as well as scientific aspects of recruiting and dosing of volunteers and collecting all the appropriate samples. Urinary contents of phenformin and 4-hydroxy-phenformin were measured by Dr Oates by the h.p.l.c. method developed in the Department. I was closely and actively involved in the development of this method. I have also been responsible for making the deductions from the data and initiating further studies as appropriate.

I have personally been responsible for the studies on all the families during the investigations on pharmacogenetics of phenformin metabolism, individual consistency of phenformin oxidation, correlation between debrisoquine oxidation and phenformin oxidation, effect of phenformin dose on its oxidation and the phenotypic differences in the metabolic response to phenformin.

The patients with and without phenformin-induced lactic acidosis were all identified and recruited by me and I phenotyped them, including analysis of urine.

The study on drug oxidation profile in maturity-onset diabetes was initiated and executed by me. All the urine samples were analysed by me and I have been solely responsible for the presentation of and deductions from the data derived. Dr Sloan gave me useful statistical advice.

The studies described in Chapter 8, including statistics, are wholly my own work. This included designing and executing the studies after recruiting the appropriate 72 patients. Their urine samples were analysed by me. The three patients described in Section 8.6 are the patients I have been managing in the Cardiology Out-patients department and all the studies on them are also my own work except i) the six electromyograms were performed by the Dept. of Diagnostic Neurology and ii) the three plasma levels of perhexiline were measured by Mr J.D.H. Cooper at Coventry and Warwickshire Hospital, Coventry.

Routine serum biochemistry was carried out by the Dept. of Diagnostic Chemical Pathology at St. Mary's Hospital, London W2.

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C H A P T E R O N E

A CASE HISTORY

A watch-repairer with perhexiline-induced neuropathy

A 55-year old male watch-repairer started experiencing retrosternal chest pain on exertion seven years ago. The pain radiated down the left arm and was relieved by resting. A diagnosis of ischaemic heart disease was confirmed. The patient had reduced his smoking from 55 cigarettes daily eight years ago, to about 10 cigarettes daily over the last four years. There was no history of hypertension or diabetes. His cardiovascular status was otherwise satisfactory and he had no abnormalities of his respiratory, alimentary, renal, neurological and musculo-skeletal systems. Biochemical investigations did not show any evidence of hepatic or renal dysfunction.

Over a period of seven years, his effort tolerance began to deteriorate progressively and a diagnosis of intractable angina was made. His therapeutic response to conventional anti-anginal agents - nitrates and beta-blockers - was not adequate. It was therefore decided to put him on perhexiline, then a recently introduced calcium antagonist, which had received very favourable reports from various clinical trials.

He was commenced on a conventional standard regime, that is 100 mg bd, and a month later, encouraged by some improvement in his angina without any untoward effects, this dose was increased to 100 mg tds. An excellent anti-anginal response was obtained and the patient's anginal episodes virtually disappeared with an accompanying increase in his effort tolerance.

Five months later, the patient then began to experience distal paraesthesia of both upper and lower limbs and weight loss. A further seven months later he presented with paraesthesia, dysphagia, weight loss of 16 kg in the preceding 12 months, tinnitus, dyskinesia, ataxia, slurred speech and blurred vision. He was unable to carry out fine movements and, therefore, work or write. He was unsteady and falling about; his vision and his speech were grossly impaired.

On examination of his nervous system, he had papilloedema and cerebellar signs. He also had evidence of sensory and motor neuropathy involving his cranial and peripheral nerves. A diagnosis of a primary occult malignancy with secondary neurological effects was made.

In order to localise the presumptive primary occult malignancy, the following investigations were performed over the ensuing three weeks. His anti-anginal treatment was continued during these investigations.

Investigations

Full blood count, urea and electrolytes, acid phosphatase.

Sputum for acid fast bacilli and malignant cells.

VDRL, TPHA and antibody studies (except anti-nuclear factor).

Serum bilirubin, albumin and globulin.

The results of all the above investigations were normal.

Chest Xray: Normal.

Bronchoscopy: Normal.

EMI scan: Normal.

Air encephalogram: Normal.

Isotope brain scan: Normal.

Barium enema: Normal.

Barium swallow confirmed dysphagia due to dysfunctional swallowing mechanism with considerable spill of the barium into the trachea.

Lumbar puncture: Normal except for raised CSF protein.

Anti-nuclear factor: Positive 1/100.

Serum aspartate transaminase (AST) 126 U/l.

Serum alkaline phosphatase 101 U/l.

Electrocardiogram showed evidence of ST segment depression and T wave flattening.

These investigations failed to pinpoint any malignancy. Nerve conduction studies were performed and showed marked slowing of conduction in the right ulnar nerve at 29 m/sec with a distal latency of 5.5 msec. Similarly, prolonged distal latency in the right lateral popliteal nerve with a conduction velocity of 22 m/sec confirmed neuropathy. A biopsy of the left sural nerve showed gross depletion of myelinated nerve fibres but there were no specific changes on light microscopy. A fluorescence angiogram showed early leak at the right optic disc confirming the presence of papilloedema.

In view of the occasional reports that had appeared in the literature in the preceding months at that time, it was then suspected whether the clinical presentation of the patient could be due to perhexiline-induced adverse drug reaction.

Perhexiline was discontinued and the patient began to regain his lost weight. There was mild to moderate improvement in his neurological status, but not complete resolution, over the next six months. His angina recurred and he was considered for coronary artery bypass grafting. His coronary artery disease was considered amenable to surgical management. However, the insertion of coronary bypass grafts was deferred until there was further improvement in his neurological status, and he was discharged to outpatients for follow up.

When further reviewed two years after discharge, the patient was still suffering from moderate to severe dysphagia, dyskinesia, ataxia and paraesthesia. His angina was also not responding satisfactorily to other drugs used. His current medication consisted of:

Nifedipine 10 mg tds

Atenolol 100 mg tds

Warfarin 12 mg daily

Isosorbide dinitrate 20 mg tds

Dipyridamole 100 mg tds

Multivite tab one tds

Distalgesic tabs 2 bd and

Glyceryl trinitrin tab one sublingually prn

Consequent upon his physical status, the patient was confined largely to his home and had lost his job. He found it difficult to swallow his tablets and eat or drink satisfactorily. His wife had to commence working part-time and his daughter returned home to assist in his management. The patient and his family are bewildered and full of a sense of misfortune.

How does one ever begin to estimate the socio-economic consequences of such a tragedy? It is obvious that a drug which has benefitted numerous other patients has had devastating effects on this patient. He exemplifies how our modern awareness of a statement, made about 2000 years ago, is derived from adverse drug reactions:

"What is food to one man
may be a fierce poison to others"

Lucretius (99-56 B.C.)
De Rerum Natura, IV

C H A P T E R T W O

INTRODUCTION TO ADVERSE DRUG REACTIONS AND THEIR IMPACT

2.1 Introduction

The case history outlined in the preceding pages is only one of the many examples to illustrate the tragic consequences of the adverse effects of drugs in susceptible individuals. It also highlights the morbid and economic sequelae of adverse drug reactions with subsequent disruption of the patients' social life-style.

Over the last few decades, there has been an explosive increase in the number of drugs available for combating human suffering. The World Health Organisation (1970) has defined a drug as "any substance or product that is used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the recipient". However, the advances in the therapeutic options available have not been without concurrent increase in the harmful effects of these drugs. Barr (1955) clearly recognised this well over a quarter century ago. It is recognised that, even discounting the accidental or intentional attempts at overdoses, the therapeutic use of every known drug is associated with adverse drug reactions in many individuals.

Many definitions of adverse drug reactions have been advanced. The World Health Organisation's definition has been modified by Karch and Lasagna (1976). These workers have defined adverse drug reaction as "any response to a drug which is noxious and unintended and which occurs at doses used in man for prophylaxis, diagnosis or therapy, excluding therapeutic failures". This definition has the attraction of including idiosyncratic reactions as well as hypersensitivity and

excessive pharmacological effects but excluding drug overdoses, abuses, therapeutic failures and patient non-compliance.

2.2 Frequency of adverse drug reactions

For obvious reasons, it has not proved possible to quantify the exact incidence of adverse drug reactions associated with any particular drug. Often the appearance of an undesired effect is not recognised as being due to a drug. Leaving aside the natural reluctance of most doctors to accept that a patient may have been harmed by their treatment, there are five main reasons for this (Vere, 1976):

1. The reaction may be so odd and bizarre that an often used and apparently innocent drug escapes suspicion.
2. The drug induced disorder closely mimics a common natural disease.
3. There is a long delay in the appearance of the adverse effect.
4. The drug evokes a relapse of a natural disease or evokes a disorder in a naturally susceptible subject.
5. The clinical situation is so complex that its drug related components pass unnoticed.

In many cases, the medical practitioner does not report all the drug reactions that are encountered because "this effect is well known now". Often the practitioner, for a variety of reasons, may feel reluctant to report an adverse drug reaction.

It is estimated that in the United Kingdom, only 10% or less of the adverse drug reactions are reported by doctors (Inman, 1977; Ashley, 1980). The system of collection of data on drug reactions relies on the voluntary co-operation of doctors to use the yellow card system. In Sweden, where the reporting of all drug reactions to a central adverse drug reaction committee is compulsory, it is thought that the overall reporting frequency is in the order of 30% (Bottiger and Westerholm, 1973), with greater reporting of severe or fatal reactions (Bottiger, Furhoff and Holmberg, 1979).

2.3 Consequences of adverse drug reactions

In view of the poor recognition and reporting of adverse drug reactions, it is difficult to assess the morbid, the mortal and the economic consequences of drug reactions. A few surveys are, however, available from some centres.

Adverse drug reactions have recently been reported to occur in 40% of patients receiving drugs in general practice (Rawlins, 1981), and 10-15% of hospital patients (Hurwitz and Wade, 1969; McMahon et al, 1977). A frequency as high as 28% has also been reported (Miller, 1974). They account for 2.5% of all the consultations in general practice (Mulroy, 1973). Various studies available suggest that the frequency of hospital admissions due to drug reactions is in the range of 1.8-5.7% (Anonymous, 1981). George and Kingscombe (1980) recently reported that of the 250 consecutive admissions to the professorial medical unit, 19.6% had adverse drug reactions. Of these, 2.4% occurred in hospital and 17.2% before admission. In 14.8%, drug toxicity was the sole cause for hospital admission. It has been estimated that in the U.K., some 5% of the beds in the general hospital are occupied by patients suffering to a variable extent from the attempts to treat them (Dunlop, 1969). The Food and Drugs Administration estimated that in the United States, about 14% of all hospital beds were occupied by patients under treatment for adverse reactions caused by drugs (Melmon, 1969). A similar study in

Belfast demonstrated that about 12% of the patients either were hospitalised or had their hospitalisation prolonged because of adverse drug reactions (Hurwitz, 1969). A recent joint study from Jerusalem and Berlin reported that 4.1% of medical admissions in Jerusalem and 5.7% in Berlin were due to side-effects of the drugs (Levy et al, 1980). Steel et al, (1981) found that 36% of 815 patients on a general medical service of a university hospital had iatrogenic illnesses and in 9% of all these patients, the incidence was considered to be major, in so far as it threatened life or produced considerable disability. These workers, of course, recognised that the high incidence they found may be due to the fact that the data collected for their study were derived from a tertiary care hospital where admissions are necessarily pre-selected, making the presence of drug reactions fortuitously more dramatic.

The mortality figures resulting from drug reactions also make sad reading for the physician. A study from New Zealand reported that in 2.7% of cases, drugs contributed to deaths occurring in the hospital (Smidt and McQueen, 1972), while the Boston Collaborative Drug Surveillance Program reported an overall mortality rate of 0.44% for drug reactions in hospitalised patients (Jick et al, 1970). The Jerusalem/Berlin study (Levy et al, 1980) noted that in Jerusalem 4.9% of all admissions due to drug reactions (that is 0.2% of all admissions) died as a result of suspected drug

reactions, while Steel and his colleagues (1981) reported that iatrogenic illness contributed to the death of 2% of their 815 patients. In the U.S.A.,deaths occurring as a result of adverse drug reactions have been thought to be between 30,000 to 140,000 per year (Koch-Weser, 1974), the disagreement on exact figure being due to the debate on methods used to arriving at the estimate. In Sweden, the adverse drug reaction committee received 25-30 reports of fatalities due to drug reactions every year from 1966-1975 (Bottiger, Furhoff and Holmberg, 1979). The total ten year adverse drug reaction reports were 11,596 and of this, 274 were fatal, an incidence of 2.4% of all adverse drug reactions. After carefully analysing all the surveys, Karch and Lasagna (1975) concluded that the range of fatal drug reactions is 0-0.31% of all hospital medical in-patients.

In the context of drug reactions, it is curious to note that while a surgeon carefully selects his patient for surgery and talks in terms of 0.1-1% mortality figures for his operation, the physician does not appear to exercise such restraints and cautions in prescribing drugs to his patients. An example of this is provided by phenformin, an orally active hypoglycaemic biguanide drug, used in the treatment of diabetes. Following its introduction, this drug was widely prescribed for maturity-onset diabetes. In susceptible patients, the drug is known to produce lactic acidosis which has a mortality of 50-70%. This toxicity

accounted for 21 of the 274 (8%) deaths reported to the Swedish adverse drug reactions committee. Curiously, and sadly, the prescribing pattern in latter years does not reflect any attempts to identify the at-risk individuals.

Very few estimates of the cost of drug reactions are available. In the Belfast study quoted above (Hurwitz, 1969), it was calculated that the additional costs due to adverse drug reactions amounted to 4.5% of the total cost of hospitalisation and treatment of all the patients during the study period. In the U.S.A., the economic losses due to adverse drug reactions were calculated in 1971 to amount to 3,000 million dollars annually (Melmon, 1971).

2.4 Recognition, reporting and prevention of adverse drug reactions

The magnitude of the problems caused by adverse reactions due to drugs is now clearly, but slowly, recognised. It is important that utmost care be exercised for their prevention, whenever and wherever possible. The progression of drug reactions in other cases must be halted through early recognition.

Early recognition of drug reaction requires an efficient system for reporting any unusual events associated, or suspected to be associated, with drug usage and subsequent widespread dissemination of information thus collected. The physician should report all the drug reactions to a locally based centre for onward passage to a national body. In turn, the physicians will be kept fully informed on the safety aspects of the drug in use. This system has been operating in the West Midlands with considerable success.

Whether or not the reporting of drug reactions should be made compulsory is a matter of debate, but there must be a lesson in the figures available; in Sweden, with a population of about 8 million, the Swedish adverse drug reactions committee received 11,596 reports from 1966-1975, an annual reporting rate of 145 per million. In the U.K., with a population of 56 million, the Committee on Safety of Medicines received 100,000 during the period 1964-1981 (Goldberg, 1981), an annual reporting rate of 105 per million. The reporting rate in Sweden is about 30%, while it is 10% in the U.K.

It appears that the lower level of reporting is due to the tedium of form-filling and further enquiries and therefore, whatever system is employed for the purpose, it would have to incorporate a mechanism to avoid this. It may be that a pharmacy-based drug safety officer could co-ordinate the reporting of drug reactions to a national body and keep the doctors fully informed on the drug safety aspects. Professor Rawlins of the University of Newcastle has recently suggested that there is a case for devolution of some of the functions of the Adverse Reactions Subcommittee of the Committee on Safety of Medicines (Anonymous, 1979).

Before a drug is fully released for general use in the market, new drugs are often made available on a restricted basis. This involves accurate data to be kept for the drug used on a named prescription basis. However, such a system has the drawback in that it will detect only short-term toxic effects. Often, certain drug reactions escape detection because they make an appearance only after long-term use. This was, for example, the case with practolol-induced oculo-cutaneous syndrome. Since the patients in society at large are first likely to approach their general practitioner in case of a problem, it appears that the most effective means of detecting drug toxicity will be at general practitioner level, particularly if the practitioner maintains a high index of suspicion all the time.

In order to detect long-term events, Inman has recently set up a system of post-marketing surveillance of adverse drug reactions in general practice (Inman, 1981a; Inman, 1981b). It is hoped that the system involving general practitioners would allow early detection of any adverse drug event with an incidence of more than 1 in 1000 patients. Valuable information has already been obtained in its first trial by this system. Benoxaprofen was detected to cause bladder problems and internal bleeding (Anonymous, 1982). Dollery (1981) has emphasized the usefulness of randomised controlled clinical trials as a source of information about side-effects and toxicity. The Medical Research Council's hypertension trial is a very good example. By collecting information about withdrawal from randomised treatment and administering symptom questionnaires, some interesting new data were derived about the long established anti-hypertensive drugs bendrofluazide and propranolol. Both these methods demonstrated that bendrofluazide can cause impotence in the male, an important side-effect that had not previously been recognised (Greenberg, 1981).

The above methods, however, only confirm the association of certain reactions with particular drugs, and they also increase the practitioner's awareness of recognition when they occur. These methods are orientated to detecting drug reactions and not preventing them. Greater challenge and rewards lie in the prevention of drug reactions. Prevention of adverse drug reactions can only be achieved if:

a) individuals at risk can be identified and

b) the mechanisms of adverse drug reactions are appreciated.

The general mechanisms of adverse drug reactions are reviewed in the following Chapter, while the identification of the individuals at risk is discussed later.

C H A P T E R T H R E E

MECHANISMS OF ADVERSE DRUG REACTIONS

3.1 Introduction

In order to understand the mechanisms of adverse drug reactions, a classification of drug reactions is necessary. Various classifications have been devised but most of these have had some drawbacks. Rawlins and Thompson (1977) have recently suggested a useful classification of adverse drug reactions into two types; those which form part of a drug's normal pharmacological action (Type A) and those which represent abnormal response (Type B). The features of these two types are shown in Table 1.

Type A reactions are augmentation of either the primary or secondary pharmacological actions of drugs. Provided the detailed pharmacology of the drug is known, the kind of reaction is predictable. It is therefore dose-dependent and although the morbidity is high, mortality is low. These reactions are, by and large, the common types to be encountered. These reactions are commonly encountered in individuals who are nearer the extreme ends of the pharmacological dose-response curves. Indeed, it has been estimated that over 80% of major adverse drug effects represent excessive drug effects in susceptible individuals (Caranasos, Stewart and Cluff, 1974; Ogilvie and Ruedy, 1967).

Type B reactions, by contrast, are bizarre. They are qualitatively abnormal effects, apparently not related to a drug's known primary or secondary pharmacological effects.

Table 1: Classification of adverse drug reactions

FEATURES	TYPE A	TYPE B
Pharmacology	Augmented	Bizarre
Predictable	Yes	No
Dose-dependent	Yes	No
Morbidity	High	Low
Mortality	Low	High

(After Rawlins, 1981)

They are less predictable and often not dose-dependent. These reactions generally occur far less frequently and although the morbidity due to them is low, they have higher mortality.

This classification has minor drawbacks in so far as it apparently fails to find a slot for drug reactions due to withdrawal of a drug like hypercoaguability that follows abrupt warfarin withdrawal or excessive beta-receptor activity following abrupt withdrawal of beta-blockers. The classification,however, recognises that as pharmacological knowledge of a drug increases, various Type B reactions in future may need to be reallocated. Nevertheless, the classification provides a useful background against which to discuss the mechanisms of adverse drug reactions.

Drug reactions are mediated either by the metabolic or the immunologic mechanisms. In general, Type A reactions have a metabolic basis, while the majority of Type B reactions have an immunologic basis to them. The distinction is not so clear-cut but it does have the attraction of simplicity. Moreover, there is an element of overlap. For example, when a drug is antigenic per se - for instance anti-sera and polypeptides - the development of immunological effects is predictably Type A whilst certain Type B reactions, for example, practolol-induced oculo-cutaneous syndrome, may be due to the presence of antibodies in human serum which react with the product of drug metabolism,

acting as a hapten (Amos, 1980). Drug-induced systemic lupus erythematosus is another example of the latter kind (Harpey, 1973; Assem, 1977).

Immunological mechanisms, responsible for Type B reactions, account for a small number of total adverse drug reactions. The morbidity is therefore low. These reactions can sometimes, though not invariably, be predicted especially if a detailed history is obtained. Sometimes one is warned on the probability of this reaction by the patient's history of eczema and atopy. Patient's past medical history or family history provides valuable information. Motulsky (1957) considered that it is possible that the potential for antibody formation due to haptens, and therefore susceptibility to drug reactions, is conditioned by genetic factors. At present, however, little is known about the pathogenesis of such reactions. When the reaction does occur, complete withdrawal of the drug is necessary.

Metabolic mechanisms, responsible for Type A reactions, form the basis of a wide variety of drug reactions. The final trigger to the precipitation of an adverse drug reaction by these mechanisms is relatively inappropriate levels of the parent drug or its metabolites, either in the blood, tissues or at the receptor sites. It is important to stress that the drug/metabolite levels are inappropriate only relatively because such levels could result from one or both kinds of the following two factors:

1. Pharmacodynamic.
2. Pharmacokinetic.

The inappropriate levels of a drug or its metabolites, operating either pharmacokinetically or pharmacodynamically, could arise as a result of misadventure or more often, are genetically determined. Causes included under misadventure comprise overdoses, which may be either accidental or intentional, multiple drug therapy or failure to take full account of co-existing multiple pathologies in any one patient. Overdoses may also result from prescribing errors such as in dose or duration or from patient error. Often, patients are on multiple drug therapies because either one drug alone is ineffective or the patient has multiple pathologies. The risk of a drug interaction arises exponentially with the number of drugs given simultaneously (Smith, Siedl and Cluff, 1966). This is particularly so when one drug modifies the pharmacokinetics of the other with a low therapeutic-to-toxic ratio. Co-existing pathologies also play a large part in determining drug response. The factors responsible are blood flow through various organs, which is often dictated by the cardiac output and the state of vasculature and disease-related variations in drug absorption, distribution, metabolism or excretion. In many diseases, qualitative and quantitative changes in drug receptor function can be discerned, giving rise to altered drug responses. Some of these causes are considered later.

It follows, however, that adverse drug reactions from such causes should be predictable as well as preventable, given that a high index of suspicion and vigilance is always maintained.

More often than not, however, adverse drug reactions make an appearance despite the consideration of concurrent drug therapy and co-existing multiple pathologies. It is now recognised that such reactions are genetically determined. Motulsky recognised, as far back as 1957, that drug reactions may be caused by otherwise innocuous genetic traits or enzyme deficiencies.

3.2 Pharmacodynamic factors

Alterations in tissue receptor function, qualitative or quantitative, account for abnormal drug responses in a number of cases. The best example of iatrogenic pharmacodynamic response is to be found with digoxin. A patient can be adequately digitalised and show normal response on a maintenance dose. However, digoxin toxicity due to increased receptor sensitivity soon appears when the serum potassium levels are allowed to drop below a critical level (Dubnow and Burchell, 1965). Similarly, nephrotoxicity of, for example, cephalothin is easily potentiated by concomitant administration of frusemide (Lawson et al, 1972) or gentamicin (Bobrow, Jaffe and Young, 1972). A relatively larger number of abnormal pharmacodynamic responses are known to be genetically determined. The response could be either an increased or a decreased pharmacological effect or more often, an atypical response is produced.

A. Increased response

1) Chloramphenicol sensitivity:

Although chloramphenicol causes dose-dependent reversible bone marrow suppression in all subjects, probably due to mitochondrial injury, some subjects are hypersensitive and develop marrow aplasia (Yunis, 1973). Twin studies have suggested a genetic contribution (Nagao and Mauer, 1969).

Studies involving marrow cultures from susceptible individuals and family members have confirmed this (Yunis and Harrington, 1960; Yunis, 1973).

2) Atropine hypersensitivity:

Harris and Goodman (1968) have shown that subjects with Down's syndrome are particularly sensitive to the mydriatic and cardio-acceleratory effects of atropine, but not to the vagotonic effects.

3) Ethanol sensitivity:

Increased sensitivity to the effects of ethanol, in the presence of its normal metabolism, has been described by Wolff (1972) in certain oriental groups. This is particularly seen in mongoloid stock of Japan, Taiwan and Korea. Marked facial flushing and intoxication reflect the hypersensitivity.

B. Decreased response

1) Mydriatics:

Marked racial differences in mydriatic response to ephedrine and related eye drops have been reported. Negroes are virtually insensitive to the locally applied mydriatic doses of these drugs, compared to the caucasians who respond by pupillary dilatation (Chen and Poth, 1929). These effects are thought to be due to altered beta-receptor function in the iris.

2) Coumarin anticoagulants:

O'Reilly (1970) described two large pedigrees in which an autosomal dominant transmission of warfarin resistance was found. The anticoagulant dose in affected individuals is 5-20 times that required in normal subjects. The absorption, distribution, metabolism and excretion of warfarin were all normal in these individuals. Vitamin K epoxide reductase is thought to have decreased affinity for Vitamin K and warfarin in these individuals, the affinity being more markedly low for warfarin.

C. Atypical response

A few examples of atypical pharmacodynamic responses are now known and these are all genetically determined. Examples of such responses include haemolysis following administration of oxidant drugs to individuals with glucose-6-phosphate dehydrogenase deficiency (Marks and Banks, 1965). This was first reported with the anti-malarial drug primaquine within a year of its introduction (Cordes, 1926). Subsequently, many other drugs have been reported to produce this response. Other examples of atypical pharmacodynamic responses are acute abdominal crisis in intermittent porphyria following administration of certain enzyme-inducing drugs (Brodie, 1977), malignant hyper-pyrexia following certain inhalational anaesthetics (Kalow, 1972), glaucoma induced by steroids (Armaly, 1968) and

methaemoglobinaemia occurring in individuals with haemoglobin-M given a variety of drugs (Kiese, 1966).

Drug reactions arising from pharmacodynamic interactions, except digoxin toxicity, account for only a very small number of the total (Mucklow, 1978) and are predictable and preventable if appropriate and careful family history is elicited.

3.3 Pharmacokinetic factors

The pharmacokinetic factors are the major determinants of the levels of drug and its metabolites. Therefore, these factors also determine the duration and intensity of the actions of drugs, and hence the drug response. Drug absorption, distribution, metabolism and excretion dictate the pharmacokinetic profile of a drug and are much more often responsible for the variation in individual responses to drugs than are the differences in target organ sensitivity consequent upon altered receptor function. The sub-division of pharmacokinetic factors into absorption, distribution, metabolism and excretion affords a further convenient means of describing mechanisms of adverse drug reactions. Of these four, metabolism is by far the most important parameter responsible for wide inter-individual variation in drug response (Brodie and Reid, 1967; Rawlins, 1975). Shannon (1946) in fact recognised this as long ago as 1945/46 when he showed that the major variable in the clinical screening of anti-malarial drugs was the individual rate of drug metabolism and that the drug action correlated much better with the plasma levels than the drug dosage.

1. Drug absorption:

Most drugs, given orally, are absorbed from the stomach or the small intestine. A number of factors can modify the absorption of a drug (Grover, 1979).

The drug formulation is an important characteristic; the particle size, with its physico-chemical characteristics, can affect the bioavailability of a drug. Often, concurrently administered drugs alter the absorption of a drug either by chelation or competition for common transport processes of absorption. The question of bioavailability becomes very important in cases of drugs like digoxin or phenytoin with low therapeutic-to-toxic ratios. Sometimes drug toxicity is attributable to the adjuncts or vehicles used or contaminations introduced during synthesis of a drug. Ingredient-vehicle interactions are particularly important in cases of solutions used for delivering slow intravenous infusions of a drug. Contamination of a drug can also result from decomposition of active ingredients into toxic substances through improper storage. Nowadays, the formulation-related drug toxicity should be rare in view of the strict quality control imposed by various statutory bodies.

Other predictable factors altering drug absorption are gastric emptying time, intestinal motility, intestinal pathology or changes in gut enzymes.

Theoretically, the absorption of any drug could be under genetic control but up to date, only one well documented example of this is known. This concerns the absorption of Vitamin B_{12} in juvenile pernicious anaemia.

The deficit is thought to be related to intrinsic factor. It is absent in one form of this condition (McIntyre et al, 1965), is normal but unable to promote Vitamin B_{12} in the other form (Katz, Lee and Cooper, 1972), while the third form of this condition is characterised by impaired absorption of Vitamin B_{12}-intrinsic factor complex.

2. Drug distribution

When an orally administered drug is absorbed, it passes through liver and is then distributed throughout the body. It may be confined to fluid compartments and/or be sequestrated by tissues. Many drugs are highly protein bound and protein binding determines its distribution. It is the unbound fraction that is responsible for exerting the pharmacological effect of a drug. Alteration in distribution, therefore, can lead to abnormal drug responses and can arise from a number of reasons.

Levels of plasma proteins will greatly determine the absolute quantity of free drug. Occasionally, various drugs share a common protein binding site. In such cases, it must be appreciated that a change in protein binding from 98% to 96% could increase the active fraction by two-fold; an exaggerated drug response would result since the free fraction is the active fraction. The effects due to decreased protein binding tend to be exaggerated if the displaced drug has smaller apparent volume of distribution. An example of

this is the displacement of warfarin by phenylbutazone with potentiation of the anticoagulant activity (Aggler et al, 1967). Rapid alteration in body fluid volumes or plasma proteins, for example in congestive cardiac failure, pleural effusions or ascites, can also give rise to changes in drug distribution.

Drug distribution is also subject to genetic control but only a few examples are known. In hereditary analbuminaemia, protein binding is absent and therefore an exaggerated drug response occurs due to a greatly increased free fraction of the drug. Hypoalbuminaemia, which can be hereditary or acquired, would similarly result in increased free fraction of a number of drugs, for example, prednisolone (Lewis et al, 1971). In Down's syndrome, altered protein binding results in increased levels of free salicylate (Ebadi and Kugel, 1970). Congenital abnormalities of alpha-globulin, the thyroxine binding globulin, have been reported but these have not been shown to have any clinical significance.

In presence of normal plasma proteins, large inter-individual differences in the distribution of a number of drugs have been reported; these drugs include oxazepam, diazepam and particularly, nortriptyline (Smith and Rawlins, 1973). Although the physical basis of these differences is not at present clearly defined, twin studies with nortriptyline suggest a substantial genetic control (Alexanderson, 1972). Nortriptyline, as will be seen later, is a particularly interesting drug since its metabolism too is under genetic control.

3. Drug excretion

Adverse drug reactions are more common in patients with renal diseases and the factors that contribute to producing drug reactions in these patients include decreased renal clearance of drug and/or its metabolites, altered receptor sensitivity, plasma protein binding, volume of distribution, impaired renal metabolism of certain drugs or acid-base electrolyte imbalances. Many polar drugs are excreted unchanged in the urine and renal clearance becomes a major determinant of the plasma half-life of these drugs (Prescott, 1979). For drugs which are metabolised to more polar metabolites prior to their renal elimination, impaired renal function contributes to metabolite-related pharmacological and/or toxic effects (Orme, 1977). It must be remembered that the possibility of metabolite-induced inhibition of the metabolism of the parent drug will further compound the risk. Often a vicious circle is set up and the renal function is adversely affected by the drug, its metabolites or concurrent drug combinations. Obviously, unless renal function is carefully considered and monitored during the patient's management, adverse drug reactions will predictably occur in due course.

There are at present no well documented examples of genetically controlled renal elimination of drugs. The best known example of genetically controlled drug elimination is

Dubin-Johnson's syndrome in which bilirubin glucuronide transport into the bile is impaired (Dubin, 1958; Arias, 1961). The trait is inherited in an autosomal recessive fashion. In patients with Dubin-Johnson's syndrome, oral contraceptive pills precipitate jaundice presumably due to competition with bilirubin glucuronide transport system. It is now appreciated that of all the known routes of systemic drug elimination, drug metabolism per se is the most important.

4. Drug metabolism

Drug metabolism is a mechanism by which lipid soluble drugs are rendered polar to facilitate their renal elimination (Williams and Millburn, 1975). Since the number of lipophilic drugs far exceeds that of hydrophilic drugs, it follows that considerably greater numbers of drugs have to be metabolised to render them hydrophilic prior to their renal elimination (Williams, 1967). It is therefore not surprising that the metabolism of a drug is the major pharmacokinetic determinant of its pharmacological effect, be it therapeutic or toxic (Brodie and Reid, 1967; Williams, 1967). The metabolism of a drug can lead to the production of metabolites which a) are inactive, b) are toxic or c) exert secondary pharmacological effects of the drug (Williams and Millburn, 1975). Many drugs have to be metabolically activated for their primary pharmacological effects (Brodie and Reid, 1967).

A decrease in the rate of metabolism gives rise to high plasma levels of parent drug and low levels of normal metabolites; occasionally alternative pathways are called upon, or excessively loaded,to yield qualitatively and/or quantitatively different metabolites. An increase in the rate of metabolism gives rise to low plasma levels of parent drug with rapidly accumulating metabolites. For the majority of drugs, metabolism occurs primarily in the liver (Brodie and Reid, 1967; Williams, 1967). As mentioned previously, Shannon had recognised the role of drug metabolism in drug response as early as 1945/46.

Drug metabolism can be modified by a number of factors, including age and sex, hepatic blood flow, consumption of heavy meal or alcohol, smoking habits and concurrent drug administration (Prescott, 1979). The effect from concurrent drugs is particularly likely with those drugs which are known to induce, inhibit or compete for drug metabolising enzymes. In any case, the effects are not too potent (up to 400% changes) and adverse drug reactions that may result are easily recognised or predicted and can be averted by carefully planned drug therapy. The role of hepatic dysfunction per se is difficult to assess. No well designed studies are available and what evidence exists, is often contradictory, inconsistent or inconclusive (Shand, 1977; Williams and Mamelok, 1980). It has even been concluded that patients with advanced cirrhosis metabolise drugs surprisingly normally (Anonymous, 1974).

Even when all the aspects are carefully considered, serious side-effects may appear and it is now widely recognised that of all the pharmacokinetic parameters, drug metabolism is under marked genetic control (Rawlins, 1975). As Motulsky (1957) predicted, the individually heritable traits are responsible not only for variations in drug metabolism, and hence variations in drug response, but also for susceptibility to some disease states.

In the next Chapter, routes of drug metabolism are reviewed. The consequences of genetic control of one such minor route on the pattern of responses to drugs, metabolised by this route, are also subsequently illustrated.

CHAPTER FOUR

DRUG METABOLISM

4.1 Introduction

The vast majority of drugs and many environmental agents are lipid soluble and therefore cannot be eliminated from the body in their original form. These compounds, because of their lipid solubility, would remain in the body for a long time if no mechanism existed for their biotransformation into more polar forms to facilitate their elimination (Williams and Millburn, 1975).

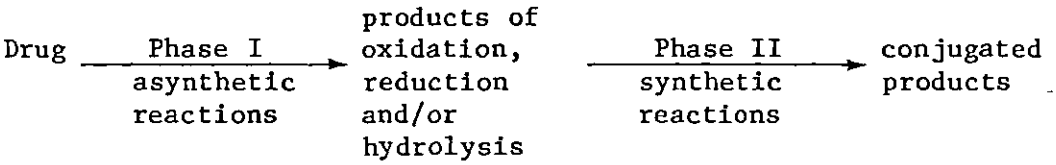
The enzyme systems responsible for drug biotransformation are located primarily, but not exclusively, in the liver (Brodie and Reid, 1967). They are also present to a minor degree in virtually all the tissues of the body, but mainly in the gut, lung and kidney.

The nature and the variety of drug metabolic reactions are complex and wide. They can, however, be conveniently divided into two phases - Phase One and Phase Two (Fig. 1).

Most drugs are metabolised by this biphasic process but there are drugs which are metabolised by only one of the two phases described (Williams, 1967; Williams and Millburn, 1975).

During Phase One metabolism, the lipid soluble drug is rendered more polar by insertion or unmasking of a polar group such as a hydroxyl group. This can occur at many centres within the drug molecule; for example, carbon, nitrogen, phosphorus or sulphur. The other mechanism is the removal of an alkyl radical from oxygen, nitrogen and sulphur alkyl ethers to generate a polar group. All these reactions are

Figure 1 Biphasic drug metabolism



Consequence:

i) Activation	i) Detoxification
ii) Inactivation	ii) Lethal synthesis
iii) Altered activity	

Major reactions in man

Phase I: Oxidation

- C-oxidation
- S-oxidation
- N-oxidation
- O-de-alkylation
- N-de-alkylation

Reduction

$R^1-N=N-R^2$	(azo compounds)
$R-NO_2$	(nitro compounds)
R^1-CO-R^2	(ketones)
R-CHO	(aldehydes)
$\equiv NO \rightarrow \equiv N$	(N-oxides)

Phase II: Conjugations with:

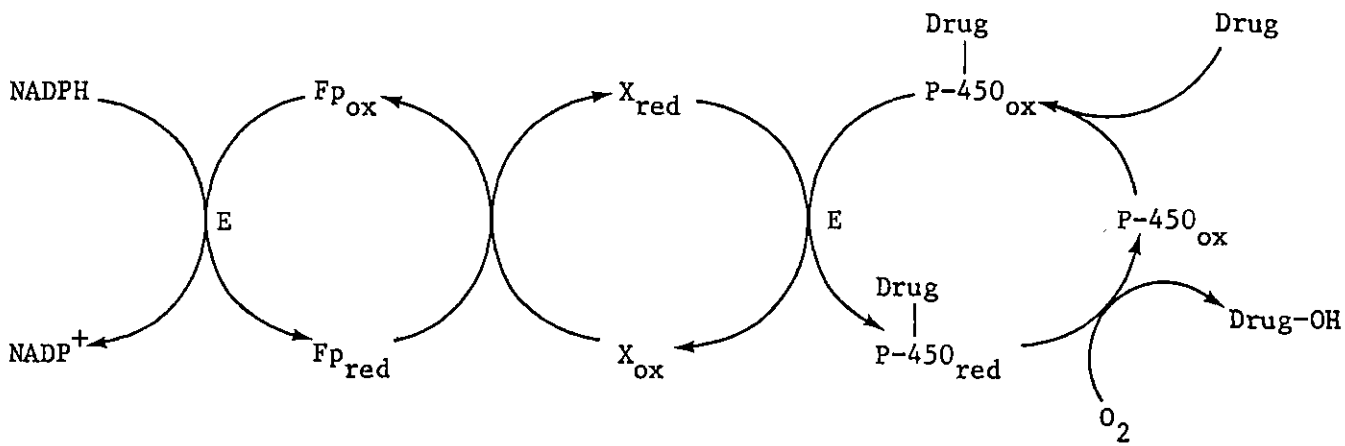
- glucuronic acid
- acetyl radical
- sulphate radical
- glycine
- methyl radical
- glutamine

termed oxidation reactions. The insertion of a hydroxyl radical at an aromatic, aliphatic or alicyclic carbon centre is the major Phase One oxidation reaction.

The majority of these oxidative reactions are carried out by enzymes located in the endoplasmic reticulum of the hepatic parenchymal cells (Fouts, 1971). This structure contains enzymes which metabolise compounds which are foreign to the body. When the liver is homogenised, the endoplasmic reticulum is disrupted giving rise to small vesicles which can be separated by high speed centrifugation to give the fraction called microsomes. Microsomal oxidation has a specific requirement for NADPH and oxygen. The system fits into the category of mixed function oxidases which catalyse the consumption of one molecule of oxygen per molecule of substrate. The oxidising system contains at least two catalysts, namely the NADPH-oxidising flavoprotein known as NADPH-cytochrome P-450 reductase and a CO-binding haemoprotein called cytochrome P-450. A scheme for electron transport chain during the microsomal oxidation is shown in Fig. 2.

Other Phase One metabolic reactions involve reduction or hydrolysis of a compound. It was at one time thought that Phase One reactions always detoxicate a drug but it is now clearer that the consequences, following Phase One metabolism, may give rise to active and/or toxic metabolites or compounds with altered activity (Williams, 1967).

Figure 2 Schematic electron transport chain during microsomal drug oxidation



Fp = Flavoprotein

ox = Oxidised

red = Reduced

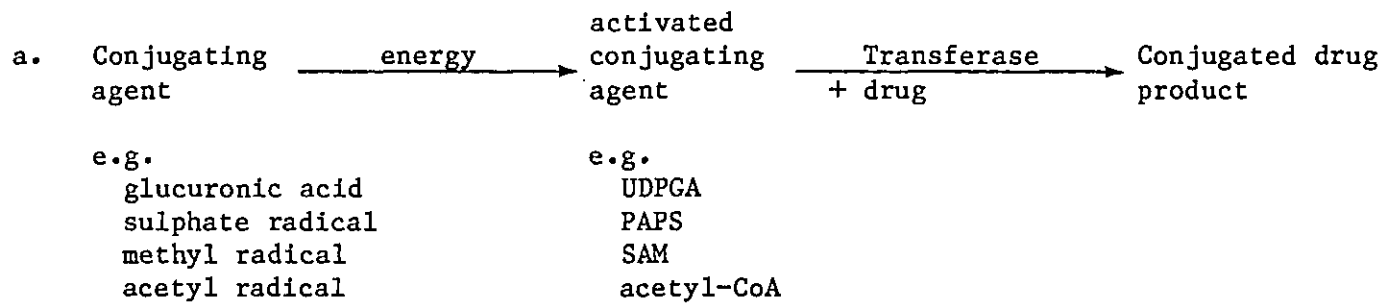
X = Non-haem iron pigment necessary in some tissues but probably not in hepatic microsomes

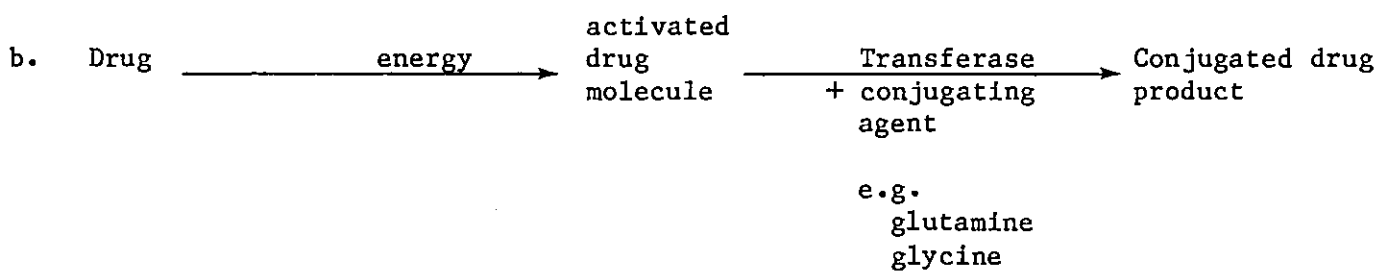
E = NADPH-cytochrome P-450 reductase

[After Gillette and associates (Holtzman et al, 1968)]

Phase Two metabolism involves conjugation or synthetic reactions. The conjugation is carried out with functional groups already present or those introduced by Phase One reactions. Many conjugating moieties are employed for the purpose and they vary with species. Glucuronic acid conjugation is the most widespread of the conjugation reactions and it can occur with compounds containing reactive groups such as hydroxyl, carboxyl, amino and sulphydryl radicals. The other common conjugating reactions that are used are acetylation, sulphation, glycine conjugation and mercapturic acid synthesis. By and large, the products resulting from Phase Two metabolism are non-toxic, though exceptions are known. The conjugating reactions require ATP for energy, activated nucleotide and transferring enzymes (Williams and Millburn, 1975). Schematically, the reactions can be represented as in Fig. 3, depending on whether the activated nucleotide contains the drug or the conjugating moiety.

Figure 3 Schematic mechanisms for drug conjugation reactions





[After Williams, 1967]

4.2 Differences in drug metabolism

It has now been recognised that the metabolism of a drug or an environmental agent plays an important role in the therapeutic response as well as its toxic effects. It is further appreciated that this metabolism is carried out by enzymes. Since enzymes are the functional expressions of the genetic make-up of an organism, it is not too difficult to appreciate that these enzymes vary in nature and amount from species to species, strain to strain, and from individual to individual (Brodie and Reid, 1967; Williams, 1967; Williams and Millburn, 1975).

A. Species variation

One of the most widely studied drug metabolic reaction is aromatic hydroxylation. This reaction is carried out by cytochromes P-450 in the microsomal fraction of the liver. Aromatic hydroxylation of amphetamine is the main route of drug metabolism in the rat but this is not so in man, monkeys, rabbits and guinea pigs (Dring, Smith and Williams, 1970). While coumarin is largely metabolised to 7-hydroxy-coumarin in man, rat does not form this metabolite (Shilling, Crampton, and Longland, 1969; Creaven, Parke and Williams, 1965). It is obvious that although the same metabolic reaction, namely aromatic hydroxylation, is involved and these chemicals lend themselves to the effect of the reaction, the species vary in their handling of these drugs.

Similarly, ephedrine can undergo N-de-methylation, hydroxylation or de-amination. While de-amination is the major route in rabbit, de-methylation and hydroxylation are major routes in dog and rat respectively (Axelrod, 1953).

A very striking example of species difference in drug handling is provided by the rate of metabolism of p-hydroxy-metabolite of phenylbutazone (oxyphenbutazone). Oxyphenbutazone has a plasma half-life of about three days in man, whereas it is only 30 minutes in dogs (Burns, 1962). Similarly, the conjugation reactions of Phase Two vary with species (Williams, 1967). Man utilises only glucuronic acid, glycine, glutamine and sulphate conjugations, methylation and acetylation as the major pathways. Mercapturic acid synthesis, involving the intermediate formation of a glutathione conjugate, may be less extensive in man than in some of the lower animals. Conjugation with ornithine occurs in birds and reptiles and in effect, replaces conjugation with glycine used by mammals in the conjugation of aromatic acids.

B. Strain differences

Weber and his colleagues (1976) examined the acetylation of p-aminobenzoic acid (PABA) and sulphamethazine (SMZ) in various species and strains. They found that the acetylation of PABA in inbred Long-Evans rats was two-fold greater than that in inbred Sprague-Dawley rats (Tannen and Weber, 1979).

They also showed that amongst the various strains of mice studied, no differences were discerned in regard to the hepatic N-acetyltransferase activity towards PABA (high activity) or SMZ (only slight activity). However, blood N-acetyltransferase activity towards PABA was distinctly polymorphic with A/J mice having no activity. The A/J mice excreted less urinary acetyl-SMZ (10% or less), while other strains, including C57BL/6J, excreted considerably more (> 20%) of this metabolite. These two strains were, therefore, designated as slow and fast acetylators respectively. The A/J mice were found to be more susceptible to developing antinuclear antibodies on procainamide, a drug also metabolised by acetylation. A later study by Tannen and Weber (1980), involving mating of A/J and C57BL/6J mice, confirmed that the acetylation phenotype, as measured by the ratio of urinary excretions of acetyl-SMZ to SMZ, is independent of blood acetylation polymorphism and is governed by simple Mendelian law of inheritance with incomplete dominance of the low urinary ratio over high. Their findings (Tannen and Weber, 1979) also suggested the presence of multiple forms of N-acetyltransferase in liver and blood.

Similarly, metabolic differences among various strains of rabbits have been shown and wide variations in the ability to effect acetylation of PABA have been confirmed. Strains

of rabbits designated slow acetylators generally have higher blood PABA N-acetyltransferase activity and significantly lower hepatic isoniazid N-acetyltransferase activity than those designated rapid acetylators (Weber et al, 1976; Szabadi et al, 1978).

Significant strain differences have been shown to occur in drug oxidation in rat inbred strains. Rats have generally been thought to be very good hydroxylators of drugs. Al-Dabbagh and his colleagues (1981) recently studied the alicyclic hydroxylation of debrisoquine in seven strains of rats, namely Wistar, Lewis, Fisher, RN, A-Gus, PVG and DA. These workers demonstrated that compared to other six strains, female rats of the DA strain exhibited a very significant and marked impairment in effecting this oxidative reaction. These differences also extended to the metabolism of other drugs such as O-de-ethylation of phenacetin.

Similarly in various strains of mice, a large variation in hexabarbitone sleeping time has been found, resulting from variation in the rate at which the drug is metabolised to inactive metabolites (Jay, 1955; Williams and Millburn, 1975).

C. Inter-individual differences in humans

Inter-individual differences in drug metabolism are of considerable importance in man. This was noted by Shannon (1946) and further elaborated upon by Motulsky (1957).

In 1957, Motulsky laid down the conceptual basis of a subject which Vogel(1959) termed "pharmacogenetics". Kalow (1962) wrote the first textbook on pharmacogenetics. Motulsky recognised that drug toxicity may be caused by otherwise innocuous genetic traits or enzyme deficiencies and further that the detection of such hereditary biochemical traits that precipitate drug toxicity may contribute to the progress of human genetics in general. Such traits may be related to susceptibility or resistance to diseases other than adverse drug reactions. He noted that "since a given gene may be more frequent in certain ethnic groups, any drug reaction that is more frequently observed in a given racial group, when other environmental variations are equal, will usually have a genetic basis", and recommended that the systematic investigations of adverse drug reactions should therefore include careful history of the ethnic or racial extraction of the patient.

Motulsky concluded that hereditary, gene-controlled enzymic factors determine why, with identical exposure, certain individuals become "sick", whereas others are not affected and he thought it increasingly probable that many of common diseases depend on genetic susceptibility. Haldane (1954) predicted that the future of biochemical genetics, as applied to medicine, lies largely in the study of diathesis and idiosyncrasies.

Pharmacogenetics deals with heritable variations in clinically important drug responses as well as diathesis to diseases. In patients with pharmacogenetic lesions, there are genetically determined qualitative or quantitative alterations in drug handling ability or receptor sites at which drugs act. These alterations are expressed clinically as drug toxicity, therapeutic failure, atypical drug response or diathesis to disease.

These genetically determined differences in the rate of drug metabolism in humans are studied by different methods. Existing clinical data can be analysed on an empirical basis but a better approach would be by studies of twins. Variations in drug response in fraternal and identical twins are determined and the results enable the genetic component to be elucidated. Such studies are ideally suited to polygenically controlled characteristics such as height and blood pressure. Family studies, in addition to confirming the genetic control, allow the mode of inheritance to be determined.

At a population level, the nature of genetic control expresses itself in the pattern of distribution. When a characteristic is polygenically controlled, a unimodal Gaussian distribution is obtained. In such cases, separation of individuals into different phenotype classes can be difficult and would involve extensive studies. When a characteristic is monogenically controlled, a bimodal

distribution may be discerned. In such cases, two phenotypes are appreciated and in most instances, family studies allow the classification of the proband into one of the three genotypes.

Genetically controlled difference in drug metabolism, resulting in increased metabolic clearance of drug, has been described in one of 1029 male subjects studied by Neitlich (1966). This subject had decreased sensitivity to succinylcholine, a drug whose action is terminated by hydrolysis by plasma psuedocholinesterase. Further studies showed three other family members of the subject to have the same abnormality. The presence of the enzyme with this increased activity is determined by an autosomal autonomous dominant variant allele. Other variant alleles have also been described and one of these is responsible for greatly decreased activity of plasma psuedocholinesterase. This results in devastatingly increased sensitivity to succinylcholine, often leading to fatal apnoea (Evans et al, 1952; Kalow and Genest, 1957; Viby-Mogensen and Hanel, 1978).

Twin studies have shown that the rate of ethanol metabolism in normal volunteers is determined almost entirely by genetic factors. In one in vitro study, it was noted that livers from 4% of the subjects in England had alcohol dehydrogenase activity 5-6 times higher than normal. In Switzerland, the corresponding figure was 20%. In vivo the activity in these individuals was shown to be higher only

by a factor of 1.5-fold, suggesting the presence of other influences (von Wartburg and Schürch, 1968). Considerable inter-ethnic differences also exist in ethanol susceptibility. It has recently been shown that the metabolism of alcohol is polymorphically controlled. The rates at which alcohol is metabolised varies from 0.101 g/kg/h in Canadian Indians, 0.110 g/kg/h in Eskimos to 0.145 g/kg/h in caucasians (Fenna et al, 1971). These differences correlate well with differences in susceptibility to the effect of alcohol.

Acatalasia is an autosomal recessive disorder in which the production of enzyme catalase is deficient or absent (Aebi and Wyss, 1978). The gene frequency among different groups vary from .05% to 1.4%. Variants of this disorder have now been described but basically, catalase is responsible for inactivation of hydrogen peroxide. As a result, when hydrogen peroxide is released locally by oral bacteria or during topical treatment of oral disorders, oral ulceration and loss of teeth result.

Several inherited disorders in the glucuronidation of bilirubin have been described (Boobis, 1979). All of these result in side-effects when drugs that are normally conjugated to glucuronic acid are given (Arias et al, 1969). Of these, the Crigler-Najjar syndrome, inherited as an autosomal recessive disorder, is the most severe one. Severe central nervous system disturbances result from profound (unconjugated) hyperbilirubinaemia. The defect is a total

absence of a form of UDPG transferase. In vivo, conjugations of paracetamol, cortisol, menthol, chloral hydrate and salicylamide are impaired. In vitro, p-nitrophenol conjugation is normal. A less severe form of these disorders, inherited as an autosomal dominant trait, has been described by Jervis (1959). A low level of UDPG transferase activity is present. In vitro, glucuronidation of p-nitrophenol is impaired. The condition improves dramatically on administration of phenobarbitone. This contrasts remarkably with Crigler-Najjar syndrome. One mild form of bilirubin glucuronidation disorder is known as Gilbert's syndrome. There is mild chronic elevation of unconjugated bilirubin in serum and the syndrome may be asymptomatic without obvious jaundice. Inheritance is autosomal dominant in nature. The defect appears to be in the transport mechanism of bilirubin into hepatocytes since the lowest UDPG transferase activity in Gilbert's syndrome is still ten-times higher than that necessary to clear the normal endogenous production of bilirubin.

While the above examples refer largely to the metabolism of endogenous substrates, idiosyncrasies to exogenously administered drugs, resulting from inter-individual differences in drug metabolic ability, have been described.

Kutt et al (1964a, 1964b) first reported such an example when studying a pedigree in which some family members were unable to effect p-hydroxylation of phenytoin and Vasko et al (1980) described another similar family recently.

Shahidi (1968) described a family in which certain members were unable to effect the O-de-ethylation of phenacetin. These members formed large quantities of alternative metabolites, 2-hydroxy-phenacetin and 2-hydroxy-phenetidine, the latter metabolite being responsible for marked methaemoglobinaemia in these individuals.

Solomon (1968) reported an extremely rare example of dicoumarol sensitivity thought to be due to a deficiency of mixed function oxidase activity. The single patient reported had a plasma half-life of bis-hydroxycoumarin of 82 hours, compared to a normal value of 27 hours. Family members were not studied but it was noted that the patient's mother developed spinal haematoma while on a very low dose of warfarin treatment. The possibility of a hereditary defect was considered.

Kalow et al (1977) recently reported a polymorphism in the metabolism of amylobarbitone. There was a twenty-fold difference in the excretion of a metabolite between the affected and unaffected individuals. The metabolite is now identified as N-glucoside (and not N-hydroxyl) of amylo-barbitone. The defect in N-glucosidation of amylobarbitone has been shown to follow autosomal recessive mode of inheritance.

Phenylbutazone, a widely used anti-inflammatory agent, has been shown to be hydroxylated at two molecular sites. Several workers have demonstrated the inter-individual

differences in the plasma half-life of this drug in humans. The probable genetic control, responsible for this variability, was suggested by Vesell and Page (1968) in a study of twins. Whittaker and Price Evans (1970), in a larger population study involving family members of some volunteers, confirmed that phenylbutazone metabolism in man is under polygenic control.

Recently, genetically determined control of alicyclic 4-hydroxylation of debrisoquine has been described and the effects of the responsible alleles have been extensively studied (Mahgoub et al, 1977; Idle and Smith, 1979). This polymorphism has opened up new areas of research into drug metabolism and drug response. Before this is further discussed, however, it seems convenient to review the clinical impact of a genetic polymorphism in a Phase Two conjugation reaction, namely the acetylation polymorphism.

C H A P T E R F I V E

POLYMORPHIC DRUG ACETYLATION AND ITS CLINICAL IMPLICATIONS

5.1 Introduction

Acetylation is a conjugation reaction mainly of amino groups (-NH2) in drugs and foreign compounds. It can also occur at functional centres such as hydroxyl(-OH) and sulphydryl(-SH) groups. The amino compounds undergoing acetylation are aliphatic amines, aromatic amines, many alpha-amino acids, hydrazines, hydrazides and aromatic sulphonamides (Williams and Millburn, 1975).

The acetylation of these various types of amino groups may be carried out by different transacetylases (N-acetyltransferases) with the assistance of activated acetyl-CoA.

5.2 Polymorphic acetylation of isoniazid

Isoniazid is a hydrazide which was synthesized in 1912 by Meyer and Mally. It is one of the most powerful, cheapest and best tolerated anti-tuberculous drug (Grunberg et al, 1952; Ellard, 1976). The primary metabolic route which determines the rate at which isoniazid is eliminated from the body is its acetylation to acetyl-isoniazid. It became obvious soon after the introduction of the drug that there were marked inter-individual (Hughes, 1953) and inter-ethnic (La Du, 1972) differences in the rate at which this drug was metabolised to acetyl-isoniazid, a metabolite which is devoid of any anti-tuberculous activity.

When isoniazid is administered in a standard weight-related dose, an individually different but consistent rate of decrease in the plasma level of isoniazid is observed. The frequency distribution of metabolic indices of a population displays a bimodal distribution (Biehl, 1957; Evans, Manley and McKusick, 1960). The individuals are thus either rapid acetylators with a plasma half-life of about 90 minutes (range 35 - 110 min) or slow acetylators with a plasma half-life of about 180 minutes (range 110 - 270 min). Various studies have confirmed that the metabolism of isoniazid in slow acetylators is not inducible (Zysset, Bircher and Preisig, 1981).

Population, family (Evans, Manley and McKusick, 1960) and twins (Bönicke and Lisboa, 1957) studies have shown that this acetylation polymorphism is controlled by two alleles at a single gene locus. There are, thus, three genotypes

but most methods used allow the recognition of only two phenotypes. The phenotype for slow acetylation is transmitted as an autosomal recessive Mendelian characteristic (Knight, Selin and Harris, 1959).

Further studies have shown that the same locus which controls acetylation of isoniazid also determines the rate at which a number of other drugs containing amino function are acetylated. These drugs include procainamide, phenelzine, sulphamethazine, dapsone, hydralazine and the amino metabolite of nitrazepam (Evans, 1977). It is apparent now that acetylation must be a complex reaction because although the above drugs which have structural dissimilarity are polymorphically acetylated, minor change such as removal of one or both methyl groups of sulphamethazine to yield sulphamerazine or sulphadiazine respectively, may result in the almost complete replacement of polymorphic acetylation by monomorphic pattern of acetylation (Gordon et al, 1974). In addition, drugs such as sulphanilamide or p-aminobenzoic acid are monomorphically acetylated.

Inter-ethnic differences in the relative proportions of slow and rapid acetylators have now been confirmed. Slow acetylators constitute from 82% of Egyptians to 5% of Canadian Eskimos. Data on other populations are shown in Table 2.

Table 2: Inter-ethnic variations in frequency of slow acetylator phenotype

Population	% slow acetylators
Eskimos	5
Japanese	10
Chinese	22
Lapps	28
Burmese	36
Nigerians	49
East Africans	55
Norwegians	56
Germans	57
Finns	58
South Indians	59
Canadians	59
Czechs	60
British	62
Sudanese	65
US - Italians & Greeks	60-70
Swedes	68
Non-Ashkenazi Jews	69
Baghdad Jews	75
Egyptians	82

(Compiled from various sources)

5.3 Clinical significance of acetylation polymorphism

The clinical implications of polymorphic drug acetylation are being greatly appreciated. Each drug polymorphically acetylated produces its adverse reaction more commonly in one or the other phenotype. Table 3 shows the acetylation phenotype susceptible to the adverse drug reactions of some of the drugs undergoing metabolism by this route.

Similarly, polymorphic drug acetylation could explain the lack of therapeutic efficiency seen more often among rapid acetylators when standard doses are employed. Zacest and Koch-Weser (1972) showed that rapid acetylators required larger doses of hydralazine for their blood pressure control. Similarly, higher doses of isoniazid against tuberculosis (Menon, 1968), dapsone against dermatitis herpatiformis (Forstram, Mattila and Mustakallio, 1974) and phenelzine against depression (Johnstone and Marsh, 1973) are required in individuals of rapid acetylator status. It is worth inquiring, and studying prospectively, whether toxic drug reactions can be prevented and therapeutic efficiency increased by determination of acetylator phenotype prior to commencing drug therapy.

Finally, acetylation phenotype is thought to be implicated in diathesis and resistance to certain diseases. It has been suggested that rapid acetylation may play a role in protection against bladder cancer caused by certain aromatic amines, for example, benzidine and 4-amino-biphenyl.

Table 3: Adverse drug reactions and susceptible acetylation phenotype

Drug	Adverse drug reaction	Susceptible Phenotype	Reference
Isoniazid	Neuropathy	Slow	Devdatta et al, 1960
	Phenytoin-toxicity (nystagmus, ataxia etc.)	Slow	Kutt, 1971
	Hepatitis	Rapid	Mitchell et al, 1975
	Systemic lupus erythematosus (SLE)	Slow	Zingale et al, 1963
Phenelzine	Drowsiness, dizziness, blurred vision	Slow	Evans, Davison and Pratt, 1965
Hydralazine	SLE + antinuclear antibodies	Slow	Perry et al, 1970
Dapsone	Haematological reactions	Slow	Ellard et al, 1974
Salazopyrine	Haematological reactions + cyanosis	Slow	Das et al, 1973
Procainamide	*SLE + antinuclear antibodies	*Slow	Woosley et al, 1978

* = Evidence not conclusive

Higher incidence of bladder cancer in slow acetylators has been noted. Platzer et al (1978) were able to demonstrate that among 27 patients with Gilbert's syndrome, 21 were slow acetylators. This prevalence of 78% was significantly different from the 51% slow acetylators in the control group. This suggested a new association of acetylation polymorphism with a disease. Recently, Bodansky et al (1981) suggested an association between rapid acetylation status and insulin-dependent (Type 1) diabetes.

Although acetylation polymorphism has now been known and well established for about two decades, clinicians, by and large, have been slow or reluctant to exploit it to the advantage of the patient. It is, therefore, not surprising that reports of drug toxicity and therapeutic failure continue to appear.

5.4 Hints on probability of oxidation polymorphism

Whilst acetylation polymorphism has served as a prototype model of genetic control of drug metabolism, and has explained a number of events in clinical practice, it has to be admitted, however, that Phase Two reactions or conjugations are less numerous; a far greater number of drugs are metabolised by Phase One reactions and particularly by oxidation. It is perhaps, on this account, that clinical pharmacologists and clinicians may have suspected that the importance of inter-individual differences are grossly exaggerated. However, clinical evidence that genetic factors controlling drug oxidation may be responsible for unexpected drug responses have been present in the literature for about the last 25 years at least.

A wide variability in plasma levels of chlorpromazine among patients on an identical dose regime has been reported. After a twice daily dose of 300 mg for several weeks, the plasma levels of chlorpromazine 12 hours after the evening dose varied from 45-477 ug/l (Curry and Brodie, 1967). In the past, considerable attention has centred on chlorpromazine-jaundice. Ethnic differences (Beeley, 1975) in these toxic effects are clearly discerned from experiences in Kenya. In a study at the hospital in Mathari, this toxic effect was conspicuously absent in the Africans, despite prolonged treatment with high doses. A few that were seen were confined to the Europeans using the same batch of the drug. Similar experiences have been reported from Hong Kong, Nigeria and Ghana (Lambo, 1957).

Kutt et al (1964b) reported an unusual family; a 24-year old male suffering from post-traumatic epilepsy was prescribed phenytoin 100 mg three times a day. He developed ataxia and nystagmus, amongst other toxic effects. His plasma levels were found to be 80 ug/ml and it took about 20 days before his plasma levels fell to zero after stopping the drug. Very little p-hydroxy-phenytoin was excreted by him compared to normal individuals. Family studies revealed impaired ability to effect phenytoin hydroxylation in his brother and mother; other family members metabolised phenytoin normally. Since then, various other similar family studies have been reported.

Shahidi (1968) described a 17-year old girl who was being investigated for cyanosis; a diagnosis of phenacetin-induced methaemoglobinaemia was confirmed. The patient's urine contained far greater than normal quantities of 2-hydroxy-phenacetin and 2-hydroxy-phenetidine. Family studies revealed that the girl and her sister had relative inability to effect oxidative de-ethylation of phenacetin to yield paracetamol, the active analgesic metabolite. Because of this inability, phenacetin was metabolised by an alternative pathway to produce unusually large amounts of 2-hydroxy-phenacetin and 2-hydroxy-phenetidine and these metabolites were responsible for methaemoglobinaemia. The production of 2-hydroxy-phenacetin and 2-hydroxy-phenetidine, and not of paracetamol, was enhanced following three days of treatment with phenobarbitone; resulting in very pronounced methaemoglobinaemia in the patient as well as her sister.

An insight into the probable genetic control of a wide variety of drugs is gained from the equi-potent dose range of these drugs. As drugs are metabolised in the liver, some individual differences in drug metabolising ability reflect themselves as inter-individual differences in plasma levels of drugs on a standard dose. Alternatively, variable doses are required to produce a pre-determined plasma level. This variation in pre-systemic drug metabolising ability is variously referred to as "first pass effect", "pre-systemic metabolism" or "pre-systemic elimination". The phenomenon of first pass metabolism, based on individual drug metabolising ability, is responsible for far more extensive variations in plasma levels of drugs than are any of the known environmental influences such as age, smoking, sex or alcohol habits, diurnal rhythm, body weight or temperature. It is not surprising, therefore, that the pharmacological effect of a drug that is subject to pre-systemic or first pass metabolism does not correlate with dosage. Clinically, first pass metabolism is expressed as a wide variation in equi-potent dose requirements (Table 4) or wide variations in plasma levels on a standard dose (Table 5).

Brodie and Reid (1967) have stated that there are very few liposoluble drugs whose rates of metabolism in man are not highly variable. Considering that a large number of drugs in common use are lipid soluble and are metabolised by oxidation prior to their elimination, it is surprising that

Table 4: Equi-potent dose range for some of the drugs

Drug	Dose range (mg)
Imipramine	10-225
Debrisoquine	20-400
Propranolol	10-960
Alprenolol	100-800
Atenolol	12.5-200
Oxprenolol	60-600
Nortriptyline	10-150
Amitriptyline	10-250
Phenytoin	100-600
Perhexiline	200-1200
Phenformin	25-2500

(Compiled from various sources)

Table 5:

Inter-individual variations in plasma levels of some of the cardioactive drugs

Drug	Observed variation
Guanfacine	2-fold
Debrisoquine	3-fold
Pindolol	4-fold
Sotalol	4-fold
Verapamil	4-fold
Diltiazem	4-fold
Oxprenolol	5-fold
Mexiletine	5-fold
Flecainide	7-fold
Metoprolol	7-fold
Lidoflazine	17-fold
Propranolol	20-fold
Alprenolol	20-fold
Urapidil	29-fold
Encainide	56-fold

(Compiled from various sources)

the polymorphic control of drug oxidation has not been uncovered much earlier and systematically investigated. The risks of giving a standard dose of any one of the drugs to all of the patients are clearly obvious. Variations in first pass metabolism underline the need to individualise drug treatment.

More than a decade ago, a report prepared by the Committee on Problems of Drug Safety (1969) of the Drug Research Board; National Academy of Sciences - National Research Council, United States of America, recognised the role of genetic factors controlling drug metabolism in rational and safer drug therapy. The Committee listed five urgent needs (quoted verbatim below):

1. Extensive exploration of pharmacogenetic variations in man is needed.
2. The relationship of known pharmacogenetic examples to the metabolism of other drugs should be investigated.
3. Improved methods are required to detect pharmacogenetic traits so that more extensive studies can be made.
4. The possibility that persons who metabolise one drug slowly may also metabolise other drugs slowly should be clarified.
5. The relative roles of genetic and environmental factors in determining individual differences should be further explored.

Clearly, a wide variety of drugs are metabolised by oxidation and this route too appears to be under genetic control. A simple test or a safe biochemical probe, allowing assessment of an individual's ability to effect metabolic oxidation of a range of drugs, would be one major step in the right direction.

The following chapters are offered as a small contribution to answering the urgent needs outlined above.

CHAPTER SIX

POLYMORPHIC CONTROL OF DEBRISOQUINE OXIDATION

6.1 Introduction

Debrisoquine hemi-sulphate, an anti-hypertensive agent, was first synthesized in 1961. It is a colourless, crystalline water-soluble substance. It is chemically 3,4-dihydro-2-(1H)-isoquinoline carboxamidine. The drug is related structurally and pharmacologically to other guanidine anti-hypertensive agents, for example, bethanidine, guanoxan and guanethidine.

The drug was found to possess hypotensive properties in several species of normotensive as well as hypertensive animals. Pharmacological studies with debrisoquine confirmed that it exerts post-ganglionic sympathetic blockade by interfering with the physiological release of noradrenaline (Moe et al, 1964). Subsequent clinical studies showed that the drug exhibited similar anti-hypertensive activity in humans (Abrams et al, 1964).

Debrisoquine differs from guanethidine and bretylium in not depleting the peripheral stores of noradrenaline and it is further unlike bretylium in not altering the uptake or release of exogenous noradrenaline (Moe et al, 1964).

After oral administration of debrisoquine, an initial pressor phase seen with guanethidine and bretylium is thought not to occur, although it has been observed following an intravenous injection (Athanassiadis et al, 1966).

The hypotensive effect following an oral dose occurs within 4 hours and lasts anything from 9-24 hours, with a maximum effect between 2 and 8 hours. The effect is more marked in the standing position (Pocelinko, Robert and Abrams, 1964).

Haemodynamic studies have shown that the hypotensive effects of debrisoquine are due to reduction in peripheral vascular resistance while cardiac output and renal flow are well maintained but some diminution of glomerular filtration rate has been shown to occur in the upright position (Kitchin and Turner, 1966). These effects of debrisoquine have been disputed by Onesti et al (1966) who believe that cardiac output and renal flow are reduced and that the hypotensive effect of debrisoquine is primarily due to a reduction in cardiac output and only to a minor extent, due to reduction in arteriolar tone.

Debrisoquine was first introduced in the U.K. in 1966. At that time, the drugs in common use for the treatment of hypertension were guanethidine, bretylium, bethanidine, reserpine, diuretics and methyldopa. These drugs were fraught with side-effects , particularly postural hypotension, dizziness, diarrhoea, failure to ejaculate, tiredness, somnolescence and in the case of methyldopa, haemolytic anaemia, hepatitis and jaundice.

Clinically, debrisoquine proved to be an effective and welcome anti-hypertensive agent. Kitchin and Turner (1966) reported a study in which 45 patients completed the protocol. Of these, 31 had severe and 14 had moderate hypertension. Twenty-two patients had received previous hypotensive therapy while 23 were new patients. Debrisoquine was found to be satisfactory in controlling blood pressure in these patients. The effect was achieved rapidly and predictably. These workers noted that compared to other hypotensive agents, side-effects were less frequent, less severe and less unpleasant. The only problem encountered was a higher incidence of drug tolerance, necessitating the addition of a diuretic.

Athanassiadis et al (1966) reported a series of 55 patients, including 12 pregnant women, in whom the drug was equally effective. They too confirmed fewer side-effects with debrisoquine compared to guanethidine. The side-effects that were encountered with debrisoquine consisted of postural dizziness (n=9), exertional dizziness (n=8), diarrhoea (n=7), failure of ejaculation (n=2), tiredness (n=4), muscle pain (n=2), blurred vision (n=1), nausea (n=2) and nasal stuffiness(n=2). There were 29 patients who were totally free of side-effects.

Athanassiadis et al (1966) also noted that the effective daily dose of debrisoquine ranged from 10 mg to 360 mg. Experience in Sheffield Hypertension Clinic also confirmed that, in the 100 patients attending the clinic, the daily dose of debrisoquine, required to control the standing blood pressure, varied from 10 mg to 360 mg (Silas, 1978).

Angelo and her colleagues, in a study which included healthy volunteers and hypertensive patients, demonstrated a correlation between the response to debrisoquine and the amount of unchanged drug (seven-fold variation) excreted in the urine. The differences in response could not be related to the differences in drug absorption. When given a standard oral dose of 40 mg of debrisoquine to 11 patients, Silas et al (1977) noted a fall in the mean standing systolic pressure varying between 0.3 and 44.4 mm Hg. The systemic availability of debrisoquine was the major determinant of response to drug.

Studies with radio-labelled debrisoquine in man have shown that the compound is well absorbed after its oral administration and variable absorption is not responsible for variation in drug response. Various studies have confirmed that 75-80% of the drug is excreted by the first 24 hours (Table 6).

The wide range of effective dose of debrisoquine, found in the two studies described above, is not surprising when the metabolism of the drug is considered. It is evident that the drug is subject to significant first pass metabolism which varies widely between individuals (Angelo et al, 1975; Silas et al, 1977; Silas, 1978).

Table 6:

Recovery of urinary debrisoquine and related products

Study	Dose	Collection Period	n	Recovery
Angelo et al, 1976	Single	24-h	4	71 - 77 %
Idle et al, 1979	Single	24-h	5	67 - 78 %
		72-h		70 - 87 %
Silas, 1978	Chronic	24-h	3	74 - 79 %
		72-h		84 - 90 %
Schwartz and Baukema, 1966	Single	192-h	2	80 %

6.2 Metabolism of debrisoquine

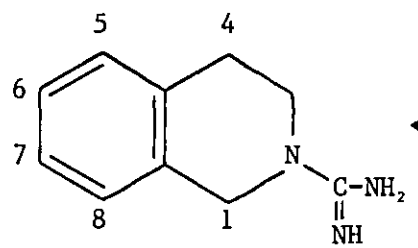
Earlier studies (Allen et al, 1975; Allen, Brown and Marten, 1976) with debrisoquine showed that the drug was metabolised by two pathways; one involved oxidation at alicyclic and aromatic ring structures, while the other involved cleavage of ring to give two acidic metabolites; 2-(guanidinoethyl) benzoic acid and 2-(guanidinomethyl)-phenylacetic acid.

The metabolism of debrisoquine has been studied in great detail by Idle and his colleagues (1979), using radio-labelled drug (Fig. 4). These workers quantitatively accounted for all the radio-activity and confirmed that the oxidative pathway was by far the dominant pathway. It produced five hydroxylated metabolites, namely

4-hydroxy-debrisoquine (by alicyclic hydroxylation)
and 5-hydroxy-debrisoquine
6-hydroxy-debrisoquine
7-hydroxy-debrisoquine
8-hydroxy-debrisoquine (all by aromatic hydroxylation)

The total amount of polar metabolites formed by all the other routes, including the two carboxylic acids described by Allen and his colleagues (1975, 1976), amounted to 3.9 ± 4.7% of the total dose (range 0-11.2%).

Figure 4 Metabolism of debrisoquine in man



Debrisoquine

major oxidation

minor

very minor ring opening

very minor

	Reported values of excretion in 0-24h urine
4-hydroxy-debrisoquine	5.4 - 33.4%
5, 6, 7, 8-hydroxy-debrisoquine	2.7 - 13.7%
unchanged debrisoquine	18.6 - 54.8%
2-(guanidinomethyl)-phenylacetic acid	0 - 11.2%
2-(guanidinoethyl)-benzoic acid	

[Modified from Idle et al, 1979]

4-hydroxy-debrisoquine was clearly found to be the major metabolite amongst the monohydroxylated products of debrisoquine. By contrast, the products of aromatic hydroxylation of debrisoquine formed a small fraction of the total oxidative metabolites. It was also confirmed that the formation of 4-hydroxy-debrisoquine was subject to large inter-individual variation, while those of 5-, 6-, 7- and 8-hydroxy-debrisoquines showed far smaller inter-subject variability.

This group of workers (Idle and Smith, 1979) have also demonstrated the individual consistency in the rate at which oxidative clearance of debrisoquine is effected. It became evident that this inter-individual variability was conveniently determined by measuring excretions of debrisoquine and 4-hydroxy-debrisoquine in urine (Mahgoub et al, 1977).

6.3 Polymorphic oxidation of debrisoquine

Mahgoub et al (1977) described a simple method by which to measure an individual's ability to effect debrisoquine oxidation.

The individual is given 10 mg of debrisoquine orally in the morning, after voiding the bladder. Urine is collected in bulk, without any preservative, for a period of up to 8 hours. During this period, the subject is allowed food and drink as usual but alcohol is not permitted. After measuring the total volume of urine voided, an aliquot is stored frozen at -20°C. Subsequently, prior to analysis, the urine is allowed to thaw and analysed by electron-capture gas-chromatography for its contents of debrisoquine and 4-hydroxy-debrisoquine. The details of the method of analysis have been described elsewhere (Idle et al, 1979).

From the urinary contents of debrisoquine and 4-hydroxy-debrisoquine, the parameter termed the metabolic ratio is calculated as follows:

$$\text{Metabolic ratio} = \frac{\%\text{ oral dose excreted as unchanged debrisoquine}}{\%\text{ oral dose excreted as 4-hydroxy-debrisoquine}}$$

in the 0-8 h period

A low metabolic ratio reflects extensive drug oxidising ability, while a high ratio represents impairment to effect debrisoquine oxidation.

These workers carried out an initial population study involving 94 healthy staff and students from St. Mary's Hospital Medical School. They showed that 4-hydroxylation of debrisoquine, as judged by the frequency distribution of debrisoquine metabolic ratio, is bimodally distributed. The frequency of the defect responsible for poor 4-hydroxylation was about 3% (3 out of 94 individuals). The metabolic ratio as an individual characteristic was also confirmed on repeat testing of randomly selected volunteers from the two groups. The bimodal distribution, together with results from the three family studies, suggested a two alleles/single gene-locus mechanism controlling debrisoquine oxidation (Mahgoub et al, 1977).

Subsequent extensions of population and family studies amongst white British caucasians have confirmed these initial conclusions and shown a much higher prevalence of the defect (Price Evans et al, 1980). About 8.9% of this population are unable to effect the debrisoquine oxidation and have been termed poor metabolisers (PM), while the remainder are termed extensive metabolisers (EM), with an antimode at the metabolic ratio of 12.6. Pedigree studies showed that the trait for poor metabolic ability is inherited as an autosomal recessive character. Although two phenotypes are recognised, three genotypes exist with heterozygotes behaving as extensive metabolisers. The two alleles have been termed the D^H allele for extensive metabolic trait and the D^L allele

for poor metabolic trait. The dominance of the EM phenotype over the PM phenotype is estimated at about 30%. The median metabolicratio was about 1 and no association of either phenotype with age or sex was found. Over the period, the studies have continued to be extended and the distribution from the pooled data on 961 healthy volunteers drawn from white British caucasians is shown in Fig. 5.

The biochemical expression of the genetic control that is responsible for polymorphic debrisoquine oxidation, in common with other drug oxidations generally, involves the hepatic cytochrome P-450 discussed earlier (Davies et al, 1981; Kahn et al, 1982).

Various ethnic studies have been carried out and the results show wide inter-ethnic differences in the frequency of poor metabolisers. These results are shown in Table 7. It can be seen that there is approximately twelve-fold inter-ethnic variation in the frequency of the D^{L} allele responsible for impaired/poor metabolic ability. The study, involving Saudi Arabs (Islam, Idle and Smith, 1980), also further confirmed that the metabolic ratios within a population were not significantly altered by sex, period of urine collection, or urinary recovery of the orally administered dose.

Figure 5 Metabolic ratios in the normal British white population

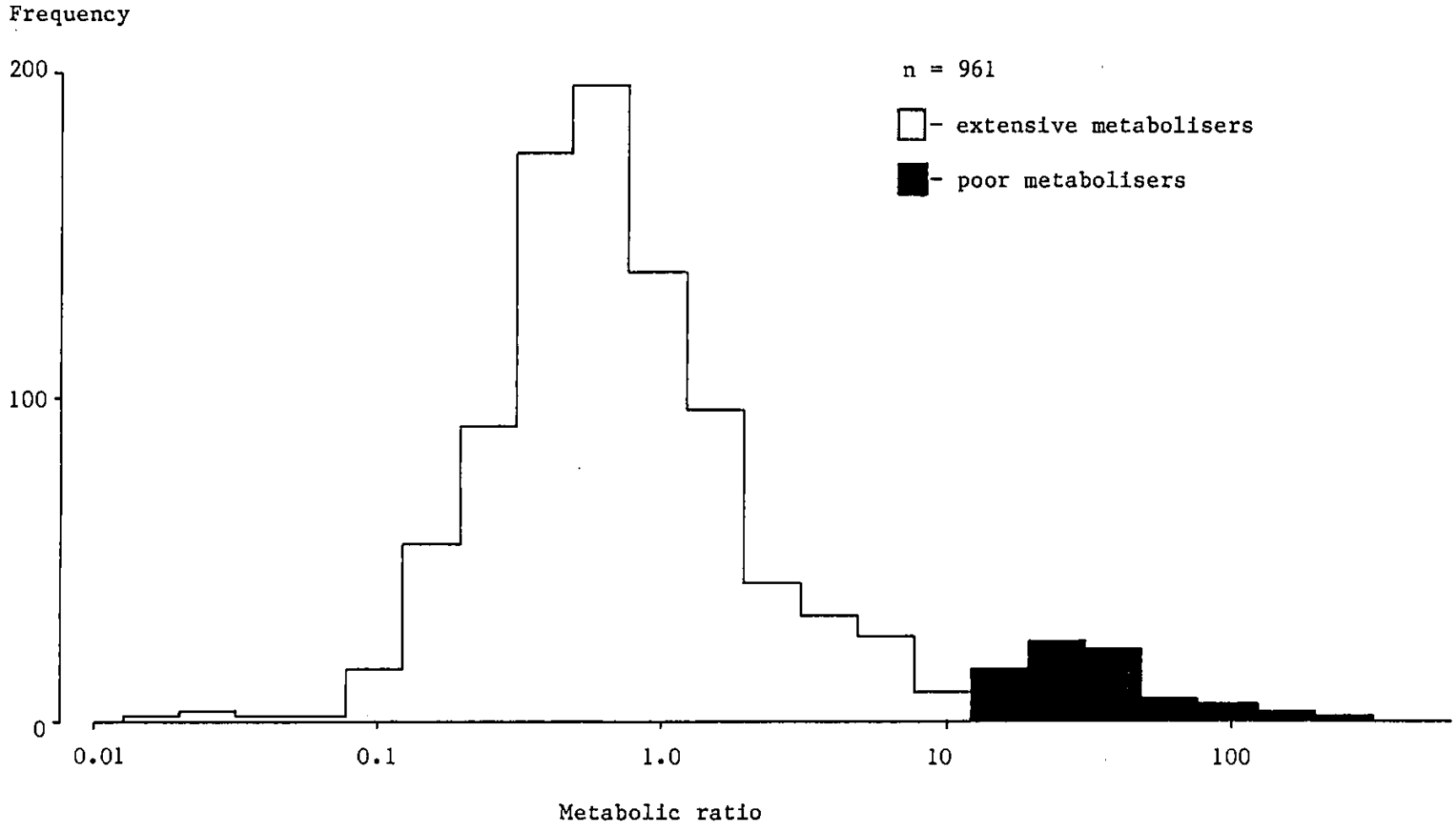


Table 7: Ethnic frequency of poor metabolising phenotype : Global heterogeneity

G R O U P	No. studied	No. PM's	% PM's (95% confidence range)
Europe:			
Sweden	155	5	3.2 (1.1- 6.6)
U.K.	961	79	8.2 (6.6-10.1)
Switzerland	58	5	8.6 (2.9-17.4)
Far East:			
Malaya	49	1	2.0 (0 - 8.0)
India	146	3	2.1 (0.4- 5.0)
Bangladesh	91	2	2.2 (0.2- 6.3)
China	34	1	2.9 (0 -11.4)
Middle East:			
Saudi Arabia	102	1	1.0 (0 - 3.8)
Egypt	72	1	1.4 (0 - 5.4)
Iraq	260	9	3.5 (1.6- 6.1)
Africa:			
Nigeria	273	11	4.0 (2.1- 6.7)
Xhosa - S. Africa	101	6	5.9 (2.2-11.5)
Ghana	80	5	6.3 (2.1-12.7)
Gambia	49	6	12.2 (4.8-23.1)

(Compiled from various sources)

6.4 Debrisoquine hydroxylation phenotype and hypotensive response to debrisoquine

It is worth inquiring if polymorphic hydroxylation of debrisoquine could explain the wide variation in effective equi-potent daily dose of debrisoquine. Studies by Angelo et al (1975) and Silas et al (1977) had already suggested that the hypotensive response to debrisoquine was related to the systemic availability of debrisoquine as judged by the urinary excretion of unchanged drug. Angelo et al (1975) further concluded that the rate of hepatic metabolism of debrisoquine was the major factor determining the systemic availability of the drug.

Idle et al (1978) studied 7 healthy normotensive human volunteers who were previously phenotyped for their debrisoquine hydroxylation status using 10 mg debrisoquine as described by Mahgoub et al (1977). These included three poor metabolisers and four extensive metabolisers. On the study day, each volunteer took orally 20 mg debrisoquine and the lying and standing blood pressures were measured at hourly intervals for 8 hours. The data published have been translated into mean arterial pressures. The results are tabulated in Table 8 and shown graphically in Fig. 6.

It is evident that in extensive metabolisers, the standing blood pressure is consistently higher than the lying blood pressure, suggesting an intact sympathetic reflex control.

Table 8 Mean arterial pressures in a panel given 20 mg debrisoquine orally.

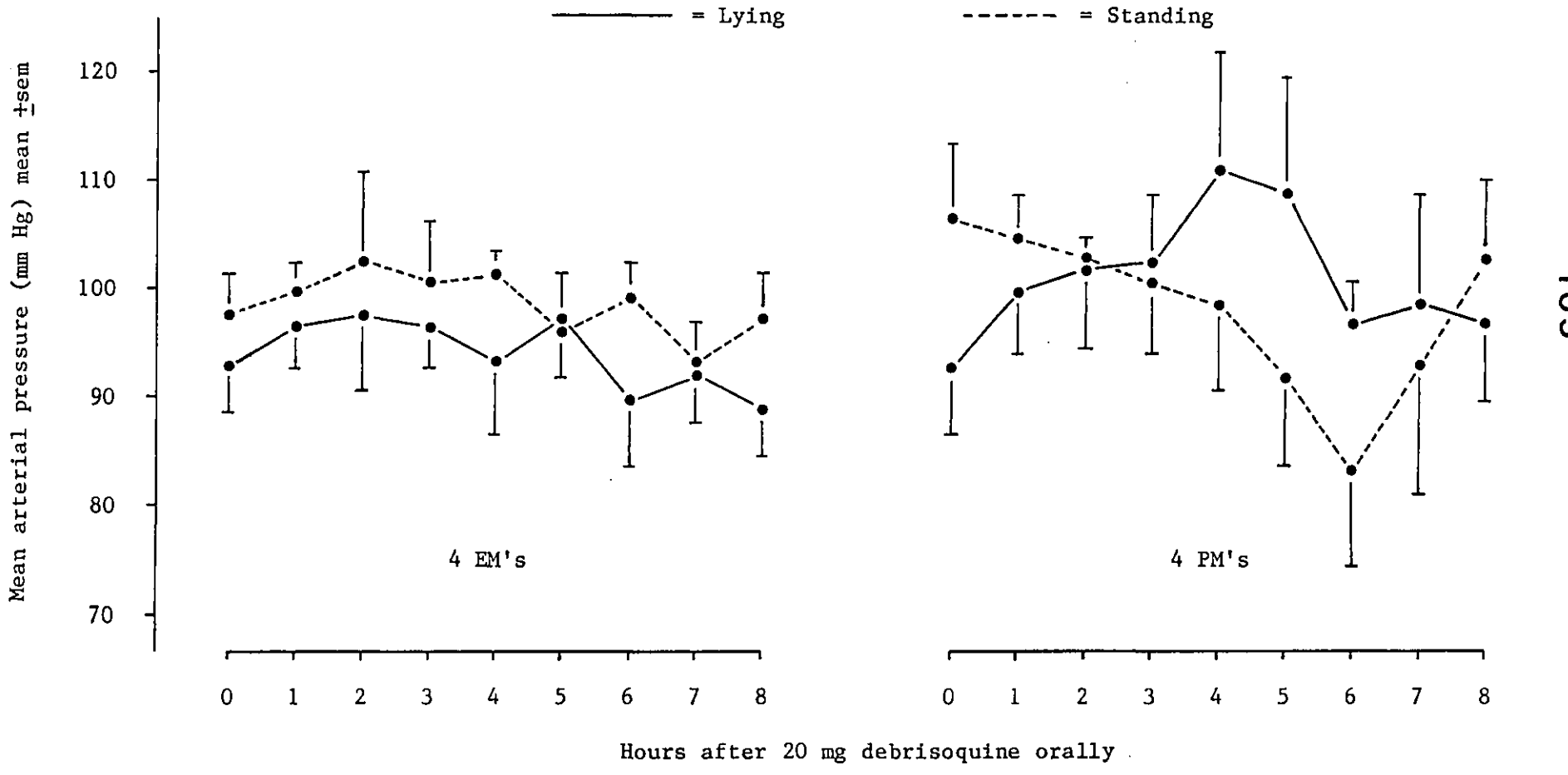
Volunteer	Ratio	Phenotype	BP*	Time (h)								
				0	1	2	3	4	5	6	7	8
1	0.4	EM	L	93	108	103	97	93	97	97	100	93
			S	108	107	110	107	107	108	110	97	110
2	1.4	EM	L	103	98	113	98	87	107	103	93	80
			S	97	98	120	113	103	93	100	100	97
3	0.5	EM	L	82	88	82	105	83	97	78	80	85
			S	93	92	93	90	102	93	97	83	87
4	0.7	EM	L	92	92	93	87	110	85	83	95	97
			S	93	102	87	92	97	90	90	90	93
Mean±sem		EM	L	93±4	97±4	98±7	97±4	93±6	97±5	90±6	92±4	89±4
			S	98±4	100±3	103±8	101±6	102±2	96±4	99±4	93±4	97±5
5	19	PM	L	82	97	95	90	90	88	93	80	88
			S	93	97	100	87	83	77	73	70	88
6	20	PM	L	93	90	95	108	117	113	105	110	92
			S	112	107	107	108	108	105	100	105	110
7	20	PM	L	103	112	115	110	125	125	92	107	112
			S	115	112	102	107	105	93	77	105	112
Mean±sem		PM	L	93±6	100±6	102±7	103±6	111±11	109±11	97±4	99±10	97±7
			S	107±7	105±4	103±2	101±7	99±8	92±8	83±8	93±12	103±7

[Modified after Idle et al, 1978]

* Mean arterial pressure = Diastolic pressure + 1/3 pulse pressure

L = Lying S = Standing

Figure 6 Phenotypic differences in hypotensive response to a single oral dose of 20 mg debrisoquine



This contrasts sharply with poor metabolisers in whom postural changes in blood pressure become evident between 3-7 hours, suggesting some loss of sympathetic reflex control. This loss was most marked in volunteers 5 and 7 and interestingly, both these volunteers experienced symptoms of postural hypotension over the period of 4-6 hours post-dosing. In another study (Ritchie et al, 1980), it was shown that when given 10 mg debrisoquine orally, the 3 volunteers of poor metaboliser phenotype had significantly higher plasma levels of debrisoquine at all time points, from 1 hour post-dosing, and they experienced about 3-4-fold greater overall exposure to the parent drug (as judged by area-under-curve) when compared with 4 volunteers of extensive metaboliser phenotype (Table 9). Bioavailability studies showed that the presystemic elimination in the two of the extensive metabolising volunteers was 77% and 58% of the drug absorbed, while it was only 2.3% in one PM volunteer studied (Idle et al, 1982).

Thus, individuals of poor metaboliser phenotype are susceptible to developing debrisoquine-induced postural hypotension.

The direct relationship between hydroxylation phenotype and the pharmacological response to debrisoquine is obvious, underlining clearly the practical application of prior phenotyping to the safe and effective therapeutic use of debrisoquine. Interestingly, a pilot study from South Africa (Seedat, 1980) demonstrated that 10 mg debrisoquine

Table 9: Oxidation phenotype and presystemic metabolism of debrisoquine

Oxidation Phenotype	Peak Plasma Concentration (ng/ml)	Mean Plasma Concentration (ng/ml)	AUC ng/ml. min.
EM (n=4)	21.9 ± 2.9	11.5 ± 0.7	105.5 ± 7.0
PM (n=3)	72.7 ± 11.8	37.7 ± 4.2	371.4 ± 22.4

Dose of debrisoquine : 10 mg orally

Results are mean values ± S.D.

AUC = area under curve

(After Ritchie et al, 1980)

produced a mean diastolic fall of 12 mm Hg (from 115 mm Hg) in 9 hypertensive Africans but only of 6 mm Hg (from 117 mm Hg) in 9 hypertensive Indian patients. This dose of debrisoquine, it was concluded, did not produce any significant fall in blood pressure and no untoward effects were noticed. The author did, however, justifiably attribute the difference in responses observed between the Africans and the Indians to genetically determined inter-racial differences in debrisoquine hydroxylation.

6.5 Debrisoquine hydroxylation phenotype and oxidation of other drugs

Oxidation of debrisoquine to 4-hydroxy-debrisoquine and other hydroxylated metabolites occurs in hepatic microsomal fraction, mediated by cytochrome P-450. The reaction is oxygen and NADPH-dependent. Since this microsomal fraction is known to be necessary in oxidative metabolism of a wide variety of drugs, it is important to determine the type of drug oxidations which display polymorphism paralleling debrisoquine hydroxylation.

It seems appropriate at this stage to recall the fourth urgent need recognised by the Committee on Problems of Drug Safety (1969), of the Drug Research Board, National Academy of Sciences - National Research Council, USA. This was:

> 4. The possibility that persons who metabolise one drug slowly may also metabolise other drugs slowly should be clarified.

In order to address oneself to this need, conveniently and without involving a large number of individuals, a phenotyped panel approach, for investigating the types of oxidative reactions probably exhibiting polymorphic metabolism in man, has been suggested (Idle et al, 1979a). The results of such studies are most encouraging; it has become evident that the D^H/D^L alleles, controlling alicyclic hydroxylation of debrisoquine, also control a number of other specific oxidative reactions.

6.5a Aromatic hydroxylation of guanoxan

Guanoxan is an anti-hypertensive agent which is related pharmacologically to debrisoquine, bethanidine and guanethidine. The four drugs are also related chemically in being guanidine derivatives. Chemically, guanoxan is 2-guanidino-methyl-1,4-benzodioxan. In man, the drug undergoes extensive oxidative metabolism by aromatic hydroxylation to yield phenolic metabolites (Jack, Stenlake and Templeton, 1972). During its earlier metabolic studies, one of the four patients was noted to excrete only the unchanged drug with no formation of phenolic metabolites. The aromatic hydroxylation of guanoxan was studied in a panel of volunteers consisting of four EMs and four PMs, as phenotyped by their ability to effect alicyclic hydroxylation of debrisoquine (Sloan et al, 1978). Each volunteer collected 0-8 hours urine after swallowing 10 mg guanoxan sulphate. The four EMs excreted a mean of 1.5% (range 1.2-1.9%) of orally administered guanoxan in its unchanged form and 29% (range 25-36%) as hydroxylated metabolites. In contrast, the four PMs excreted a mean of 48% (range 31-60%) of the dose as unchanged drug and 6.2% (range 4.2-7.6%) as hydroxylated metabolites of guanoxan. These results strongly suggest polymorphism in aromatic hydroxylation of guanoxan and that its pharmacogenetic control is common with that of 4-hydroxylation of debrisoquine.

6.5b O-de-ethylation of phenacetin

Phenacetin is an analgesic which was in wide and common use in the early 1960s. Following reports of its nephrotoxic effects (Goldberg et al, 1971), as well as its propensity to precipitate methaemoglobinaemia (Shahidi, 1968), the drug was finally withdrawn from use. Phenacetin is subject to metabolism along several pathways but the main one involves its oxidative de-ethylation to produce paracetamol, a metabolite with analgesic activity. This metabolic pathway was studied in a panel of 8 volunteers as described for guanoxan. Each volunteer swallowed 500 mg phenacetin capsule and the rate of formation of paracetamol was measured by hourly collection of urine samples, up to 8 hours and an additional 8-24 hours sample in bulk. The results demonstrated that subjects of EM phenotype were about three times faster than those of PM phenotype in effecting oxidative de-ethylation of phenacetin (Sloan et al, 1978). The differences in metabolic handling of paracetamol by the two phenotypic groups were excluded in a further study (Idle and Smith, 1979).

In another study (Ritchie et al, 1980), it was shown that the subjects of the PM phenotype instead utilised the alternative pathway available to produce large amounts of a toxic metabolite of phenacetin, namely 2-hydroxy-phenetidine. The rate of formation of this metabolite as well as that of paracetamol were studied in a panel of volunteers following ingestion of 1 g of phenacetin. The results are shown in Table 10.

Table 10: Oxidation phenotype and alternative pathways of toxic metabolite formation

(following single oral dose of 1 g phenacetin)

Metabolic Measurement	Oxidation Phenotype		2-tailed test Significance level
	EMs n=5	PMs n=7	
	% of dose excreted in 0-8 hours as each metabolite		
Paracetamol	58.1 ± 3.8	34.7 ± 2.5	$2p < 0.001$
2-OH-phenetidine	3.8 ± 1.7	9.3 ± 3.7	$2p < 0.01$
	Rate constants (h^{-1})		
K_f.paracetamol	0.251 ± 0.088	0.092 ± 0.015	$2p < 0.005$
K_f.2-OH-phenetidine	0.015 ± 0.009	0.017 ± 0.006	Not significant
	Ratio		
O-de-ethylation / 2-hydroxylation	18.1 ± 10.6	4.2 ± 1.5	$2p < 0.01$

Values are mean ± S.D. (After Ritchie et al, 1980)

These results show that the two phenotypes show marked differences in their ability to effect O-de-ethylation and further, that these differences paralleled their differences in ability to effect alicyclic hydroxylation of debrisoquine. However, these results illustrate two other important features; first, that inability to effect one pathway would impose a greater load on an alternative pathway thereby generating more of alternative metabolites which may prove toxic and secondly, all known aromatic hydroxylations are unlikely to be under the same genetic control. This is confirmed by the finding that aromatic hydroxylation of acetanilide to form paracetamol does not parallel debrisoquine hydroxylation (Wakile et al, 1979).

6.5c Sulphoxidation of S-carboxymethyl-L-cysteine

S-carboxymethyl-L-cysteine ('Mucodyne') is a mucolytic agent widely used in bronchitis. The drug undergoes a complex metabolic elimination where at least four metabolites are the result of sulphoxidation of the parent drug (Waring, 1980). Sulphoxidation of mucodyne was investigated in a panel of 8 volunteers, which included 4 EMs and 4 PMs (Waring et al, 1981). Each volunteer swallowed 750 mg of mucodyne in capsule form and 0-8 hours urine was collected in bulk. Results showed that both EMs and PMs eliminated a mean of 53% (range 35-85% for EM and 35-91% for PM) of the oral

dose in 8 hours. However, sulphoxides accounted for a mean of 22% (range 18-27%) in EMs but only 2.2% (range 1.3-4.2%) in PMs. This ten-fold difference in the output of sulphoxides between the two phenotypes suggests that sulphoxidation of mucodyne may also be controlled by the D^H/D^L alleles.

6.5d Metabolic oxidation of sparteine

Sparteine is an anti-arrhythmic and an oxytocic drug. In 1975, during the kinetic studies of this drug, two subjects who developed side-effects were noticed to have plasma levels which were 3-4 times higher than normal (Eichelbaum et al, 1979). Although the exact mechanism by which sparteine is oxidised is a matter of some debate, it is accepted that the drug undergoes metabolic dehydrogenation to produce sparteine-2-dehydro and sparteine-5-dehydro metabolites (Eichelbaum et al, 1979). The above two subjects were unable to metabolise sparteine and further extensive studies confirmed that the dehydrogenation of sparteine was polymorphic and under genetic control of a single pair of alleles. Eichelbaum et al (1979) found that 18 out of 350 subjects (5%) investigated were unable to effect dehydrogenation of sparteine. The trait for impaired dehydrogenation was inherited in autosomal recessive fashion (Eichelbaum et al, 1979). Subsequently, further studies have been reported and it has been confirmed that the

inability to carry out the dehydrogenation of sparteine has the same cause as the inability to carry out the alicyclic hydroxylation of debrisoquine. Further extensive studies have confirmed that the two oxidations are under the control of the same pair of gene alleles (Eichelbaum et al, 1982).

6.5e Stereospecific benzylic hydroxylation of nortriptyline

Nortriptyline is an anti-depressant drug whose plasma levels are controlled by genetic factors. The major metabolite of the drug is 10-hydroxy-nortriptyline, resulting from benzylic hydroxylation of the parent drug (Alexanderson and Borgä, 1973). This metabolite is excreted in the urine as two isomers, E- and Z- isomers (Bertilsson and Alexanderson, 1972).

Mellström et al (1981) studied the metabolic clearance of nortriptyline in 8 healthy, previously phenotyped, volunteers following a single oral dose of the drug. The 8 volunteers between them covered a wide range of ability to effect debrisoquine 4-hydroxylation. These workers found that the 4 subjects who had the least ability to effect debrisoquine hydroxylation excreted less 10-hydroxy-nortriptyline in urine than the other 4 subjects, and further showed that 10-hydroxylation of nortriptyline in E- position, but not in the Z-position, correlated closely with debrisoquine 4-hydroxylation status.

It therefore appears that the D^H/D^L alleles, controlling alicyclic debrisoquine oxidation, also control:

a) aromatic hydroxylation (for example, guanoxan)

b) O-de-ethylation (for example, phenacetin)

c) sulphoxidation (for example, mucodyne)

d) dehydrogenation (for example, sparteine)

It is also evident that the control may be stereo-selective in some instances (for example 10-E-hydroxylation of nortriptyline) and further, that the control may not encompass all known drugs oxidised by above pathways (for example, 2-hydroxylation of phenacetin).

The fact that a single pair of gene alleles, controlling debrisoquine hydroxylation, also control a variety of other oxidation reactions in metabolic elimination of other drugs makes it probable that:

A) the metabolism of, and the response to, a drug in a large population sample, irrespective of the ethnic origin, can be predicted from a phenotyped panel approach and

B) if the metabolism of, and the response to, a drug have been defined beforehand, the debrisoquine phenotyping test may offer a simple means of identifying at-risk individuals before the therapy with the drug is even commenced.

These two precepts are examined in the following Chapters. The proposal A) is studied prospectively using phenformin as the test drug. The proposal B) is validated by a retrospective study of patients who had developed peripheral neuropathy on long-term treatment with perhexiline and the predictive value of debrisoquine phenotyping test is verified in three patients so treated.

CHAPTER SEVEN

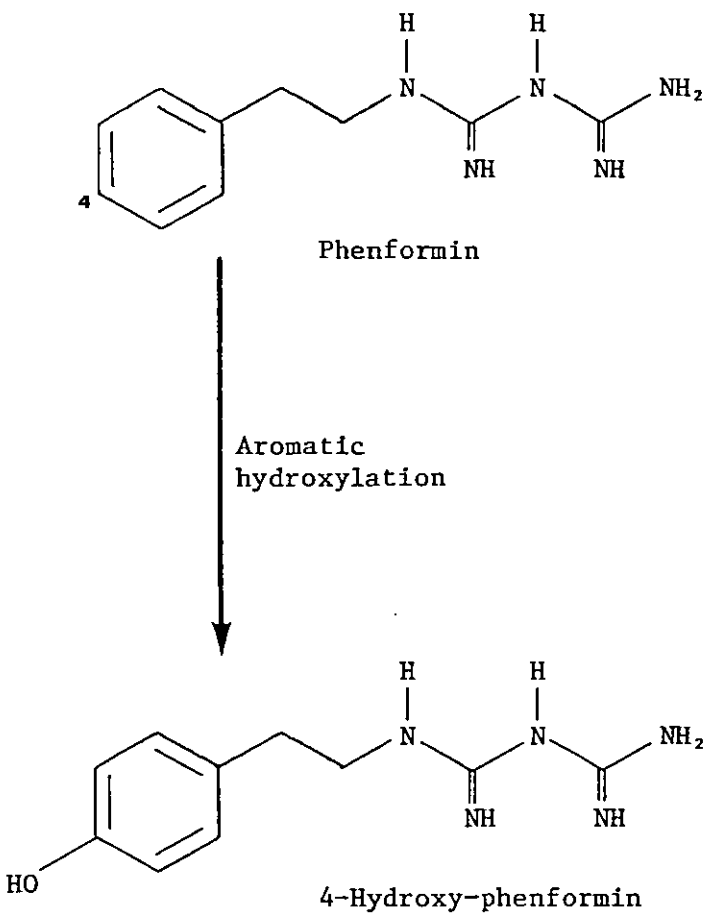
POLYMORPHIC HYDROXYLATION OF PHENFORMIN AND ITS SIGNIFICANCE

7.1 Phenformin metabolism in relation to its hypoglycaemic and hyper-lactic acidogenic properties

The hypoglycaemic action of guanidine was first described in 1918 by Watanabe (1918). Subsequently in the 1920's, two biguanides (synthalin A and synthalin B) were synthesized and introduced for the clinical treatment of diabetes mellitus. These later fell into disrepute and were withdrawn because further animal studies showed them to be hepatotoxic and just then, insulin was being introduced in clinical medicine. It was not until 1953 that substituted biguanides with hypoglycaemic actions were discovered and were made available for clinical use in 1956 for the treatment of maturity-onset diabetes mellitus (Ungar, Freedman and Shapiro, 1957; Williams *et al*, 1957). A large number of these compounds were synthesized but only three are in common use (Cohen and Woods, 1976); namely, phenformin, metformin and buformin, the use of the latter being confined largely to Germany and Switzerland.

Phenformin, one of the substituted biguanides, was introduced in the U.K. in 1957. Chemically, phenformin is beta-phenethyl-biguanide (Fig. 7), and is an orally active hypoglycaemic agent. It is more potent than the other commonly used biguanide in the U.K., namely metformin. Whereas phenformin is commonly used in the dose of 100-150 mg daily, the equi-potent dose of metformin appears to be 850-1350 mg daily (Cohen and Woods, 1976).

Figure 7 Structure and metabolism of phenformin in man



Various clinical trials (Patel and Stowers, 1964; Bloom, 1969a, 1969b) have confirmed the efficacy of phenformin, especially in combination with a sulphonyl-urea drug like chlorpropamide, in controlling the blood sugar levels in diabetic patients (Waters, Morgan and Wales, 1978). The combination was particularly effective since the two groups of drugs have different sites of action with synergistic effects (Beaser, 1958). Phenformin was also used in juvenile, brittle diabetes to supplement treatment with insulin. The drug found considerable favour with diabetologists. The sales of biguanides, in Sweden alone, with an approximate population of 7.5 million, were converted to defined-daily-doses. During the years 1975-77, the figures yielded were 7.37×10^6 for phenformin and 7.50×10^6 for metformin. These drugs were introduced in Sweden in 1961 and 1965 respectively (Bergman, Boman and Wiholm, 1978). In 1975, it is estimated that in U.K., about 30,000 patients were taking phenformin (Gale and Tattersall, 1976) and it was widely used in that country as late as 1976.

The hypoglycaemic effects of phenformin appear to be exerted by three main mechanisms (Alberti and Nattrass, 1977):

1. Inhibition of absorption of glucose from the gastro-intestinal tract.
2. Inhibition of gluconeogenesis.
3. Enhancement of glycolysis.

Clinically, the main effect is exerted by the first two mechanisms. It is generally thought that phenformin and other biguanides are without any significant effect in non-diabetic individuals (Shen and Bressler, 1977).

When given orally, phenformin appears to be concentrated in the gastric juice due to the high pKa (11) of the drug (Shapiro, quoted by Wick, Stewart and Serif, 1960). In man, 45-55% of the oral dose of drug is absorbed over a period of 24 hours (Beckmann, 1968). The drug is also concentrated in the liver (Bingle, Storey and Winter, 1970) where it undergoes oxidative biotransformation to give 4-hydroxy-phenformin (Fig. 7; Beckmann, 1968) by a significant first pass metabolism. There is minimal protein binding with reported values between 12 and 20% (Alkalay et al, 1975). The volume of distribution is calculated at about 200 litres. Beckmann (1968) reported the composite terminal plasma half-life of the drug and its metabolite to be 3.2 hours, while Alkalay et al,(1975) found the terminal plasma half-life of 11 hours for phenformin. This value is closer to a previously reported value of 9 hours for phenformin plasma half-life (Karam et al, 1974).

In man, the drug is very largely eliminated from the body by urinary excretion. Most of the drug elimination takes place in the first 24 hours, the renal excretion following first-order kinetics (Beckmann, 1968). Alberti and Nattrass (1977) quotes the renal clearance of phenformin

to be 20 ml/min. About $\frac{2}{3}$ of the absorbed dose is eliminated as unchanged parent drug while the remainder appears as its inactive metabolite, 4-hydroxy-phenformin, resulting from aromatic hydroxylation (Fig. 7; Beckmann, 1968). Species other than man further conjugate the hydroxyl function to form its glucuronide (Murphy and Wick, 1968). This does not appear to occur in humans and 4-hydroxy-phenformin appears to be the exclusive metabolite (Beckmann, 1968).

The enthusiastic use of phenformin was slightly tempered in 1963 when Tranquada and his colleagues (Tranquada, Bernstein and Martin, 1963) first reported an association between phenformin therapy and lactic acidosis.

Phenformin has been known to have lactic acidogenic properties as early as 1957 (Tyberghein and Williams, 1957). Lacher and Lasagna (1966) found elevation of blood lactate concentrations and an associated impairment in ability to metabolise the lactate load in rats treated with phenformin in a dose of 40 mg/kg for 3-8 days. In phenformin-treated diabetic patients, apart from overt lactic acidosis, small but significant effects on lactate metabolism and circulating lactate concentrations have been observed. Craig et al (1960) found a small elevation of resting blood lactate levels and increased lactate excretion in eight diabetic patients following therapeutic doses of phenformin. Importantly, the effect was noted to be dose-related. Also, phenformin decreased lactate tolerance of the six patients studied by these workers.

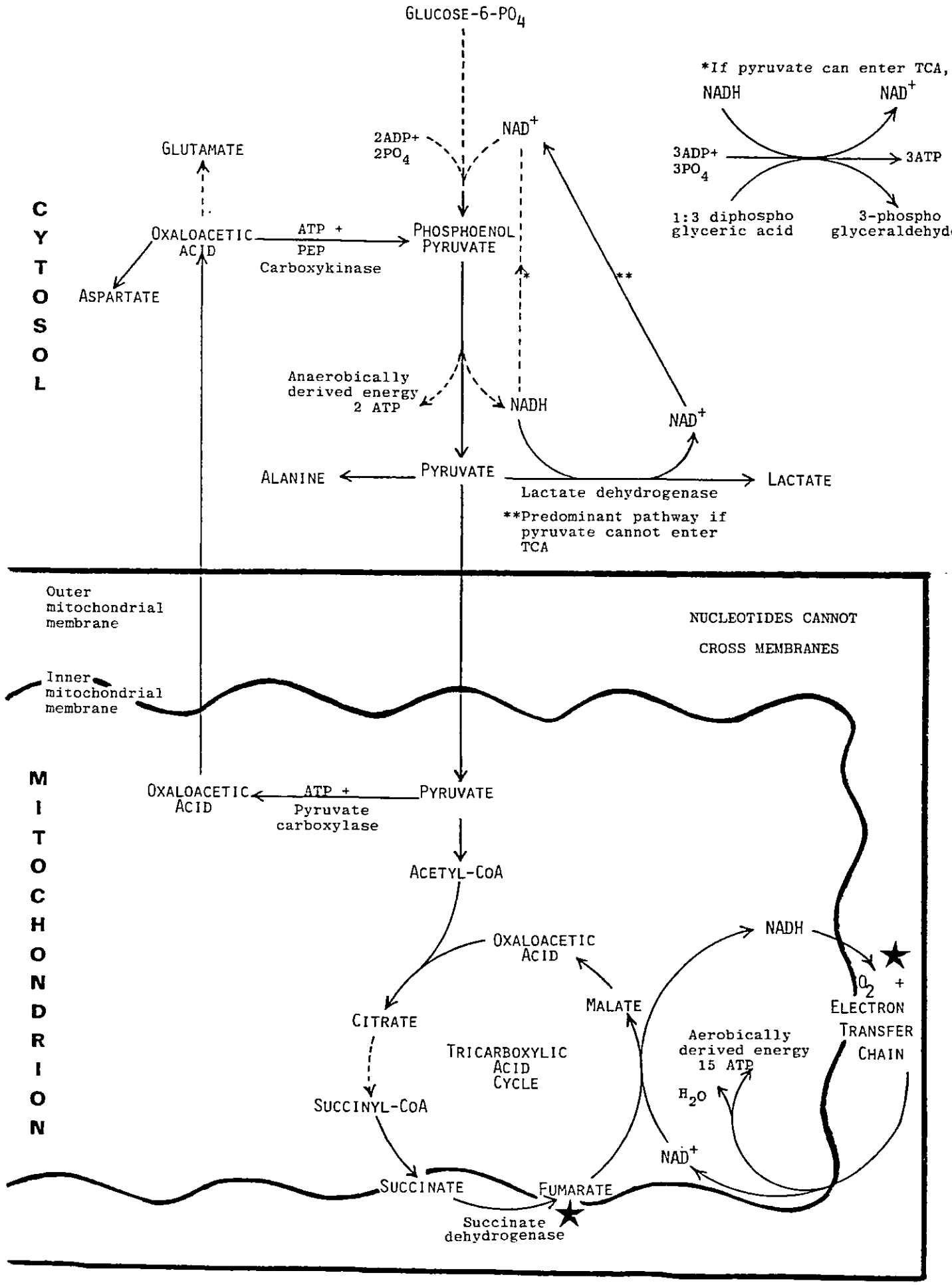
The exact mechanism by which phenformin leads to disturbance of lactate metabolism is not clear.

The metabolism of lactic acid in normal individuals (Kreisberg, 1980) is shown in Fig. 8. The anaerobic glycolysis in the cytosol leads to the production of pyruvate. This pyruvate crosses the mitochondrial membrane to enter mitochondria and is further metabolised by the tricarboxylic acid (TCA) cycle to yield aerobically derived energy stored in adenosine triphosphate. If, for any reason, oxygen debt is built up, for example during exercise, pyruvate builds up first in the mitochondria and then in the cytosol. This build up of pyruvate results in the production of lactate to convert NADH into NAD^+ which is then utilised to yield more energy by cytosolic anaerobic glycolysis. The result is a rise in blood lactate levels. When the oxygen debt is restored, lactate is re-converted into pyruvate which is then metabolised by aerobic tricarboxylic acid cycle. Normally, the tissues that produce the body load of lactate are skeletal muscles and cardiac muscles and the organ that is able to clear lactate is the liver.

It is easy to see how, if the tricarboxylic acid cycle in hepatocytes is disrupted, lactate cannot be metabolised and hence hyperlactic acidaemia would follow.

If the hypoglycaemic effect of phenformin is produced primarily by inhibition of glucose absorption and gluconeogenesis, glycolysis becomes an important route to

Figure 8 Lactate metabolism in normal man. Biochemical lesions due to phenformin are also indicated (★)



generate energy. This cytosolic pathway, which is enhanced by phenformin, is anaerobic and results in accumulation of pyruvate and NADH. Unless pyruvate can enter the TCA cycle, pyruvate is converted to lactate by LDH-dependent conversion of NADH to NAD^+ for recycling since nucleotides do not cross the mitochondrial membrane.

In vitro studies have suggested that this, in fact, is the case; additionally, phenformin also interferes with cellular aerobic metabolism (Steiner and Williams, 1958, 1959). In this respect, phenformin can be likened to other inhibitors of oxidative metabolism like cyanide and malonic acid (Handler, 1945). Administration of these drugs to animals also leads to hypoglycaemia and hyperlactic acidaemia (Albaum, Tepperman and Bodansky, 1946).

The exact mechanism by which phenformin inhibits TCA cycle is a matter of debate. It seemed that since the drug inhibited NAD^+-dependent oxidative phosphorylation at the lowest drug concentration (10^{-4} moles) employed in various in vitro studies (Ungar, Psychoyos and Hall, 1960), this was the primary site of action in TCA cycle. However, in patients taking phenformin in standard dose of 50 mg tds, the drug concentration was in the order of $1\text{-}5 \times 10^{-6}$M range (Cohen and Woods, 1976). Schafer (1976) showed that biguanides, including phenformin, bind to biological membranes and cause a positive shift of the surface electrostatic membrane potential. Phenformin binds to the

phospholipid content of mitochondrial membrane with a high affinity. This is directly related to the partition coefficient of this highly lipid soluble drug. Thus, electrostatic forces probably contribute negligibly in its partition but the high pKa of phenformin renders it highly charged at body pH and this appears to be very important for the binding. Since cytochromes, responsible for oxidative phosphorylation in the electron transfer chain, are integral parts of intramitochondrial membranes, disruption of the TCA cycle is inevitable. Malfunction of the mitochondrial electron transfer chain fails to generate NAD^+ (from NADH) on which the effective functioning of the TCA cycle depends (Jangaard, Pereira and Pinson, 1968). Pyruvate in the cytosol accumulates to high concentration which, with NADH, then shifts the equilibrium in favour of lactic acid plus NAD^+. Since cytosolic nucleotides cannot penetrate into mitochondria, NAD^+ is necessarily utilised in anaerobic glycolytic pathway to yield pyruvate plus NADH. While NADH accumulates in mitochondria, the cellular energy requirements are met by anaerobic glycolysis in the cytosol where nucleotides are recycled with resulting increase in lactic acid levels and decrease in sugar levels. In essence, phenformin dislocates cytosolic anaerobic metabolism from mitochondrial aerobic oxidative phosphorylation.

A vicious circle is then set up and it can be seen that its intensity would depend on the extent to which glycolysis is enhanced and the electron transfer chain disrupted. In turn, this would depend on phenformin binding. In addition, it has been found that phenformin inhibits succinic acid dehydrogenase (Steiner and Williams, 1958), an enzyme in the TCA cycle, involved in conversion of succinate to fumarate. This enzyme too is located in the inner mitochondrial membrane. These effects result in peripheral over-production of lactic acid which is either under-utilised or not utilised at all by liver and kidney.

The above suggests that the hypoglycaemic effects of phenformin cannot be separated from its propensity to raise lactate levels (Alberti and Nattrass, 1977) and further, that these two aspects would be related to plasma phenformin levels (Cohen et al, 1973).

Since the first description of phenformin-induced lactic acidosis by Tranquada in 1963, more reports of this association appeared. In a comprehensive review in 1970, Oliva could find only 18 published cases of phenformin-associated lactic acidosis. Cohen and Woods reported that by January 1976 there were 150 published cases of phenformin-associated lactic acidosis, while by autumn of that year, the figure had risen to at least 166 (quoted by Alberti and Nattrass, 1977) and Cohen knew of just under 300 published cases by June 1978 (Cohen, 1978). The figures quoted are,

in all likelihood, a gross under-estimate and the association being well known, many more cases must have gone unreported. Many cases may not be reported because of inadequate documentation of lactic acidosis. It may be mentioned at this stage that although no universally accepted definition of lactic acidosis exists, lactic acidosis due to phenformin is the likely diagnosis when, in a patient taking phenformin:

1. arterial pH is less than 7 and/or
2. there is an unexplained anion gap substantially in excess of 20 mmol/L.

Of course, the surest way of diagnosis is to document a lactate level in excess of 6 mmol/L (the normal being 0.4-1.3 mmol/L in venous blood; the arterial values being a little lower) (Cohen, 1978).

In Sweden, where reporting of adverse drug reactions is compulsory, it is thought that only 30% of reactions are reported. Between the years 1965-1977, 64 cases of adverse drug reactions due to phenformin were reported to the Swedish Adverse Drug Reactions Committee (Bergman, Boman and Wiholm, 1978). Of these, 50 (78.1%) were lactic acidosis. During the same period, 8 reported cases of adverse drug reaction to metformin included only one case of lactic acidosis (12.5%) - over six-fold greater incidence with phenformin compared to metformin. By June 1978, Cohen could find only 20 reported cases of lactic

acidosis associated with the use of metformin.

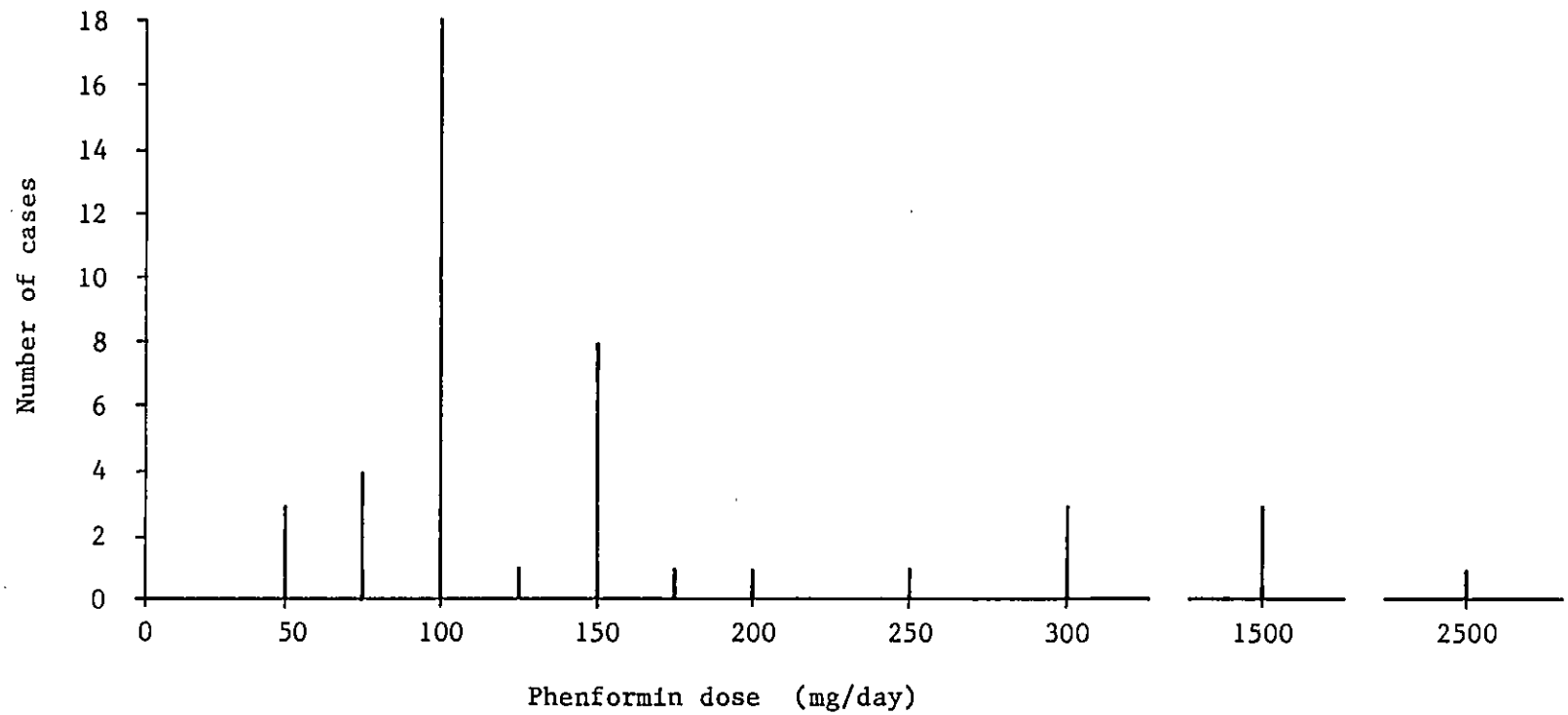
When the epidemiology of phenformin-associated lactic acidosis is reviewed, interesting facts emerge. The fact that the effect of phenformin on lactate metabolism *in vitro* (Altschuld and Kruger, 1968) and during clinical usage (Craig *et al*, 1960; Cohen *et al*, 1973) is dose-related, leads one to determine whether the patients were receiving large doses of phenformin. Cohen and Woods (1976) analysed the doses of 44 patients at the time of onset of lactic acidosis. If those cases (n=4) where a massive overdose was ingested are excluded, it appears that in most cases, n=34 out of 40, the patients were taking 150 mg per day or less of phenformin. Six patients were taking doses of 175 mg to 300 mg daily. Luft, Schmulling and Eggstein (1978), in a review of patients with lactic acidosis due to biguanides generally, showed that 281 cases out of a total of 327 were due to phenformin. Twelve were due to metformin while buformin accounted for another 30 cases. Four patients were taking phenformin and metformin concurrently. The doses of phenformin, metformin and buformin in these patients were 123 $\pm$ 4 mg, 1595 $\pm$ 182 mg and 258 $\pm$ 25 mg respectively. In a review of 34 patients whose case histories contained sufficient information, Cohen *et al* (1973) confirmed that in 24 of these patients, lactic acidosis occurred within 2 months of starting therapeutic dose of phenformin. In 17 of these patients,

onset was within 2 weeks of starting the treatment. These workers acknowledged that if lactic acidosis had been the result of long standing disease alone, such as diabetes or liver disease, the early concentration of incidence would not be expected. Cohen and Woods' (1976) analysis of the doses being taken at the time of onset of lactic acidosis is shown in Fig. 9, whilst distribution of times to onset is shown in Fig. 10. The distribution of times to onset in Fig.10 is strong evidence for causal role of phenformin.

Phenformin-associated lactic acidosis has a mortality of 50-70% (Alberti and Nattrass, 1977; Cohen, 1978) and in September 1977, the Committee on Safety of Medicines issued a warning on the use of phenformin but it stopped short of withdrawing the drug (Cohen, 1978).

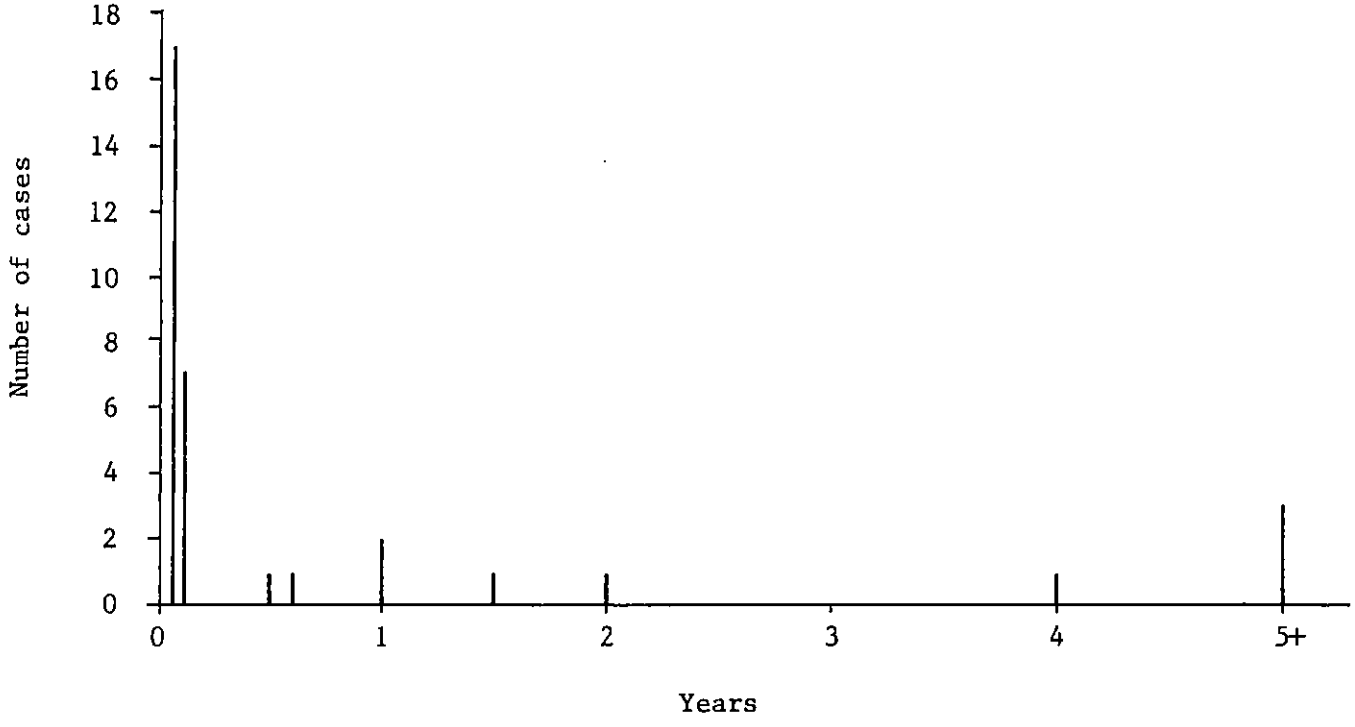
It may be argued that the relative safety of metformin reflects prescribing habits. Argument against this is provided by experiences in France and Switzerland. In France, 76% of biguanide therapy is with metformin and 24% with phenformin and yet, as in the case of Sweden, there was about six-fold greater incidence of lactic acidosis with phenformin (Isnard and Lavieuville, 1977). In Switzerland where buformin is also used, phenformin, metformin and buformin shared 8%, 23% and 69% of the biguanide market but the frequency of lactic acidosis associated with these drugs was 13%, 3% and 84% respectively (Berger, quoted by Luft, Schmulling and

Figure 9 The dose of phenformin being taken at the time of onset of lactic acidosis: an analysis of 44 cases



[Modified after Cohen and Woods, 1976]

Figure 10 Duration of phenformin therapy before onset of lactic acidosis: an analysis of 34 cases



[Modified after Cohen et al, 1973]

Eggstein, 1978). Furthermore, metformin-induced lactic acidosis seems to occur exclusively in the presence of marked renal impairment (Assan et al, 1977).

Cohen (1978) concluded that without doubt, the incidence of lactic acidosis per patient treated is much greater with phenformin. Phillips, Thomas and Harding (1977) estimated that the risk of developing lactic acidosis with phenformin may be about 50 times that with metformin. Clearly other factors are involved.

Amongst such factors, hepatic, renal and/or cardiac haemodynamic insufficiency have been implicated. The results of dysfunction of any of these organs may be further potentiated by concurrent use of other drugs or septicaemia. However, there appears to be little reason to suspect that these adverse factors operated in a disproportionately greater percentage of patients (about six-fold!) receiving phenformin than those receiving metformin (Luft, Schmulling and Eggstein, 1978). Furthermore, the severity of the acidosis as measured by arterial pH and lactate concentration does not correlate with the levels of serum urea present immediately before the development of lactic acidosis (Bengtsson, Karlberg and Lindgren, 1972). These observations suggest that renal function may not be a major determinant of the serum phenformin concentration or susceptibility to developing lactic acidosis. Age and sex distribution do not show any differences between the phenformin and the metformin groups.

The suspicions of other, as yet unrecognised, factors are confirmed by the precipitation of lactic acidosis in phenformin treated patients who have well documented normal hepatic, renal and cardiac functions (Anonymous, 1977; Conlay and Loewenstein, 1976; Blumenthal and Streeten, 1976). The suspicions are also supported by lactic acidosis in some of those normal individuals who attempted suicide with phenformin (Strauss and Sullivan, 1971; Cohen et al, 1973). Even in Sweden, it was found that the cases of lactic acidosis related to the use of phenformin had not decreased by 1975 to 1977, despite restrictions of the medication and clear definition of contra-indications for both phenformin and metformin in 1973 (Bergman, Boman and Wiholm, 1978). The relative incidences of adverse drug reactions reported for phenformin and metformin had not differed by 1977. Metformin itself does have hyper-lactic acidogenic properties (Bjorntorp et al, 1978). Clearly one suspects the presence of some other predisposing factor for the marked propensity of phenformin to cause lactic acidosis.

The onset of lactic acidosis is directly related to excessive accumulation of phenformin in plasma or tissues (Cohen, 1978) and the phenformin concentration need not be much increased for lactic acidosis to occur (Conlay et al, 1977). It is also noted that in patients with lactic acidosis, phenformin is mainly eliminated as unchanged drug.

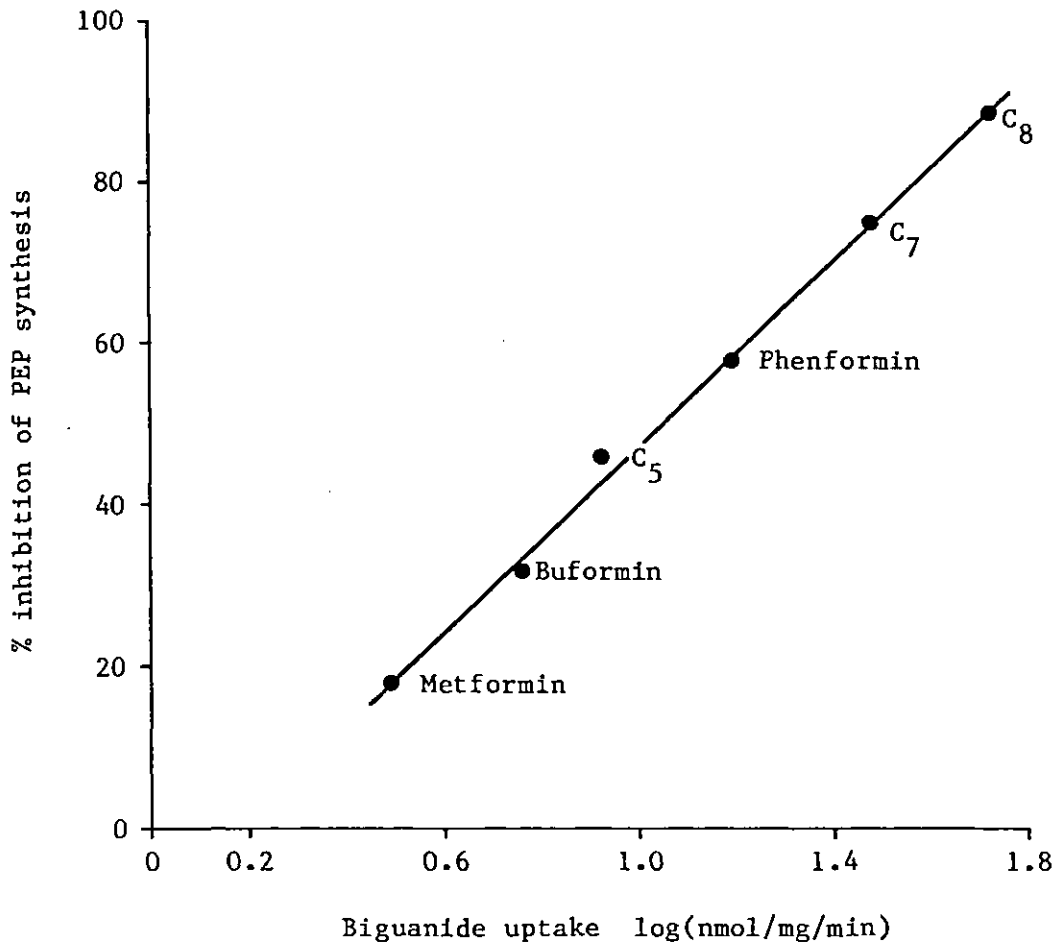
The answer as to why phenformin is so prone to producing lactic acidosis is to be found elsewhere. When the pharmacokinetics of phenformin is compared to that of metformin, the interesting differences that emerge appear to provide many answers.

One difference between the two compounds is the affinity with which each binds to mitochondrial membrane. Although all biguanides bind to this membrane, the quantitative effect is related to the length of the hydrocarbon side-chain attached to the biguanide moiety (Schafer, 1976). Since the hydrocarbon portion of the molecule is responsible for membrane binding, the larger this side-chain, the greater is the binding affinity and the greater are the metabolic effects exerted.

In vitro, the binding is quantified by the synthesis of phosphoenol-pyruvate from pyruvate which cannot enter the TCA cycle. Pyruvate is converted by pyruvate carboxylase to oxaloacetic acid which is then converted to phosphoenol-pyruvate by phosphoenol-pyruvate-carboxykinase. Figure 11 shows the correlation of biguanide inhibition of phosphoenol-pyruvate synthesis versus mitochondrial capacity for biguanide binding in pigeon liver (Schafer, 1976). It can be seen that phenformin is approximately three times as potent as metformin in inhibiting phosphoenol-pyruvate synthesis. However, it is difficult to relate this directly to the effects of these drugs on intermediary metabolism in vivo because:

Figure 11 Correlation of biguanide inhibition of phosphoenol-pyruvate (PEP) synthesis with mitochondrial capacity for biguanide binding in pigeon liver

(Schäfer, 1976)



1. Clinically much smaller doses of phenformin are usually used when compared to metformin and
2. The overall relationship between membrane binding affinity and pharmacological effect does not appear to be clear.

It is then probable that other pharmacokinetic factors play a part.

The major pharmacokinetic difference between phenformin and metformin resides in their metabolism resulting from their polarity. Metformin is highly water-soluble polar drug which is excreted unchanged in the urine (Pentikainen, Neuvonen and Penttila, 1979). Its renal clearance approximates glomerular filtration rate (125 ml/min). In contrast, unchanged phenformin has a renal clearance of only 20 ml/min and the elimination of phenformin depends very markedly on its metabolism by 4-hydroxylation in the liver, rendering it polar. It has been shown that approximately $\frac{2}{3}$ of the absorbed phenformin is excreted unchanged while $\frac{1}{3}$ is excreted as 4-hydroxy-phenformin in the urine. Further, 4-hydroxylation is the exclusive pathway in man.

Thus, metformin elimination depends on glomerular filtration rate while phenformin elimination depends on its hepatic metabolism. A correlation between plasma phenformin levels and the effect upon blood lactate has been documented

and it has been shown that in many patients with lactic acidosis, higher than expected blood levels of phenformin are detected. The overall picture that emerges from the survey suggests that these higher levels of drug may be related neither to the dose nor to the hepato-renal functions. Interestingly enough, phenformin-induced lactic acidosis seems to occur only very rarely in India (Chandalia, 1979) and possible ethnic differences in phenformin metabolism have been suspected to account for this.

It then seems possible that in certain individuals, the inherent inability to hydroxylate phenformin would predispose to its higher blood levels and hence to lactic acidosis. As long ago as 1970, Sussman et al suspected the presence of a probable inborn error in lactate metabolism unmasked by phenformin. Cohen (1978) too concluded that in many patients, it is at present impossible to predict their response to phenformin.

In conclusion, phenformin, in common with other biguanides, exerts its hypoglycaemic effects by inhibition of glucose absorption from the gut and inhibition of gluconeogenesis. However, the dislocation of the disrupted mitochondrial aerobic metabolism from the cytosolic anaerobic metabolism and the enhanced glycolysis that results lead to hyper-lactic acidaemia. Phenformin is more prone to producing lactic acidosis when compared to metformin. The propensity of

phenformin to produce lactic acidosis depends on its concentration in blood and patients with phenformin-associated lactic acidosis excrete relatively more of the unchanged drug in their urine. It is noted that phenformin concentration in blood is determined largely by metabolic clearance by aromatic hydroxylation. No obvious and consistent cause to account for high levels of phenformin in some individuals has been found up to date. Phenformin-associated lactic acidosis is an individual idiosyncrasy, based on drug metabolic factors. It displays ethnic differences in frequency. An individual predisposing factor, possibly an inborn error of metabolism, has been suspected.

Various questions, then, arise. These are:

1. Can inter-individual differences in the ability to hydroxylate phenformin be discerned by the use of a debrisoquine-phenotyped panel approach?
2. If so, does phenformin metabolism display genetically controlled polymorphic hydroxylation?
3. Is phenformin hydroxylation status of an individual a consistent characteristic?
4. If phenformin is polymorphically hydroxylated, is there an alternative pathway of metabolism present in poor hydroxylators of phenformin?
5. Can the relation between phenformin hydroxylation and debrisoquine hydroxylation be extrapolated to a larger sample of population?

6. Is phenformin hydroxylation dose-related?
7. Is there a difference in the metabolic response between the extensive and the poor hydroxylators of debrisoquine?
8. What is the debrisoquine hydroxylation status of patients who have survived lactic acidosis?

If any consistency is observed, the debrisoquine phenotype test may offer a means of identification of at-risk individuals.

9. If it is suspected that lactic acidosis is the consequence of poor hydroxylation of phenformin, what is the explanation of the apparent relatively lower frequency of phenformin-induced lactic acidosis, compared to the frequency of poor metabolisers in a population?

In order to address these questions, various studies were designed. These studies and their results are discussed in the following sections.

A simple, reliably accurate h.p.l.c. method, allowing simultaneous measurement of phenformin and 4-hydroxy-phenformin in urine, was developed in the department by Drs N.S. Oates and J.R. Idle. This is described first.

7.2 An h.p.l.c. method for simultaneous measurement of phenformin and 4-hydroxy-phenformin in the urine

A simple and reliably accurate method to measure phenformin and 4-hydroxy-phenformin simultaneously in the urine was developed to carry out these studies.

A number of methods have been described for the measurement of phenformin (Matin, Karam and Forsham, 1975; Hill and Chamberlain, 1978) but this is not the case for measurement of 4-hydroxy-phenformin. This is so because 4-hydroxy-phenformin possesses a strongly basic biguanide group and a weakly acidic phenolic hydroxyl group, rendering it difficult to extract into non-polar solvents. There were encountered technical problems with the method described by Mottale and Stewart (1975) for derivatising 4-hydroxy-phenformin and therefore an h.p.l.c. method was developed by my colleagues, Drs N.S. Oates and J.R. Idle.

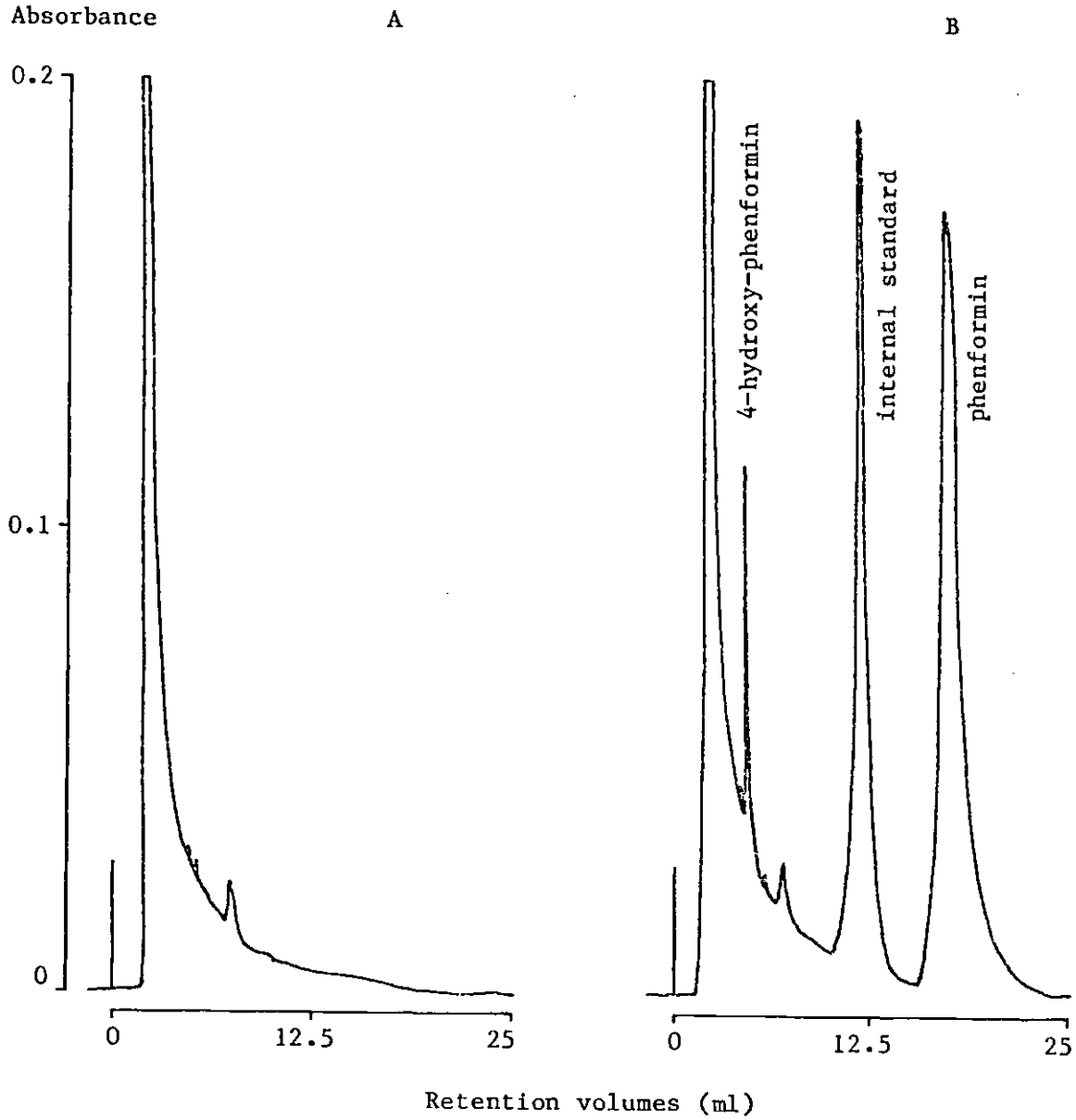
In the normal urine, there appears to be present an unidentified substance with chromatographic properties similar to those of 4-hydroxy-phenformin. This substance was separated from phenformin and 4-hydroxy-phenformin by exploiting the ability of Amberlite XAD-2 resin to absorb polar aromatic compounds such as phenformin and its metabolite. The h.p.l.c. method developed is described below:

Amberlite XAD-2 resin (BDH) was packed into small columns measuring 5 cm long x 0.5 cm diameter. These were prepared for use by washing successively with three ml each of acetone, methanol and water. One ml of a solution of phenacetin (100 ug/ml), which acted as an internal standard, was applied to each column and this was followed directly by 1-5 ml of the test urine sample or by calibration standard within the range 40-200 ug of phenformin and 4-20 ug 4-hydroxy-phenformin. The columns were then washed with 3 ml of water to remove the impurities. The elution of phenformin, 4-hydroxy-phenformin and the internal standard was carried out with 3 ml of methanol and the eluant was collected in 50 ml ground-glass stoppered tubes. Using a rotary evaporator, these extracts were then dried under vacuum at 50°C and chromatographed using an h.p.l.c. column packed with a bonded reversed phase material (Waters Radial Compression Column Pak A) through which a mobile phase of 30% (v/v) acetonitrile in 0.05M potassium dihydrogen orthophosphate was pumped (Pye Unicam LC-XPS) at ambient temperature at 3 ml/min. Detection was achieved by UV-absorption at 230 nm (Pye Unicam LC-UV). The dried residues obtained from the eluates from the XAD-2 columns were redissolved in 100 ul of the h.p.l.c. solvent, 5 ul of which was injected onto the column. The retention volumes were as follows:

4-hydroxy-phenformin 5.1 ml, internal standard (phenacetin) 12.6 ml and phenformin 18.9 ml (Fig. 12). Calibration curves were obtained for both phenformin and 4-hydroxy-phenformin by calculating the peak height ratios (component : internal standard). Over the range of concentrations used, the curves were linear and had a sensitivity of measurement down to 1 ug/ml of phenformin and 0.5 ug/ml of 4-hydroxy-phenformin. Their recovery was high giving a mean (± S.D.) value of 97% (± 2.5%) for phenformin and 95.5% (± 2%) for 4-hydroxy-phenformin.

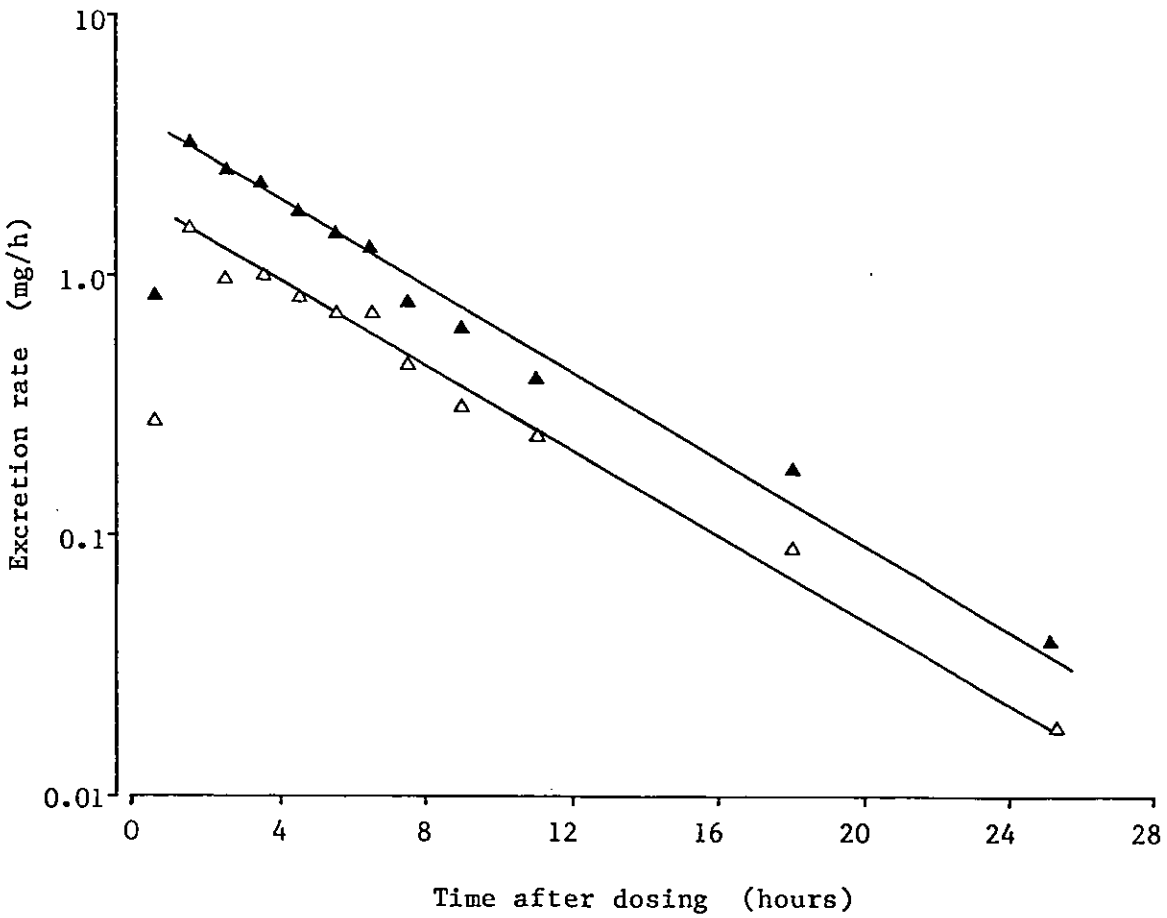
Following a single oral dose of 50 mg phenformin (Dibotin, Winthrop Laboratories, U.K.)to a healthy male volunteer, urine samples were collected hourly for 8 hours and then at 10, 13, 24 and 26 hours. The volume of each fraction was recorded and an aliquot stored at -20°C prior to analysis. The unchanged drug and its metabolite could be detected in all samples and their excretion rates are plotted on a logarithmic scale against time (Fig. 13). The existence of a significant first pass metabolism was strongly suggested by the coincidence of maximum rates of excretion for both compounds in the same urine sample (1-2 hours post-dosing). Their subsequent exponential elimination yielded the estimated elimination half-lives of phenformin and 4-hydroxy-phenformin as 3.7 hours and 3.8 hours respectively. The total observed recovery of the drug in this subject was 55.2% of the oral dose, comprising 37% as unchanged phenformin and 18.2% as 4-hydroxy-phenformin.

Figure 12 Chromatograms showing peaks for phenformin, 4-hydroxy-phenformin and internal standard



Hplc traces of: A. Blank urine
B. Urine containing
100 ug/ml phenformin
10 ug/ml 4-hydroxy-phenformin
100 ug/ml internal standard (phenacetin)

Figure 13 The rates of urinary excretion of phenformin (▲) and 4-hydroxy-phenformin (△) in one healthy male volunteer (given a single oral dose of 50mg phenformin)



7.3 Phenformin hydroxylation in a phenotyped panel of volunteers

Introduction

The metabolism of phenformin was investigated in a panel of 7 volunteers. These were previously phenotyped as extensive (EM) or poor (PM) metabolisers by their ability to effect debrisoquine hydroxylation.

Volunteers

The panel included 4 EMs and 3 PMs and the details of these volunteers are shown in Table 11. They had normal hepato-renal, cardiac and pulmonary functions. Their full blood count, urea and electrolytes, liver function tests, chest X-ray and electrocardiograms were all normal. None had taken any medication for at least one week before the study.

Method

Each volunteer, after an overnight fast, presented on the morning of the test in the laboratory. After voiding the bladder, a single 50 mg dose of phenformin (Dibotin, 25 mg x 2 tablets, Winthrop Laboratories, U.K) was swallowed orally with 150 ml of water. Timed fractional urine samples were collected over the following 24-26 hour period. After measuring the volume of each sample, an aliquot was stored frozen at -20°C for subsequent analysis.

Table 11: Details of the seven volunteers of the phenotyped panel

Volunteer	Sex	Age	Weight (kg)	Height (cm)	Cigarettes daily	Usual alcohol *consumption	Debrisoquine Metabolic Ratio	Debrisoquine Oxidation Phenotype
AZ	M	23	71	165	10	±	0.1	EM
JR	M	24	84	183	0	±	0.3	EM
LW	M	29	84	180	0	±	0.4	EM
JI	M	29	92	183	15	±	0.7	EM
RS	M	44	90	183	0	±	19.9	PM
JOG	M	29	63	170	15	±	24.5	PM
JDS	M	25	56	163	0	±	26.5	PM

* ± = only socially

The volunteers did not consume any medication or alcohol during the test period and they were allowed their usual meals at appropriate times.

For analysis, the urine samples were allowed to thaw at room temperature and the contents of phenformin and 4-hydroxy-phenformin were determined by Dr. Oates by the method described previously.

Results

The detailed results are tabulated in Appendix I. From the concentration of phenformin and 4-hydroxy-phenformin in timed aliquot samples, the following parameters were calculated for each compound and every volunteer:

1. Hourly excretion to 10 hours and at 10-12 and 12-24 hours.
2. Cumulative excretion up to 24 hours.
3. Observed and infinity recoveries.
4. Ratio of observed excretion of phenformin to 4-hydroxy-phenformin, both expressed as percentage of oral dose of phenformin.
5. Ratio of 0-8 hour excretion of phenformin to 4-hydroxy-phenformin, both expressed as percentage of oral dose of phenformin.
6. Urinary elimination rate constant.
7. Half-lives of the two compounds.

The mean excretion rates of unchanged drug and 4-hydroxy-phenformin for the two phenotyped groups are shown in Figs. 14 and 15 while the mean cumulative excretion of unchanged phenformin and total observed recovery of orally administered phenformin are shown in Figs. 16 and 17. Other parameters calculated are indicated in Table 12.

It can be seen from Table 12 that the volunteers of the EM phenotype excreted a mean total of 63% (range 57-69%) of the oral dose of phenformin, while the corresponding values for the PM phenotypes were 66% (range 61-70%). Of these, whereas the individuals of EM phenotype excreted a mean of 17% (range 13-20%) as the 4-hydroxy metabolite of phenformin, those of PM phenotype excreted only a mean of 1.2% (range 0-3.4%) as this compound. Thus, there was a 14-fold difference between the two groups in their ability to form the 4-hydroxy metabolite of phenformin. These differences between the two groups are also reflected in the ratios of percentage dose excreted as phenformin to percentage dose excreted as 4-hydroxy-phenformin over the observation period. In the EM volunteers, this ratio ranged from 1.9 to 4.0, while in the PM group, the range was 19.5 to infinity.

The mean hourly excretions of phenformin indicate that the maximum excretion for both groups occurs during the period 1-2 hours and furthermore, that apart from the quantitative differences, the mean hourly excretion of 4-hydroxy metabolite of phenformin in both groups closely parallels

Figure 14 Mean (±s.e.mean) rates of urinary excretion of phenformin and 4-hydroxy-phenformin in EM's

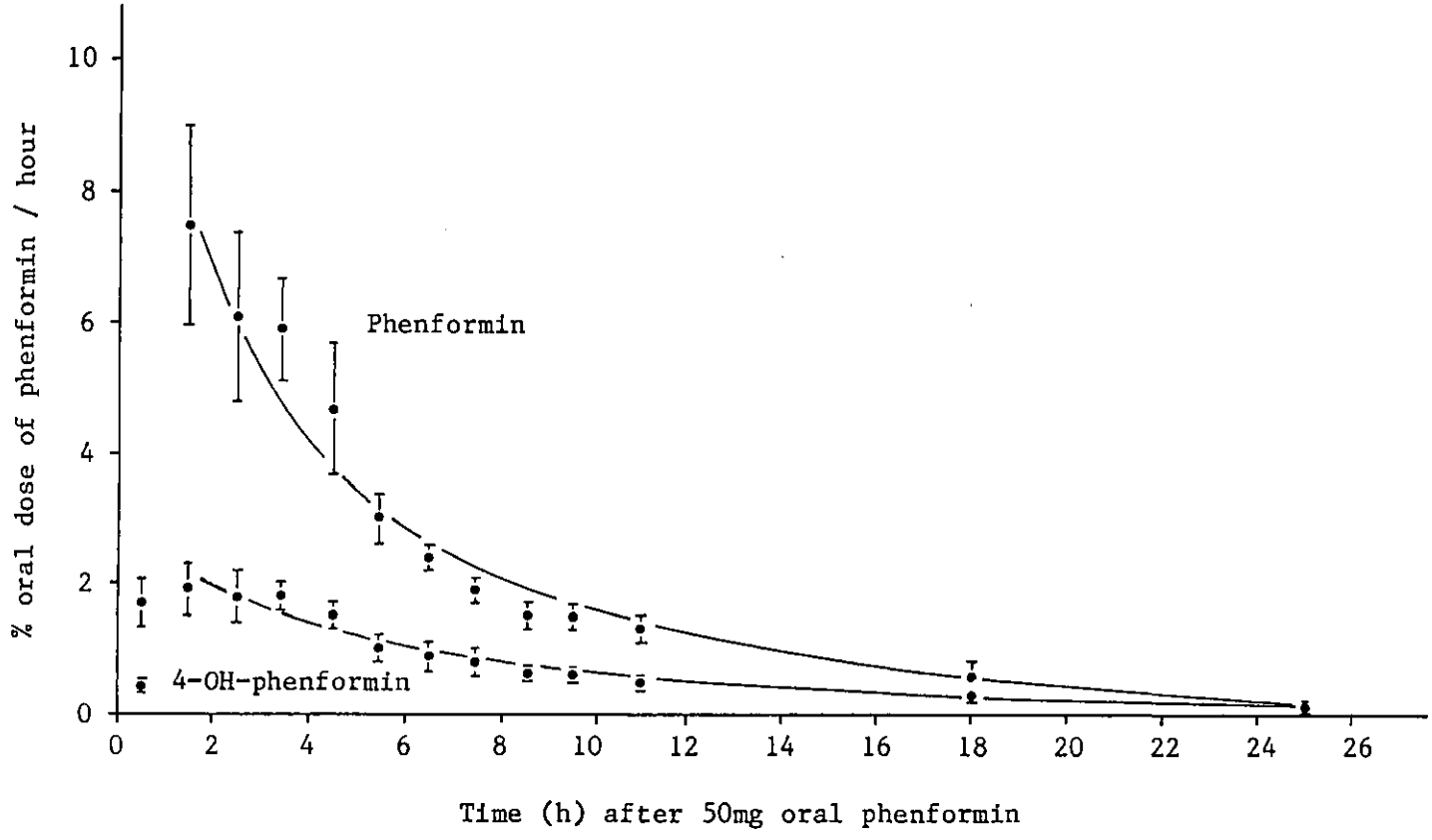


Figure 15 Mean (±s.e.mean) rates of urinary excretion of phenformin and 4-hydroxy-phenformin in PM's

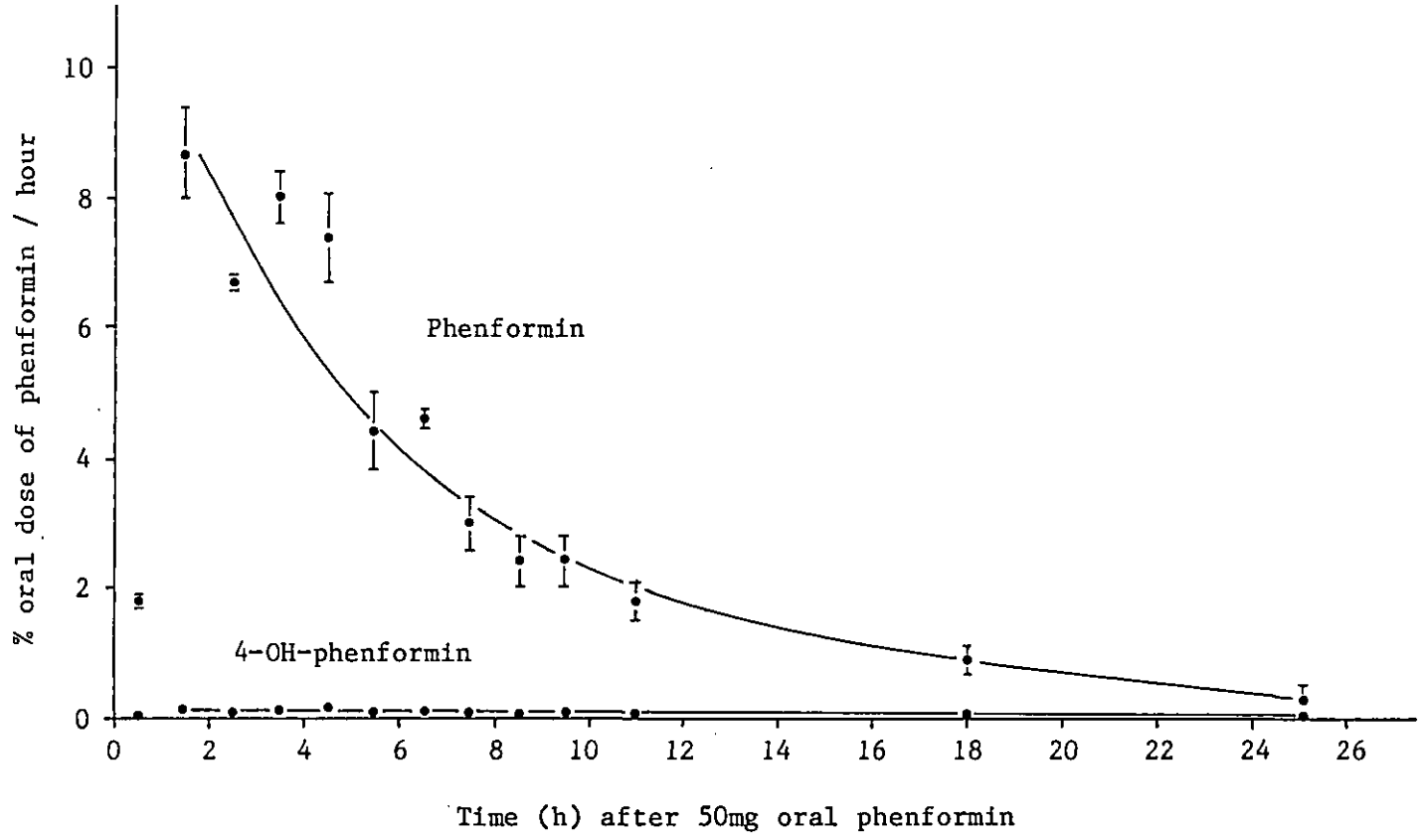


Figure 16 Cumulative urinary excretion of unchanged phenformin in the two phenotypes

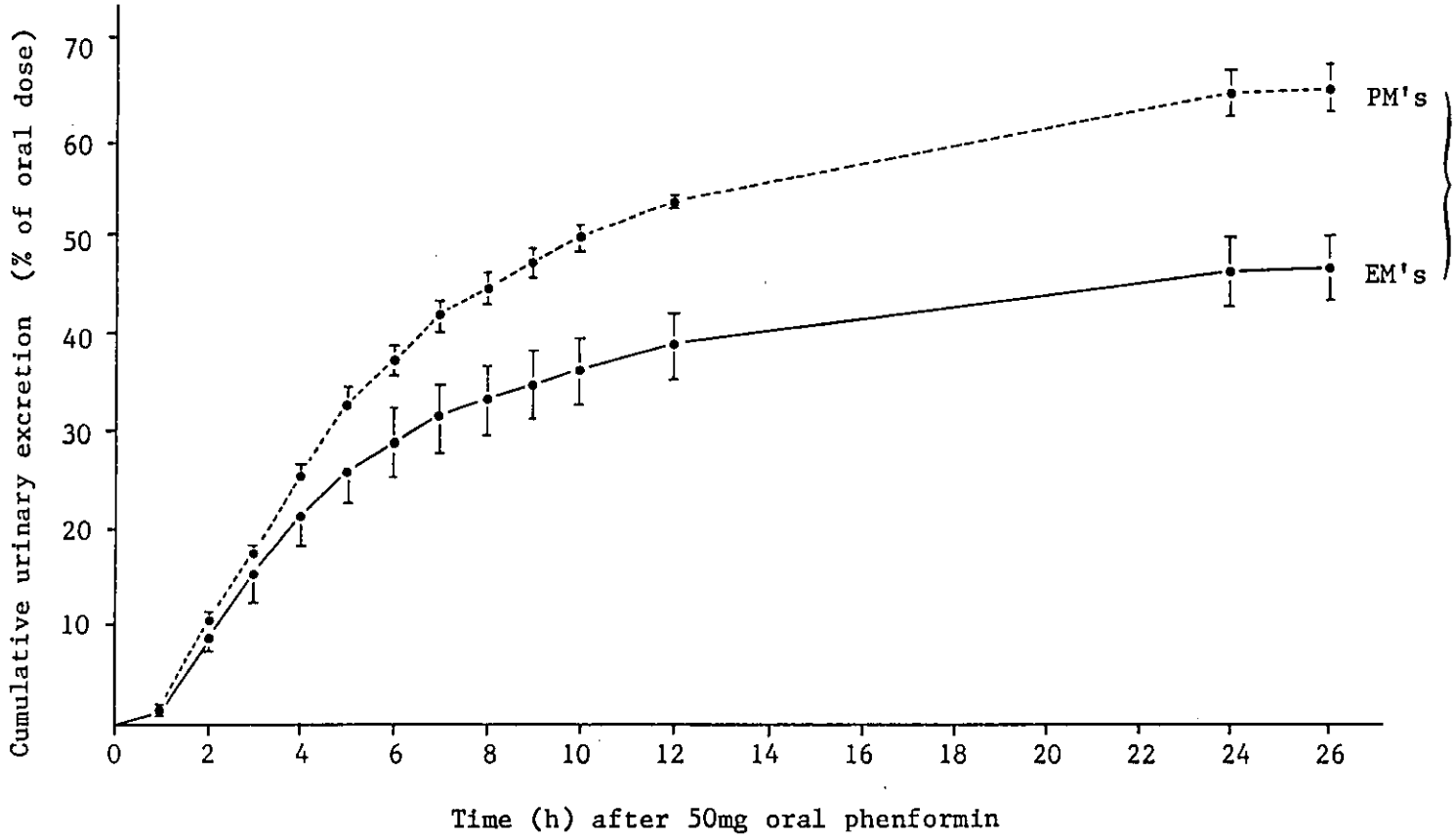


Figure 17 Cumulative urinary excretion of phenformin-related products in the two phenotypes

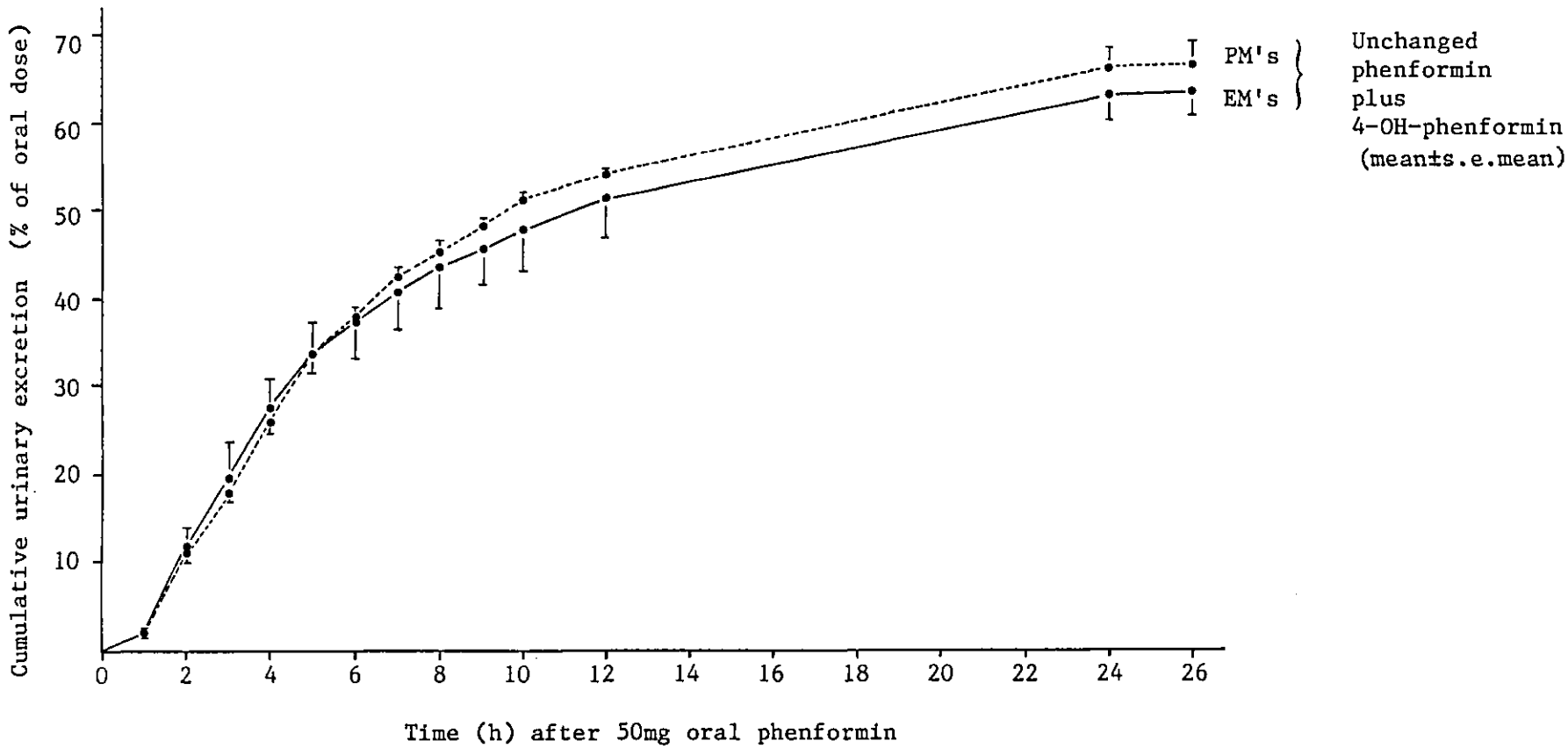


Table 12 Derived parameters of phenformin urinary elimination in the two phenotypes

		EM's				PM's		
		AZ	JR	LW	JI	RS	JO'G	JD'S
Total observed recovery (% of oral dose)	Phen	50.6	45.7	37.1	52.5	67.1	66.4	60.3
	4-OH-Phen	18.2	15.0	19.8	13.0	-	3.4	0.2
	Total	68.8	60.7	56.9	65.5	67.1	69.8	60.5
Total recovery to infinity (% of oral dose)	Phen	50.6	46.1	37.4	54.5	70.8	70.8	61.4
	4-OH-Phen	18.5	15.2	20.0	13.9	-	3.6	0.2
	Total	69.1	61.3	57.4	68.4	70.8	74.4	61.6
Ratio of $\frac{\text{Phenformin}}{\text{4-OH-Phenformin}}$ in	Observation period	2.78	3.05	1.87	4.04	∞	19.53	>100
	0 - 8 h	3.43	3.75	2.34	4.14	∞	21.7	>100
K_{el} (h^{-1})	Phen	0.249	0.203	0.185	0.127	0.128	0.108	0.151
	4-OH-Phen	0.145	0.189	0.188	0.108	-	0.121	-
$t_{1/2\ el}$ (h)	Phen	2.78	3.42	3.75	5.46	5.42	6.39	4.59
	4-OH-Phen	4.78	3.67	3.69	6.42	-	5.75	-
Total 0 - 8 h recovery (% of oral dose)	Phen	43.6	28.5	28.1	32.3	46.6	41.4	45.7
	4-OH-Phen	12.7	7.6	12.0	7.8	-	1.9	0.4
	Total	56.3	36.1	40.1	40.1	46.6	43.3	46.1

that of phenformin in each group, supporting the presence of a quantitatively variable first pass metabolism of phenformin.

As judged by the calculated infinity recoveries, these data support previous findings that most of the drug (unchanged + metabolite) elimination occurs in the first 24-hour period (Beckmann, 1968; Alkalay et al, 1975). Analysis of earlier fractions of urine in the study further indicates that even within this 24-hour period, a substantial fraction (just over two-thirds) is eliminated in the first 8 hours. It is important to note, therefore, that the differences in the ability to effect 4-hydroxylation of phenformin, as judged by the ratio phenformin to 4-hydroxy-phenformin excretions, are also discerned equally reliably if the observation period is limited to 8 hours.

The mean ($\pm$ S.D.) elimination rate constant for phenformin in EM volunteers was 0.191 ($\pm$ 0.050) while in the PM individuals, it was 0.129 ($\pm$ 0.022). This difference is not statistically significant ($p > 0.05$). Similarly, no significant difference was observed in the mean ($\pm$ S.D.) terminal elimination half-life ($t_{\frac{1}{2}}$) of phenformin between the two groups, (3.85 $\pm$ 1.14h in EMs v 5.47 $\pm$ 0.90h in PMs), as calculated from urinary measurements ($p > 0.05$).

Discussion

The results support the conclusion that phenformin is subject to a significant first pass metabolism and confirm the wide inter-individual variation in the ability to effect oxidative clearance of phenformin. Previously reported studies (Beckmann, 1968) have suggested that the urinary excretion of phenformin and 4-hydroxy-phenformin occurs in the ratio of 2:1. In retrospect, it can now be appreciated that these studies did not include individuals of poor oxidation status amongst the volunteers they used.

The difference between the two phenotypic groups in this panel is strongly indicative of polymorphic control of phenformin hydroxylation. It is important to recall that the volunteers were grouped into the two phenotypes by their debrisoquine hydroxylation status. Consequently, the marked difference between the two groups in their ability to effect phenformin hydroxylation strongly suggests that the aromatic hydroxylation of phenformin is also controlled by the same pair of gene alleles that controls alicyclic hydroxylation of debrisoquine, in addition to the oxidation of a number of other drugs mentioned previously. If this polymorphic hydroxylation of phenformin and its genetic control can be confirmed in a larger sample of population, the phenotyped panel approach, as a method of studying inter-individual differences in oxidative metabolic ability, can be justified in the investigation of a number of other drugs. The

results obtained in this study indicate that such an investigation can be conveniently and reliably carried out at a population level by an individual bulk collection of urine over only 8 hours post-dosing.

It is interesting to note that the inter-individual differences in drug metabolising ability in a small panel of subjects are not reflected in the urinary determinations of either the elimination rate constant or the terminal urinary elimination half-life of phenformin. This is not surprising since the difference between the two groups has its origin in the liver (presystemic hepatic metabolism) and not in the kidneys. The renal clearances of the parent drug or its 4-hydroxy metabolite in the two groups are essentially similar. For water soluble drugs which undergo presystemic metabolism, the most reliable method of uncovering the metabolic differences is to measure the elimination of the unchanged drug as well as its metabolite and then relate this to each other. It is by this approach that the genetic control of debrisoquine oxidation was uncovered.

In view of the differences observed in the two groups in their ability to form the 4-hydroxy metabolite of phenformin, it is important to be certain that the differences in metabolic ability are individually consistent and that the poor metabolisers are not utilising an alternative pathway to produce qualitatively and/or quantitatively different metabolites.

Some evidence against the presence of an alternative metabolic pathway in individuals of poor metaboliser phenotype is provided by the results obtained in this panel study.

If such an alternative pathway did exist, the pathway will be called upon to clear a greater load of phenformin and consequently its existence can be discerned from the poor recovery of unchanged phenformin as well as total phenformin, based on the measurement of parent drug plus 4-hydroxy-phenformin alone. The examination of Figs. 16 and 17 shows that the mean cumulative excretion of unchanged phenformin at all times and that of total phenformin (unchanged drug plus 4-hydroxy-phenformin) after the first 5 hours are consistently and markedly higher in the PM group than in the EM group. Up to the first 5 hours, hardly any difference in the total phenformin (unchanged drug plus the metabolite) elimination is apparent between the two phenotypes.

The individual consistency of phenformin hydroxylation and the absence of an alternative pathway were further examined using radio-labelled phenformin in 3 individuals from the panel. This is discussed in the next section.

7.4 Consistency of phenformin hydroxylation and the absence of an alternative pathway in a poor metaboliser of debrisoquine

Introduction

The metabolism of phenformin was studied in Sprague-Dawley rats by Murphy and Wick (1968). They confirmed that in this species, phenformin was oxidised to 4-hydroxy-phenformin and that approximately half of the hydroxylated metabolite was further metabolised by conjugation with glucuronic acid. Beckmann (1968) studied in detail the fate of biguanides in man and showed that man, like the rat and other animals, metabolised phenformin to 4-hydroxy-phenformin. Using ^{3}H-labelled phenformin for oral dosing, he found only two radioactive spots on the paper chromatography of urine - one corresponding to unchanged drug (R_F value 0.60) and the other corresponding to 4-hydroxy-phenformin (R_F value 0.47). No other metabolite of phenformin, including the glucuronide of 4-hydroxy-phenformin, was detected. Amongst his volunteers about two-thirds of the absorbed dose was excreted as unchanged fraction of phenformin while the remaining one third as the 4-hydroxy metabolite. If an alternative pathway did exist, it will operate, albeit to a considerably minor extent, even in the individuals of EM phenotype. Consequently, the recovery of radioactivity will be consistently more than the recovery of phenformin as determined by combined measurements of unchanged phenformin and 4-hydroxy-phenformin by the non-

radioactive method. In contrast to this, Beckmann (1968) found that the mean 24-hour recovery of radioactivity was 45.2% (range 43.0-48.6%, n=3), while the mean ± S.D. recovery of phenformin-related compounds was 54.4 ± 3.2% (n=9). These findings support the conclusion that in man phenformin is metabolised by an all exclusive oxidative route to a single metabolite, 4-hydroxy-phenformin. It was decided to confirm this in 3 of the volunteers used in the initial panel study.

Method

Three volunteers, consisting of 2 EMs and 1 PM, were selected from the panel and after their informed consent, 50 mg of labelled phenformin (0.73 uCi of ^{3}H-labelled phenformin) was given orally to each after an overnight fast. The urine was collected in bulk for a 24-hour period post-dosing. After measuring the volume of urine, an aliquot was analysed for the contents of unchanged phenformin and 4-hydroxy-phenformin by the h.p.l.c. method described and also by scintillation counting for the total recovery of radioactivity. A few weeks later, one of these volunteers was re-studied for the third time using 50 mg unlabelled phenformin administered orally and collecting 0-24 hours urine post-dosing.

Results

From the measurements of radioactivity and of phenformin and 4-hydroxy-phenformin, percentage recoveries of radioactivity and of the oral dose of phenformin over the 0-24 hour period were calculated for each volunteer. These are shown in Table 13. It is clear that the recovery of radioactivity, compared to the recovery of total phenformin, varied from +3.6% to -3.1% in the 2 EMs, whilst interestingly, the radioactive recovery fell short of drug recovery by -6.4% in the PM individual.

The results of percentage recoveries of phenformin as unchanged phenformin and as the 4-hydroxy metabolite, together with ratios of the former to the latter, over the various 0-24 hour periods, are shown in Table 14. The individual consistency in the ability to effect phenformin hydroxylation is evident.

Discussion

The discrepancy between the recoveries of radioactivity and of the orally administered drug ranges from +3.6% to -6.4% (Table 13) in the volunteers studied. This discrepancy is well within the combined variance of the two assays employed. Interestingly enough, the recovery of radioactivity fell short of drug recovery in the poor metaboliser by -6.4%, thereby supporting previously published findings and conclusively excluding the presence of an alternative pathway or for that matter, the further metabolism of 4-hydroxy-phenformin in man.

Table 13: Comparison of radiolabelled and h.p.l.c. recoveries of phenformin + 4-hydroxy-phenformin following a single oral dose of ^{3}H-labelled 50 mg phenformin (1.62×10^{6} dpm = 0.73 uCi)

Volunteer	Total Recovery dpm	% radioactivity recovery of oral dose	H.p.l.c. assay - mg recovered as Phen	H.p.l.c. assay - mg recovered as 4-OH-Phen	% h.p.l.c. recovery of oral dose	$\frac{\text{Radio-h.p.l.c. assays}}{\text{h.p.l.c. assay}} \times 100\%$
AZ (EM)	1.13×10^{6}	69.8	27.8	5.9	67.4	+3.6 %
JRI (EM)	1.01×10^{6}	62.8	24.7	7.7	64.8	-3.1 %
JOG (PM)	1.05×10^{6}	64.8	32.4	2.2	69.2	-6.4 %
Mean ± S.D.		65.8 ±3.6			67.1 ±2.2	-1.94%

Table 14: Individual consistency in effecting hydroxylation of phenformin

Study	Parameter*	AZ (EM)	JRI (EM)	JO'G (PM)
FIRST	Phen (mg)	25.2	25.9	32.6
	4-OH-phen (mg)	8.9	6.4	1.7
	Total (mg)	34.1	32.3	34.3
	Ratio $\frac{\text{Phen}}{\text{4-OH-P}}$	2.8	4.0	19.2
SECOND	Phen (mg)	27.8	24.7	32.4
	4-OH-phen (mg)	5.9	7.7	2.2
	Total (mg)	33.7	32.4	34.6
	Ratio $\frac{\text{Phen}}{\text{4-OH-P}}$	4.7	3.2	14.7
THIRD	Phen (mg)	22.7		
	4-OH-phen (mg)	8.3		
	Total (mg)	31.0		
	Ratio $\frac{\text{Phen}}{\text{4-OH-P}}$	2.7		

* Phen = Phenformin, 4-OH-P = 4-hydroxy-phenformin

The drug oxidation status, as an individual characteristic, is evident on examination of Table 14. A minor degree of variation is noted in these volunteers and this could have arisen from a number of other variables, such as hepatic blood flow to name but one. Individual consistency in phenformin oxidation status was examined and confirmed further during the investigation of applicability of the phenotyped panel data to demonstrate the presence of widely variable ability to effect phenformin hydroxylation in a larger population sample.

7.5 Inter-individual differences in phenformin hydroxylation: A population study

Introduction

It was previously suggested that inter-individual variations in the ability to effect oxidation of certain drugs at a population level can be discerned from the study of that drug in a phenotyped panel. The results of such a panel study, described earlier, showed that individuals of EM and PM phenotypes, as assigned by their debrisoquine hydroxylation status, showed about 14-fold variation in their ability to hydroxylate phenformin. It also suggested that aromatic hydroxylation of phenformin was controlled by the same pair of gene alleles as that which controls alicyclic 4-hydroxylation of debrisoquine. The reliability of a period of urine collection shorter than 24 hours, namely 0-8 hours post-dosing, was also confirmed by the results. In order to confirm the applicability of panel results to a larger sample, a study to investigate phenformin hydroxylation at a population level was undertaken.

Methods

The study was approved by the Ethics Committee of St. Mary's Hospital, London W2.

After giving their informed consent, 195 unrelated members of the staff and students of St. Mary's Hospital and Medical School took part in the study. Each

volunteer completed a questionnaire regarding details of age, sex, weight, height, concurrent drugs if any taken during the preceding 7 days, alcohol and smoking habits and the history of any hepatic or renal diseases.

After an overnight fast, each volunteer emptied the bladder in the morning and took a single oral dose of 50 mg phenformin. Urine was collected in bulk, without any preservative, for a period of 8 hours after the dose. The volume of 0-8 hour urine voided was recorded and an aliquot stored frozen at -20°C for subsequent analysis. During the test period, the volunteers refrained from alcohol and other drugs but were allowed their usual meals including breakfast, at least one hour after taking phenformin.

Subsequently, the urine samples were allowed to thaw at room temperature and were analysed for their contents of phenformin and 4-hydroxy-phenformin by Dr. Oates by the h.p.l.c. method described.

From the concentration of phenformin and 4-hydroxy-phenformin in each sample, percentage doses excreted as unchanged phenformin and as 4-hydroxy-phenformin in the 0-8 hour period were calculated. The ratio, termed the phenformin ratio (P.R.), was derived for each volunteer as follows:

$$P.R. = \frac{\%\text{ oral dose excreted as unchanged phenformin}}{\%\text{ oral dose excreted as 4-hydroxy-phenformin}} \text{ in 0-8h period}$$

Results

The details of the 195 volunteers are individually shown in Appendix II. These volunteers were white British caucasians and included 72 males and 123 females of whom 57 took oral contraceptive steroids.

The mean (± S.D.) age, weight and height for males were 22.8 (± 6.1) years, 73.8 (± 13.3) kg, and 177.2 (± 7.8) cm respectively, while the corresponding figures for females were 22.0 (± 4.1) years, 57.3 (± 5.7) kg, and 166.4 (± 5.5) cm.

Twelve of the males and 39 of the females were smokers. There were 13 volunteers who did not drink alcohol at all, while 174 drank only socially and 8 drank moderately, that is regularly but not excessively. None of the volunteers had any history of hepatic or renal diseases.

The percentage oral doses recovered as unchanged phenformin and as 4-hydroxy-phenformin in the 0-8 hour period, together with the phenformin ratio (P.R.) for each individual, are shown in Appendix III. During the preceding one week, 10 volunteers had been on other medication and these are also indicated in Appendix IV. The frequency distribution of the phenformin metabolic ratio is shown in Figs. 18 and 19. The total recovery of oral dose, unchanged phenformin + 4-hydroxy-phenformin expressed as phenformin, ranged from 9.8% to 89.6% with a median of 41% (mean ± S.D. = 42.3 ± 13.6%). There was a marked inter-individual variation in the ability

Figure 18 Frequency distribution of the phenformin ratios of 195 volunteers - linear scale

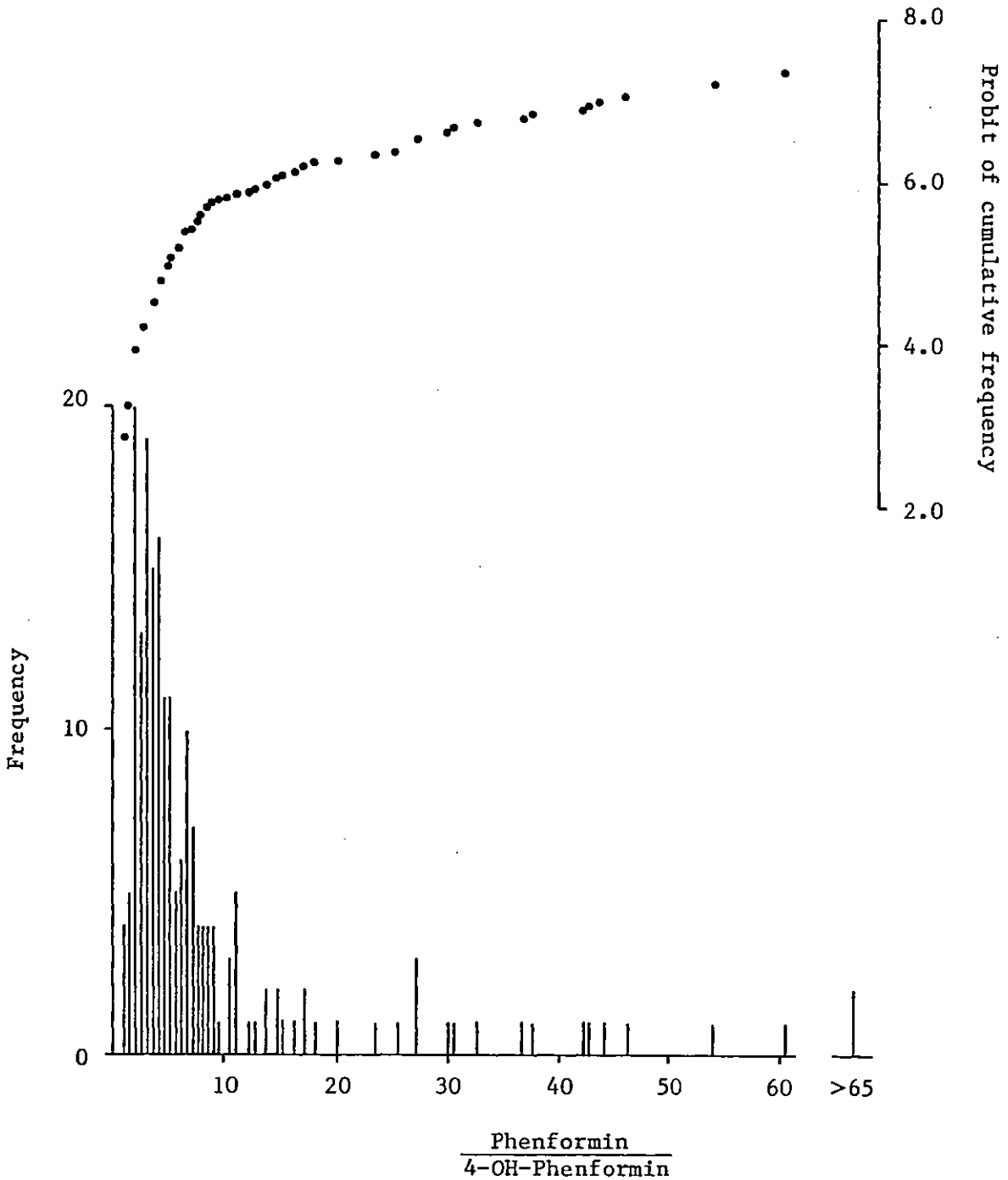
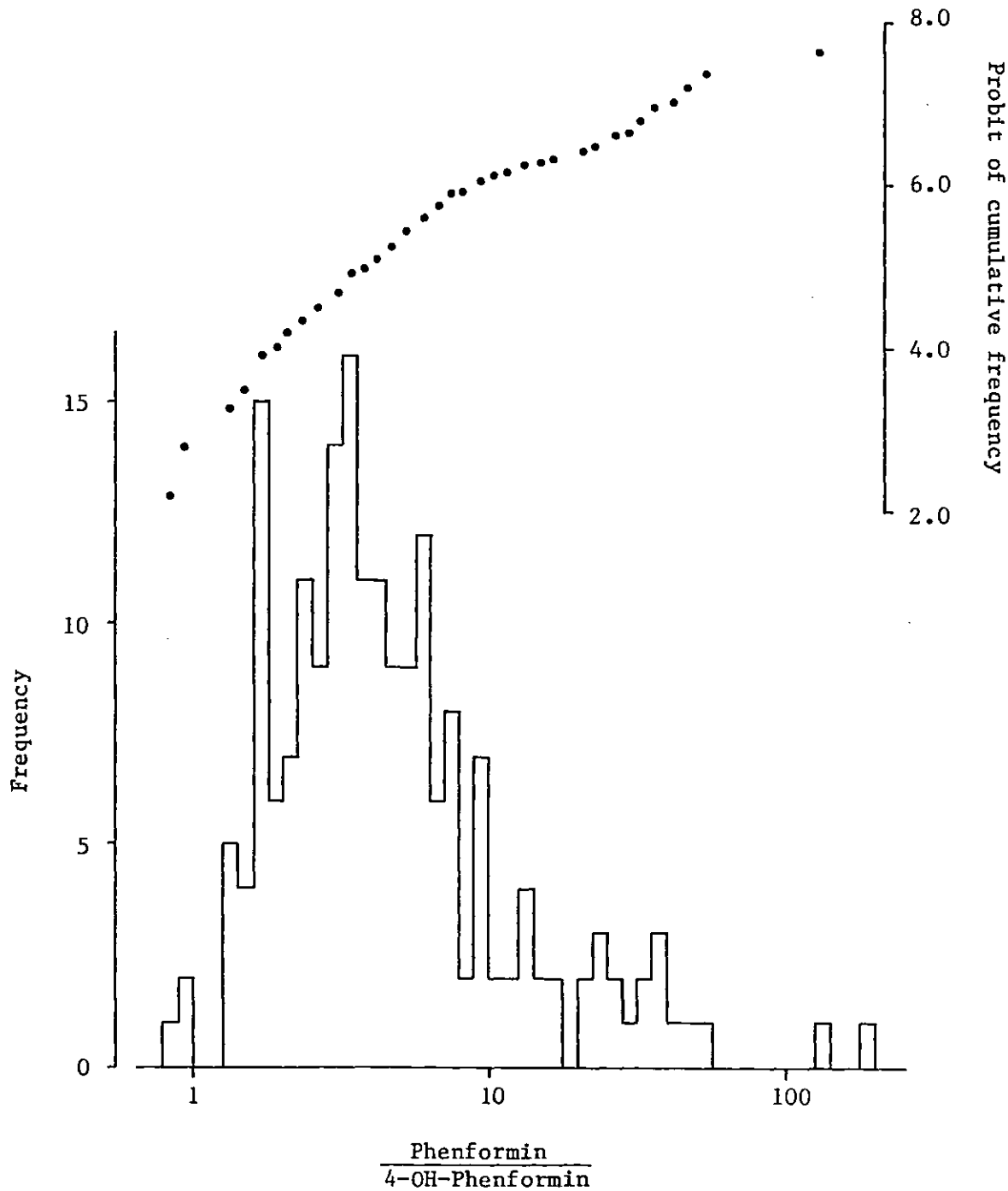


Figure 19 Frequency distribution of the phenformin ratios of 195 volunteers - logarithmic scale



to effect oxidation of phenformin. The percentage of the oral dose recovered as 4-hydroxy-phenformin ranged from 0.2-31.6%. However, this may simply be due to variable absorption of drug and hence, the need to relate the metabolite formation to the excretion of unchanged drug as well. When the ability to effect oxidation is expressed as P.R., a range of 0.9 to 184 (median value 4.3) is obtained. This indicates about 97-fold variation in the ability to effect phenformin oxidation between the individuals studied. The percentage of absorbed drug metabolised is calculated by the following formula:

$$\frac{100}{1 + \text{P.R.}} \%$$

The distribution of P.R. within the population studied deviated significantly from linearity ($\chi^2 = 862$, $p < 0.0001$). The probit analysis also deviated significantly from a linear relationship ($\chi^2 = 279$, $p < 0.001$) and revealed an inflection between the ratios of approximately 10 and 35 (Figs. 18 and 19). These provided some evidence that the population distribution is bimodal, having an antimode at approximately 20.

The percentage of individuals with a P.R. > 20 in the population studied is 9.2%, with 95%-confidence limits of 5.6 to 13.8% (<u>vide infra</u>, Section 7.6).

Discussion

The results of the study confirmed the wide inter-individual variation that exists in the ability of a population to effect oxidative clearance of phenformin. These findings strongly support the value of the judicious use of phenotype panel approach in order to uncover individual metabolic differences within a population. This, in turn, would permit a more thorough evaluation of the pattern of drug response that is likely to arise from such wide metabolic variations in the population.

The percentage of individuals studied who had a P.R. > 20 (9.2%) is very close to the percentage of individuals who are poor metabolisers of debrisoquine (8.9%) (Price-Evans et al, 1980). This lends further support to the probability that the oxidative clearances of debrisoquine and phenformin may have a common basis with a common control.

It is important to note that phenformin ratios of the 10 individuals who had taken other medications within the week preceding the study ranged from 1.7 to 45.8 with a median of 4.5 This small sample does not allow any firm conclusion but it does appear that the wide inter-individual variations in effecting drug oxidation are not drug-induced artefacts. In order to elucidate the nature of the control responsible for the marked variations between the individuals in effecting phenformin oxidation, a family study was considered necessary. Some insight into its probable genetic nature is

already evidenced in the bimodal distribution of the P.R. It was, however, first necessary to confirm that the individual phenformin oxidation status was consistent and reproducible.

7.6 Phenformin oxidation status as an individual characteristic and family studies

Introduction

The previous studies confirmed that within a population, there exists wide inter-individual differences in the oxidative handling of phenformin. The phenotyped panel studies suggested that there may be a concurrence in the oxidations of phenformin and debrisoquine. This suggestion was further strengthened by the finding in the population study that the percentage of individuals with impaired phenformin oxidation (metabolic ratio of phenformin oxidation greater than 20) was very close to that of poor metabolisers of debrisoquine - namely 9.2% and 8.9% respectively - in British white caucasians (Price-Evans *et al*, 1980). It has been shown that debrisoquine oxidation is under the control of a single pair of gene alleles, resulting in the bimodal distribution of debrisoquine metabolic ratio. The probability that a similar, if not identical, control extends over phenformin oxidation was suggested by the bimodal distribution of phenformin (metabolic) ratio. It was decided to investigate this probability by further extension of the population study described previously. This involved the investigation of the phenformin oxidation status of the immediate family members of some of the individuals included in the population study.

Methods

The investigation consisted first of studying the consistency of P.R. of a number of individuals from the population study. For this purpose, 51 volunteers who participated in the population study were re-studied in an identical manner as before.

In order to investigate the genetic nature of the control of phenformin oxidation, family studies were carried out. The immediate family members of 27 of the re-studied 51 volunteers were approached and after their informed consent, 87 relatives of these 27 probands were studied for their phenformin oxidation status. This study was approved by the Ethics Committee of St. Mary's Hospital, London W2.

Each member completed a questionnaire regarding the details of age, sex, the relationship to proband, concurrent drug treatment and past medical history.

After an overnight fast, the bladder was emptied in the morning and 50 mg phenformin swallowed orally with 150-200 ml of water. The urine was collected in bulk over the following 8 hour period and after measuring the volume, an aliquot was stored frozen for subsequent analysis of phenformin and 4-hydroxy-phenformin concentrations by the h.p.l.c. method described.

Members on concurrent drug treatment were requested to allow a drug free period extending from 10 hours before to 3 hours after the oral dose of phenformin. Alcohol was

not permitted during the test period and meals were taken as usual, including breakfast at least one hour after having taken phenformin. Wherever applicable, members refrained from "to be taken as required" medications such as analgesics and hypnotics.

Results and discussion

The results of the re-study of 51 volunteers are tabulated in Table 15 where the data of the previous phenformin test are shown together with the results from repeat tests. The status of these volunteers (Appendix I) regarding their age, weight, height, smoking and alcohol habit, drug treatment in preceding week and past medical history had not changed in the six weeks between the two tests. It is evident that the percentage recovery of drug during the second test (mean 45.6% $\pm$ 14.8, median 42.8%) is comparable to that during the first test (mean 40.5% $\pm$ 11.6, median 41.8%). In some individuals, the percentage drug recovered during the two tests was markedly different and yet, their P.R. during the two tests were remarkably close to each other. It may be pointed out at this stage that a change in the ratio from 40 to 80 signifies very little change in the amount of drug oxidised but a change from 1 to 2 is a significant change in the percentage drug metabolised; whilst the former denotes a change from 2.4 to 1.2%, the latter denotes a change from

Table 15: Re-study of phenformin oxidation status in 51 individuals from population study

Volunteer	Previous study		Second study	
	% Recovery	1st Ratio	% Recovery	2nd Ratio
2	47.4	15.9	52.4	15.1
4	39.8	2.4	32.6	3.6
5	36.4	3.0	61.2	3.7
6	22.6	4.1	24.2	3.7
8	50.2	30.4	59.2	147.0
9	24.0	1.6	64.0	1.7
10	22.0	4.3	28.4	3.4
14	33.4	5.0	31.6	3.6
15	43.4	23.1	28.0	22.5
16	30.8	1.5	34.0	2.1
20	34.4	3.4	48.8	2.7
21	21.4	1.9	57.8	1.9
23	36.8	60.3	50.2	82.6
27	19.8	8.9	44.2	7.2
28	24.6	2.1	42.8	2.6
29	26.0	1.7	29.4	2.4
30	41.8	3.1	61.4	2.8
31	42.2	10.7	54.2	8.1
32	44.8	43.8	71.0	32.2
33	40.2	1.0	42.2	1.1
39	56.6	2.4	37.8	2.3
40	16.8	27.0	18.8	24.8
53	44.0	54.0	82.8	58.1

Table 15: (continued)

Volunteer	% Recovery	1st Ratio	% Recovery	2nd Ratio
58	47.4	8.9	45.6	5.5
60	42.8	3.7	39.6	2.7
62	53.4	10.1	61.2	9.9
63	31.2	13.2	40.6	17.5
64	47.0	25.1	66.4	35.9
70	59.8	6.7	58.6	6.7
73	49.4	16.6	25.0	4.9
76	32.4	8.0	20.2	4.9
77	33.6	27.0	42.2	25.4
78	60.4	2.8	35.0	3.0
81	31.2	3.6	59.0	4.1
82	48.2	6.3	42.0	7.7
85	31.6	2.0	28.8	1.2
89	37.4	45.8	69.2	46.3
91	46.6	3.7	35.6	2.4
92	56.4	140.0	57.6	78.9
102	52.4	4.1	56.6	4.4
108	62.0	1.0	50.2	1.1
109	43.2	42.2	36.0	57.9
110	48.0	5.9	33.2	4.7
111	53.4	32.4	38.2	37.2
114	32.0	10.4	53.2	17.0
119	46.6	14.5	49.8	16.1
120	49.0	10.7	68.0	11.6
128	50.4	27.0	38.6	31.2
129	36.4	29.7	26.4	65.0

Table 15: (contined)

Volunteer	% Recovery	1st Ratio	% Recovery	2nd Ratio
132	30.0	36.5	38.8	26.7
136	55.0	4.7	52.6	3.1
Median	41.8 %		42.8 %	
Means ± S.D.	40.5 ± 11.6%		45.6 ± 14.8%	

50% to 33%. Included amongst these 51 subjects were 15 individuals with P.R. > 20 during the first estimate. Their phenformin oxidation status was found to be consistent during this second estimate. Similarly, 36 individuals with a ratio < 20 also showed consistency during both the estimates. None of the volunteers showed any significant deviation during the second estimate. The correlation between the two estimates of ratio for the whole group is shown in Fig. 20. It is evident that there is a high degree of consistency between the two estimates of all the volunteers. The calculated Spearman rank correlation coefficient (r_s) for this data is 0.97 ($p < 0.0001$). It is clear from these results that the phenformin oxidation status, like the debrisoquine oxidation status, is a consistent individual characteristic.

The relevant details of the participating members of the 27 families are shown in Appendix V. The 0-8 hour recovery of the drug, expressed as percentage of the oral dose, is also shown together with the P.R. for each. The family pedigrees are shown in Fig. 21.

The individuals with P.R. > 20 are designated as poor oxidisers of phenformin. Amongst the 27 probands and their 87 relatives, there were 21 such individuals. The family relationship of these individuals (Fig. 21), together with P.R. of all the family members, is consistent with the conclusion that the trait for impaired phenformin oxidation

Figure 20 Consistency of the two estimates of phenformin ratio in 51 individuals

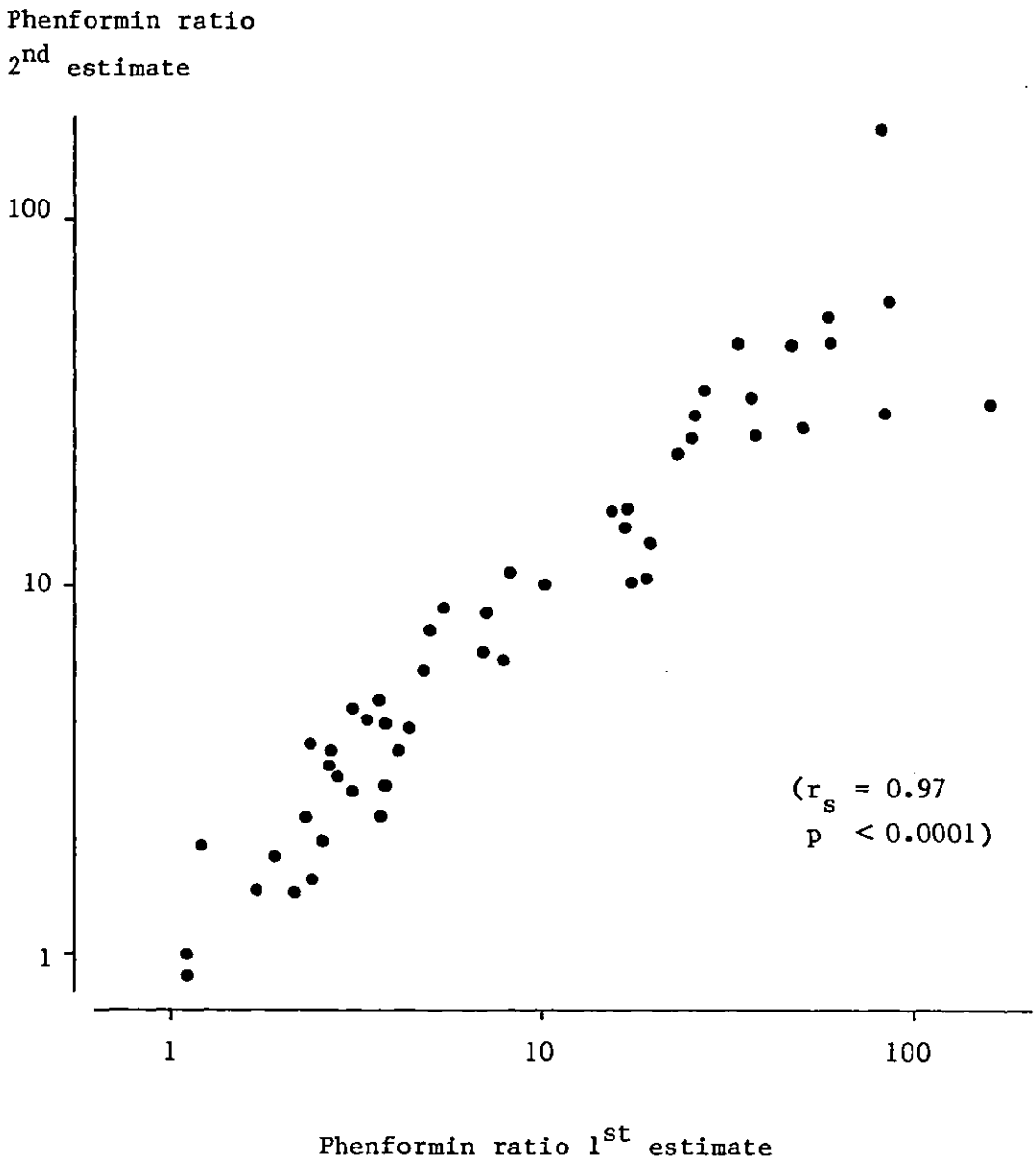
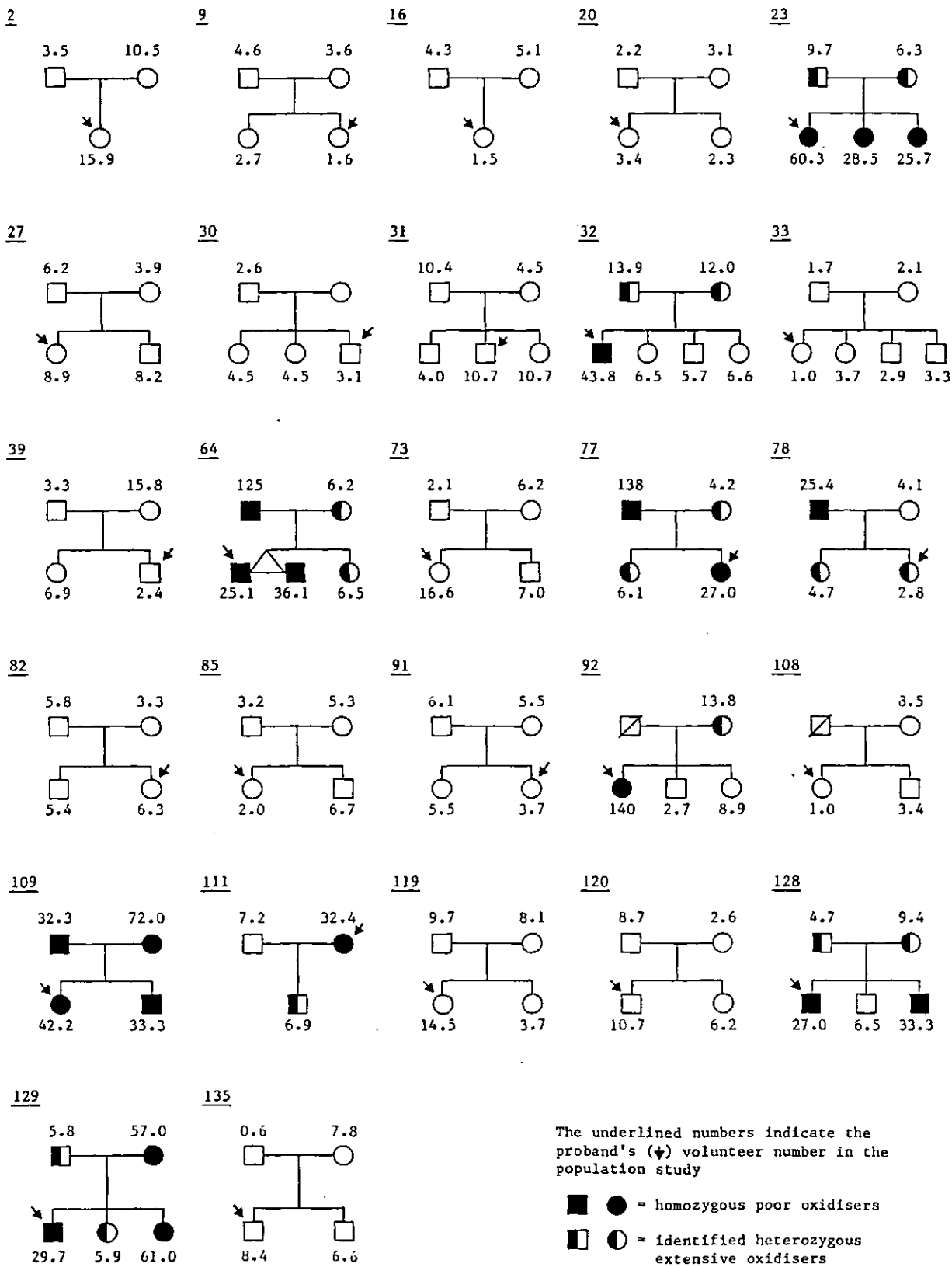


Figure 21 Phenformin oxidation ratios in 27 family pedigrees



is inherited in a Mendelian autosomal recessive fashion (Fig. 22). From this information, it is possible to calculate the frequency of the allele, responsible for impaired oxidation, by the Hardy-Weinberg equations. These equations assume the population to be in equilibrium with the gene pool. If the proportional frequencies of the alleles are designated as $p + q$, the equations are:

1. $(p + q)(p + q) = 1$ (Total population), i.e.
   $p^2 + 2pq + q^2 = 1$ and

2. $p + q = 1$ (Total gene pool)

The p^2 and q^2 represent the frequencies of the two homozygous genotypes, whilst pq indicates the heterozygote frequency.

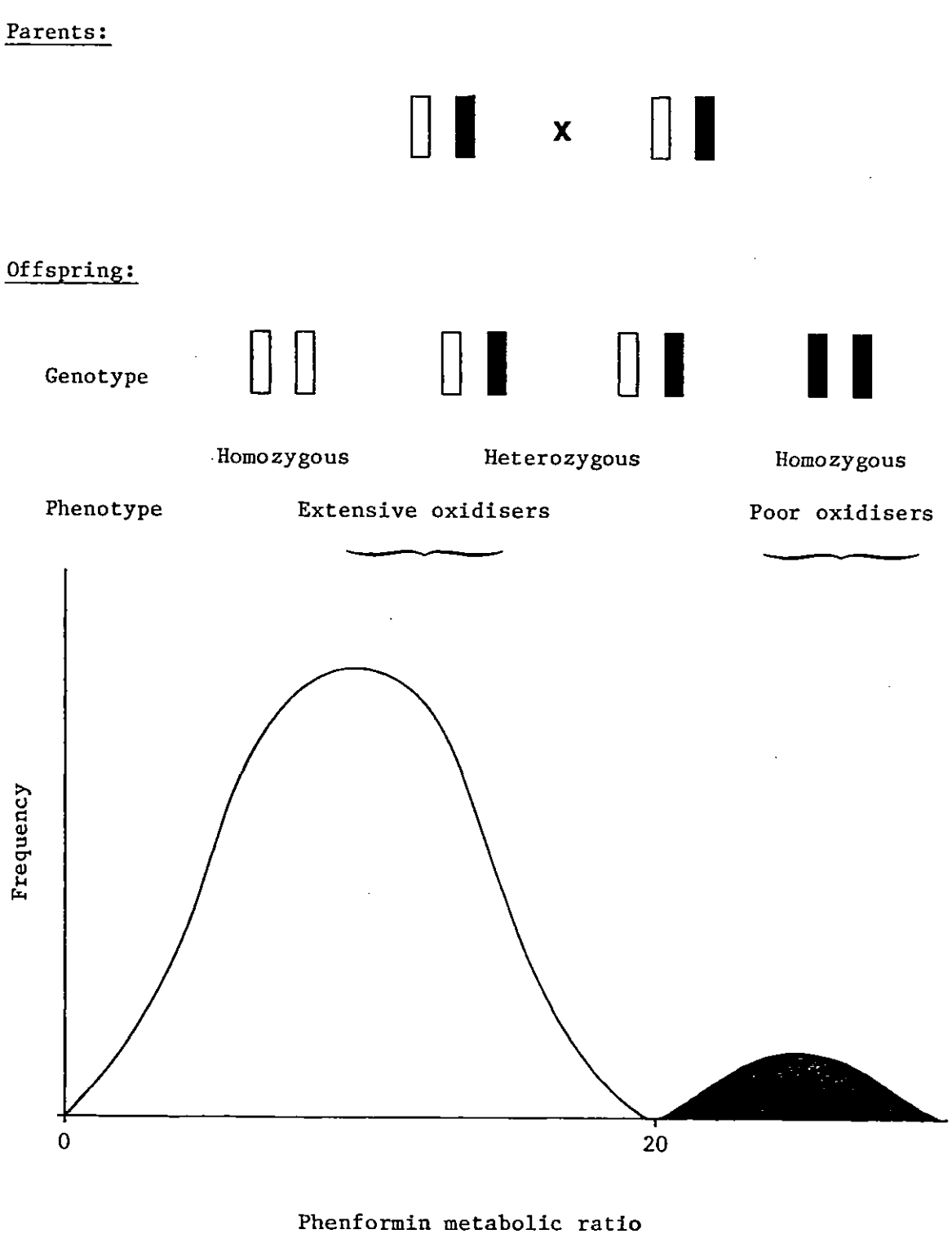
In the population study described previously, there were 18 individuals (out of a total of 195) with a P.R. > 20. Therefore, the individuals who are homozygous for the allele for impaired phenformin oxidation (q) form 9.23% of the population studied. Therefore,

$$q^2 = 0.0923$$

therefore, $q = 0.3038$ and

$$p = 0.6962$$

Figure 22 Autosomal recessive transmission of the allele for impaired phenformin oxidation



The standard error in the estimation of q based on the frequency of individuals with impaired phenformin oxidation, may be calculated from the equation described by Emery (1976), namely:

$$\text{s.e.m. of } q = \left[\frac{(1 - q^2)}{4N}\right]^{\frac{1}{2}}$$

$$\text{i.e.} \quad 0.034$$

Thus, the 95%-confidence limits of the frequency of this allele, responsible for impaired phenformin oxidation, is 0.2358 to 0.3718. The corresponding 95%-confidence limits for the frequency of the individuals who are homozygous for the allele, responsible for impaired phenformin oxidation, are 5.6-13.8%. Similarly, the frequencies of homozygous extensive and heterozygote extensive oxidisers of phenformin can be calculated and are indicated in Table 16.

The calculated frequency of the allele for impaired phenformin oxidation (0.236 to 0.372) is remarkably close to the one for impaired debrisoquine oxidation (0.239 to 0.359), in British white caucasians (Price-Evans et al, 1980). The frequencies of these two alleles for impaired oxidations do not differ significantly ($p > 0.1$).

Table 16: Probable frequencies of the three genotypes of phenformin oxidation in white British caucasians.

Phenformin Oxidation Genotype	95% confidence limits for Percentage of Population
Homozygous extensive	41.4 - 55.8
Heterozygous extensive	30.4 - 53.0
Homozygous poor	5.6 - 13.8

Price-Evans et al (1980) have estimated that the phenotype for extensive debrisoquine oxidation exerts an approximately 30% dominance over the poor debrisoquine oxidation phenotype. Similar treatment of the data obtained from the phenformin population study suggests that the phenotype for extensive phenformin oxidation exerts an approximately 35% dominance over the poor phenformin oxidation phenotype - once again suggesting that the same pair of alleles probably controls oxidations of both the drugs.

Amongst the 27 probands and their 87 relatives, a total of 16 heterozygous individuals can be identified with certainty. Their P.R's range from 2.8 to 13.9 with a median value of 6.3.

The findings of these studies suggest that phenformin oxidation status is a consistent individual characteristic and it is genetically inherited. It is controlled by a single pair of gene alleles and the allele for impaired phenformin oxidation is transmitted as an autosomal recessive trait. The allele has a frequency of 0.236 to 0.372 and poor phenformin oxidation phenotype is subject to approximately 35% dominance, in a heterozygote genotype, by the phenotype for extensive phenformin oxidation. These qualitative and quantitative aspects of inheritance for phenformin oxidation status bear a striking closeness to

those for debrisoquine, raising the possibility that both oxidations are concurrent (co-inherited) and are controlled by similar, if not identical, genetic factors. This possibility was investigated and is discussed in the next section.

7.7 Phenformin oxidation status and its relation to debrisoquine hydroxylation

Introduction

Previous studies confirmed that polymorphic pattern of phenformin oxidation is the result of a single pair of gene alleles controlling its oxidative clearance. The qualitative and quantitative aspects of the allele inheritance were remarkably close to those described for debrisoquine oxidation. These raised the strong possibility that similar, if not identical, genetic mechanisms may control the oxidative clearances of both the drugs. It was decided to investigate this by testing individuals of known phenformin metabolic ratios for their ability to metabolise debrisoquine.

Methods

After giving their informed consent, 101 volunteers who participated in the phenformin population study were recruited and studied for their debrisoquine oxidation status, as described by Mahgoub *et al* (1977). An interval of at least 4 weeks had elapsed from their participation in any previous studies.

After an overnight fast, each volunteer emptied the bladder and took a single 10 mg oral dose of debrisoquine. The 0-8 hour urine was collected in bulk and after measuring the volume, an aliquot was stored frozen at -20^{o}C

for subsequent analysis. The subjects did not consume alcohol during the test period and were allowed their usual meals, including breakfast at least one hour after dosing. The volunteers once again completed a questionnaire regarding the details of their age, sex, height, weight, usual smoking and alcohol habits, any drugs taken in the preceding one week and history of hepatic and/or renal diseases.

Prior to their analysis, the urine samples were allowed to thaw at room temperature. Their contents of debrisoquine and its major oxidative metabolite, 4-hydroxy-debrisoquine were measured by gas chromatography using an electron capture detector. The method used was the modification by Idle et al (1979) of the one described by Erdtmansky and Goehl (1975) and has been previously described in detail elsewhere. From the contents of the unchanged debrisoquine and the 4-hydroxy-debrisoquine, debrisoquine metabolic ratio (M.R.) was calculated as follows:

$$\text{M.R.} = \frac{\%\text{ oral dose excreted as unchanged debrisoquine}}{\%\text{ oral dose excreted as 4-hydroxy-debrisoquine}} \text{ in 0-8 hr period}$$

Drug recovery was calculated as percentage of oral dose of drug recovered as the unchanged drug plus its corresponding 4-hydroxy metabolite expressed as debrisoquine.

Results and Discussion

The status of these 101 volunteers regarding their age, weight, height, smoking and alcohol habits, drug treatment in the preceding week and past medical history had not changed since their participation in the population study (Appendix I).

Amongst these 101 volunteers, the mean (± S.D.) percentage recovery of phenformin during the first test was 45.9 (± 13.6) percentage with a median of 46.6%, while the corresponding figures for debrisoquine were 24.8 ± 8.4% with a median of 24.5%. The details of the phenformin metabolic ratio and recovery with corresponding values for debrisoquine for each volunteer are shown in Appendix VI. The correlation between phenformin and debrisoquine metabolic ratios is shown in Fig. 23. The oxidation of these two drugs displays a close correlation which is highly significant, (r_s = 0.785; $p < 0.0001$). This further confirms that the genetic mechanisms controlling the oxidations of the two drugs are very similar, if not identical.

There are, however, three volunteers (Nos. 77, 128 and possibly 183, Table 17) whose phenformin oxidation status does not appear to correlate with their debrisoquine oxidation status. Of these, volunteers 77 and 128 are poor oxidisers of phenformin (P.R. > 20), whilst being extensive oxidisers of debrisoquine(M.R. <12.6); the third volunteer,

Figure 23 Correlation between phenformin oxidation and debrisoquine oxidation in 101 volunteers

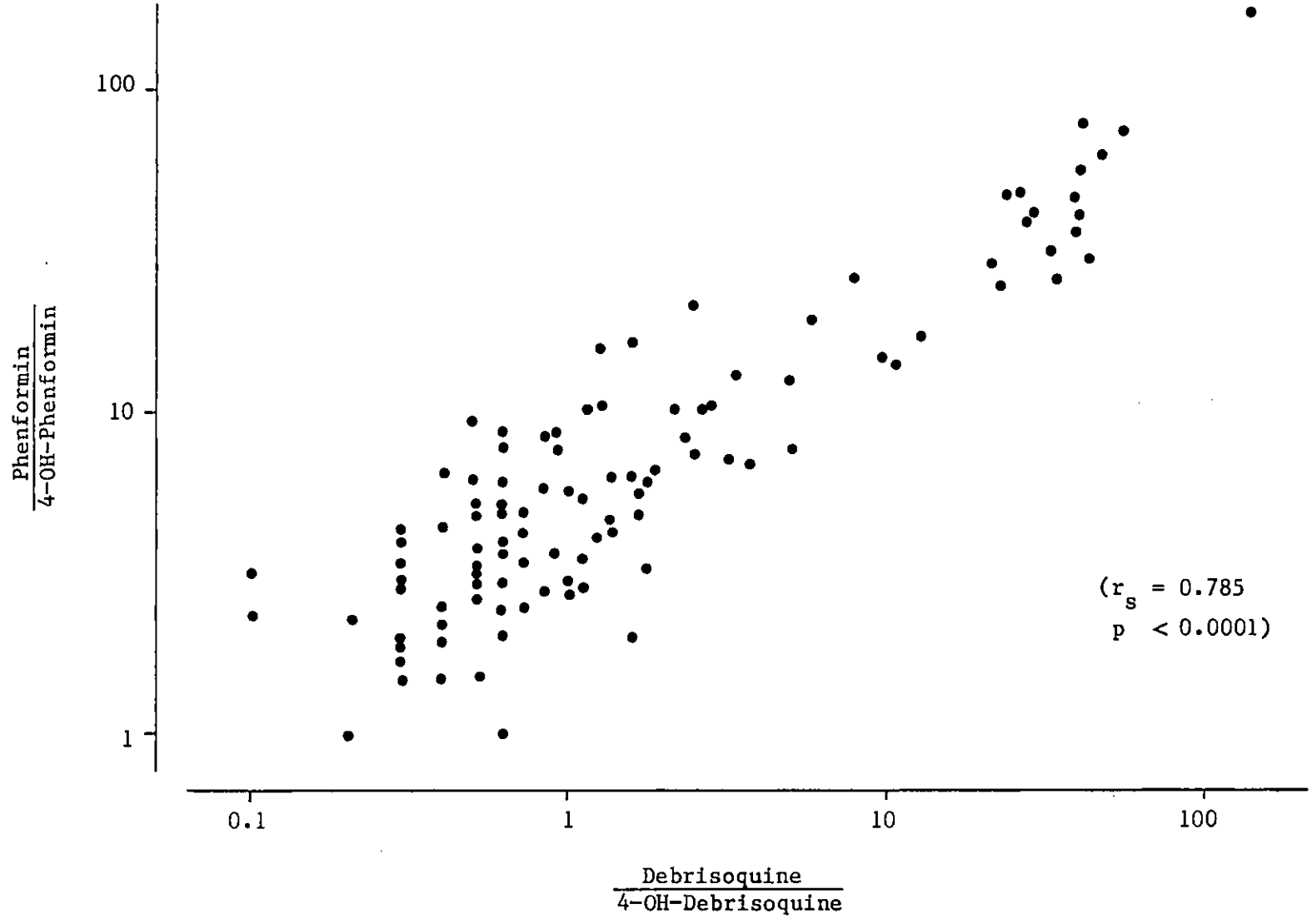


Table 17: Debrisoquine and phenformin oxidation status of three volunteers warranting further studies

Volunteer number	Phenformin Metabolic ratio (PR)	Debrisoquine Metabolic ratio (MR)
77	27.0	3.2
128	27.0	2.3
183	19.7	5.6

183, has a borderline phenformin ratio (P.R. = 19.7) and is an extensive metaboliser of debrisoquine (M.R. = 5.6). These findings suggest that the genetic mechanisms controlling the two oxidations may not be identical but very closely linked and co-inherited in view of the rarity (vide infra) of such individuals. The volunteers 77 and 128, including their family members, are subjects of further investigations to measure their abilities to oxidise a number of other drugs which have been studied so far in relation to debrisoquine.

If the linkage between the loci controlling the two oxidations is confirmed, 2/15 or 3/16 of the poor metabolisers of phenformin can be expected to display the 'linkage disequilibrium' i.e. 1.23-1.73% of the whole population.

It may be that in the case of these three volunteers, the oxidative pathway for phenformin became saturated at the dose (50 mg) employed. In order to exclude the dose-dependent kinetics of phenformin oxidation, a number of volunteers were studied on different dose schedules. This is discussed in the next section.

7.8 Effect of the dose of phenformin on its oxidation

Introduction

In the previous studies described, the dose of phenformin used was 50 mg phenformin given as 2 tablets of 25 mg phenformin formulated conventionally (Dibotin, Winthrop Laboratories, U.K.). Beckmann (1968) in his studies also used 50 mg phenformin and suggested that phenformin hydroxylation followed first-order kinetics. In view of the postulated genetic nature of the factors controlling this oxidation, it was considered of interest to study if the kinetic characteristics of this reaction were dose-dependent and to investigate if some individuals may have displayed high values of phenformin oxidation ratios at 50 mg of phenformin due to change in kinetics to zero-order type.

Methods

After giving their informed consent, 15 volunteers who took part in previous studies (Appendix I) were investigated further for their phenformin oxidation status at various dose levels of phenformin.

The doses of phenformin used were 25 mg (conventional), 75 mg (conventional) and 50 mg (slow-release formulation). The characteristics of these volunteers in terms of their age, weight, height, smoking and alcohol habits, past medical history and concurrent drug treatment had not changed in the interim period.

Each test was carried out in the usual manner with normal provisions and restrictions. It consisted of 0-8 hour urine collection post-dosing. A period of at least 72 hours elapsed between each test. For each test, the volume of urine voided was measured and an aliquot was analysed for its contents of unchanged phenformin and 4-hydroxy-phenformin by the h.p.l.c. method described. From this, the phenformin oxidation ratio was calculated as described previously.

Results

The study included 15 volunteers on whom two estimates of phenformin oxidation ratio on 50 mg conventional phenformin were available. Thirteen of these volunteers had ratios < 20, while the other 2 volunteers had their ratios > 20. These values, together with the values for the ratios on other dose schedules, are shown in Table 18. The corresponding total recovery of phenformin expressed as percentage of oral dose is shown in parentheses.

It can be seen that the overall recoveries during most of the current tests were better, if not comparable, than the corresponding previous recoveries. The two individuals who were classified as poor oxidisers of phenformin remained so during all the current tests. Of the remaining 13 individuals, 12 maintained relatively consistent phenformin oxidation ratios over the dose ranges studied. It is also

Table 18: Phenformin ratios of 15 volunteers on various dose schedules

Volunteer Number	Conventional 50mg Phenformin 1st study	Conventional 50mg Phenformin 2nd study	Conventional 25mg	Slow - Release 50mg	Conventional 75mg
2	15.9 (47.4)	15.1 (52.4)	19.7 (76.0)	14.3 (49.8)	12.9 (58.8)
4	2.4 (39.8)	3.6 (32.6)	2.3 (52.0)	2.5 (74.0)	2.1 (82.1)
5	3.0 (36.4)	3.7 (61.2)	3.0 (94.8)	2.5 (80.0)	3.0 (78.6)
14	5.0 (33.4)	3.6 (31.6)	5.9 (52.0)	5.9 (60.8)	4.3 (60.3)
21	1.9 (21.4)	1.9 (57.8)	1.1 (54.4)	1.1 (12.0)	1.1 (31.7)
28	2.1 (24.6)	2.6 (42.8)	2.1 (61.6)	2.5 (56.2)	2.0 (98.5)
70	6.7 (59.8)	6.7 (58.6)	5.3 (58.0)	9.3 (49.2)	6.2 (56.5)
76	8.0 (32.4)	4.9 (20.2)	3.4 (71.2)	7.6 (47.4)	8.0 (67.6)
77	27.0 (33.6)	25.4 (42.2)	25.0 (82.4)	34.0 (64.4)	46.0 (77.7)
78	2.8 (60.4)	3.0 (35.0)	2.6 (68.0)	4.1 (59.8)	3.6 (53.5)
81	3.6 (31.2)	4.1 (59.0)	3.1 (78.0)	3.5 (59.4)	2.6 (76.5)
102	4.1 (52.4)	4.4 (56.6)	3.4 (56.0)	3.3 (52.0)	3.8 (89.8)
110	5.9 (48.0)	4.7 (33.2)	5.0 (67.2)	4.8 (62.0)	6.8 (83.1)
111	32.4 (53.4)	37.2 (38.2)	30.0 (58.4)	35.0 (67.0)	43.0 (71.6)
119	14.5 (46.6)	16.1 (49.8)	11.2 (70.0)	14.3 (101.2)	31.0 (76.1)
Median	5.0 (39.8)	4.4 (42.8)	3.4* (67.2)	4.8* (59.8)	4.3* (76.1)
Mean ±SD % Recovery	41.4±12.2	44.8±12.7	66.7±12.4	59.7±19.1	70.8±16.6

* not significantly different from 50mg conventional dose

evident that no significant change in the median value of phenformin ratio occurs on various dose schedules. In only one volunteer (No 119) did the phenformin oxidation ratio rise from a value below 20 to a value above 20 on the highest dose employed (75 mg conventional formulation). Even this volunteer maintained relative consistency over the other dose schedules. It is to be noted, in particular, that volunteer No. 77 (the female who was a poor oxidiser of phenformin but appeared to be an extensive oxidiser of debrisoquine) maintained a ratio in excess of 20 on all the dose schedules.

Discussion

The results obtained in this study lend support to the conclusion that phenformin oxidation follows first-order kinetics. It is important to note that the poor metabolisers of phenformin remain so even at lower doses. This would, of course, be hardly surprising if the molecular expression of the genetic control were to be qualitative rather than quantitative and hence, applicable at all doses. It may be noted in this context that Davies et al (1981) did not find any quantitative differences in the contents of microsomal cytochrome P-450 from the liver biopsies of the extensive and poor metabolisers of debrisoquine. This suggests qualitative defects in presence of normal quantities of cytochrome P-450. This phenomenon of qualitative,but not

quantitative, abnormalities is well known amongst other haemoproteins such as haemoglobinopathies. The above results also suggest that whilst in the majority of individuals phenformin oxidation follows first-order kinetics over the range of drug dose (25 mg to 75 mg), the characteristic of the kinetics may change to zero-order type when high doses are employed in an occasional individual. This dose-dependent kinetics has been described for a number of other drugs such as phenytoin (Cummings, Martin and King, 1967) and the data obtained in this study suggests that there may be an individual threshold of high dose at which the oxidation kinetic characteristics will change from first-order to zero-order. The relatively consistent phenformin oxidation ratios of the individuals in this study at the 25 mg and 50 mg doses of phenformin, justify the conclusion reached on phenformin oxidation, its polymorphic population distribution and its genetic control and exclude any dose-related artefact at 50 mg dose of conventionally formulated phenformin tablets.

Since the individuals of impaired oxidation ability are not able to effect oxidative clearance of phenformin, it could be argued that they would be exposed to higher plasma levels of phenformin and consequently, would experience augmented pharmacological effects of phenformin. This possibility was investigated and is discussed in the next section.

7.9 Phenotypic differences in the metabolic response to phenformin

Introduction

The propensity of phenformin to produce hyperlactic acidaemia has already been referred to and the mechanisms for this property have also been reviewed. These mechanisms are consistent with the finding that changes in blood lactic acid levels are directly related to the phenformin concentrations in plasma (Nattrass, Sizer and Alberti, 1980) and/or hepatocytes. It has already been noted that the drug response correlated better with plasma levels of drugs than with orally administered dose of that drug. This has now been shown to be due to inter-individual differences in the metabolic elimination of the drug during its passage through the liver, resulting in wide inter-individual differences in the systemic exposure to the drug.

Phenformin is subject to genetically controlled wide inter-individual variations in its presystemic metabolic elimination by liver. It has been shown that the hepatic oxidation of phenformin bears high concordance to that of debrisoquine with a probable common genetic control. It was, therefore, decided to study if the metabolic response to phenformin showed any differences within a panel of volunteers previously phenotyped with debrisoquine for their oxidation status.

Methods

After giving their informed consent, 8 volunteers participated in the study. These volunteers were previously phenotyped for their debrisoquine oxidation status and consisted of 4 extensive and 4 poor metabolisers of debrisoquine.

None of the volunteers had taken any medication for three weeks preceding the study. All volunteers had normal hepato-renal and cardiac functions and none had history of diabetes. While all the volunteers consumed alcohol socially, in mild to moderate quantities, alcohol was forbidden for the period of 10 hours before the test and up to its completion.

After an overnight fast, each volunteer presented himself/herself on the morning of the test. The bladder was emptied and 20 ml of venous blood drawn for control values of drug, blood glucose, lactate and pyruvate concentrations. The volunteers then took a single oral dose of 50 mg of phenformin swallowed with 150 ml of water. Post-dosing, 20 ml of venous blood was drawn at 0.5, 1, 1.5, 2, 3, 4, 6 and 8 hours for the measurement of drug levels and also blood glucose, lactate and pyruvate concentrations. Fractional urine samples were collected at 1, 2, 4, 6, 8, 10, 12, 24 and 26 hours post-dosing. After measuring the volume of urine voided during each period, a 20 ml aliquot

was stored frozen at -20°C for later analysis of its phenformin and 4-hydroxy-phenformin contents by the h.p.l.c. method described.

The blood for plasma level was collected in lithium heparinised bottles and immediately centrifuged for removal of plasma. The plasma was stored at -20°C for subsequent analysis of its concentration of phenformin. These measurements were made by the Research and Development Section of M/s Winthrop Laboratories at Alnwick, U.K. The method is briefly described below.

2 ml of plasma, 3 ml of distilled water and 50 ul of the internal standard (p-methoxy-phenformin-monohydrochloride, 100 ug/ml) were mixed thoroughly into a 20 ml centrifuge tube. 500 ul of trichloroacetic anhydride (50% w/v aqueous) were added to this, mixed and centrifuged for 5 min at 2,000 r.p.m. The supernatant was transferred to a second centrifuge tube and 8 ml of chloroform-methanol (85:15 v/v) were added. After shaking for 1 min, the tube was centrifuged at 2,000 r.p.m. for 5 min and the organic phase was discarded. 2 ml of 5M sodium hydroxide was added to the remaining aqueous phase, mixed and extracted with 8 ml of chloroform-methanol. The mixture was shaken for 3 min and centrifuged at 2,000 r.p.m. for 5 min. The organic phase was transferred to a pear-shaped flask and evaporated to dryness by a rotary evaporator. The residue was dissolved in 1 ml of trimethylamine (1M in ether) and derivatised with 25 ul of

tri-fluoroacetic anhydride (which was previously re-distilled over phosphorous pentoxide). After standing for 30 min, 200 ul of the reaction mixture was transferred to a conical tube containing 100 ul of ether-washed saturated sodium tetraborate solution. This was thoroughly mixed and 2 ul of the ether layer is injected into the gas liquid chromatogram column within 30 seconds.

The gas chromatograph is equipped with electron capture detector. A 5 mm internal diameter x 2 metre glass column was packed with 10% OV-17 on 100-120 mesh chromasorb W-HP. The packed column was conditioned for 15 hours at 350°C under helium (flow 40 ml/min). The column operating conditions were as follows: column temperature 220°C; detector temperature 300°C and carrier gas (argon-methane 5%) flow rate 50 ml/min.

The retention times for phenformin and p-methoxy-phenformin were found to be 2.4 and 5.4 mins respectively under the chromatographic conditions. No plasma interfering peaks were observed in either phenformin or internal standard locus. A linear calibration curve (Fig. 24) was obtained for plasma over the concentration range 20-110 ng/ml. The precision of the assay was confirmed by running a series of spiked samples on a blind basis. A good correlation (Fig. 25) was obtained between the actual and observed concentrations with a high correlation coefficient ($r^2 = 0.99$). The minimum quantifiable limit, defined as the estimated concentration whose

Figure 24 Calibration curve for the determination of phenformin in plasma

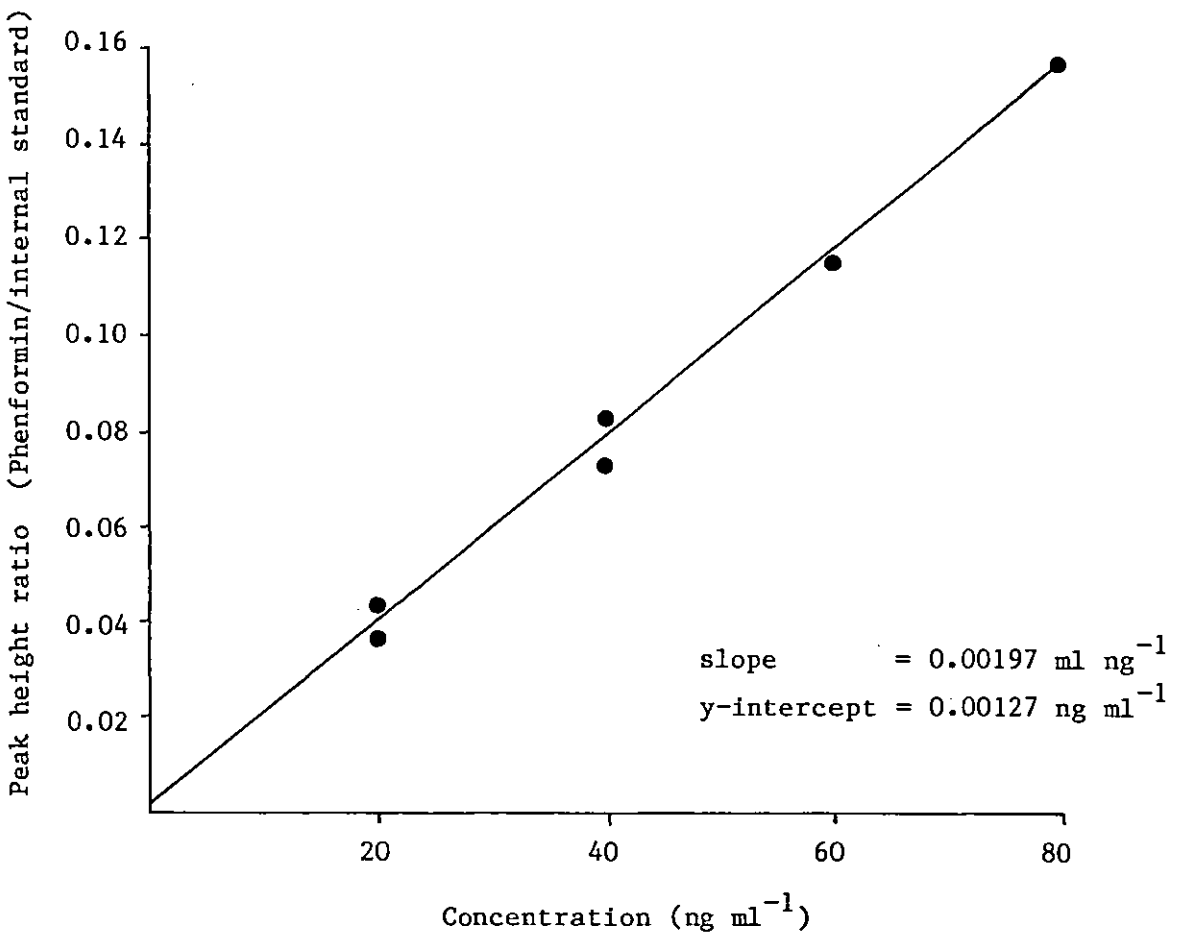
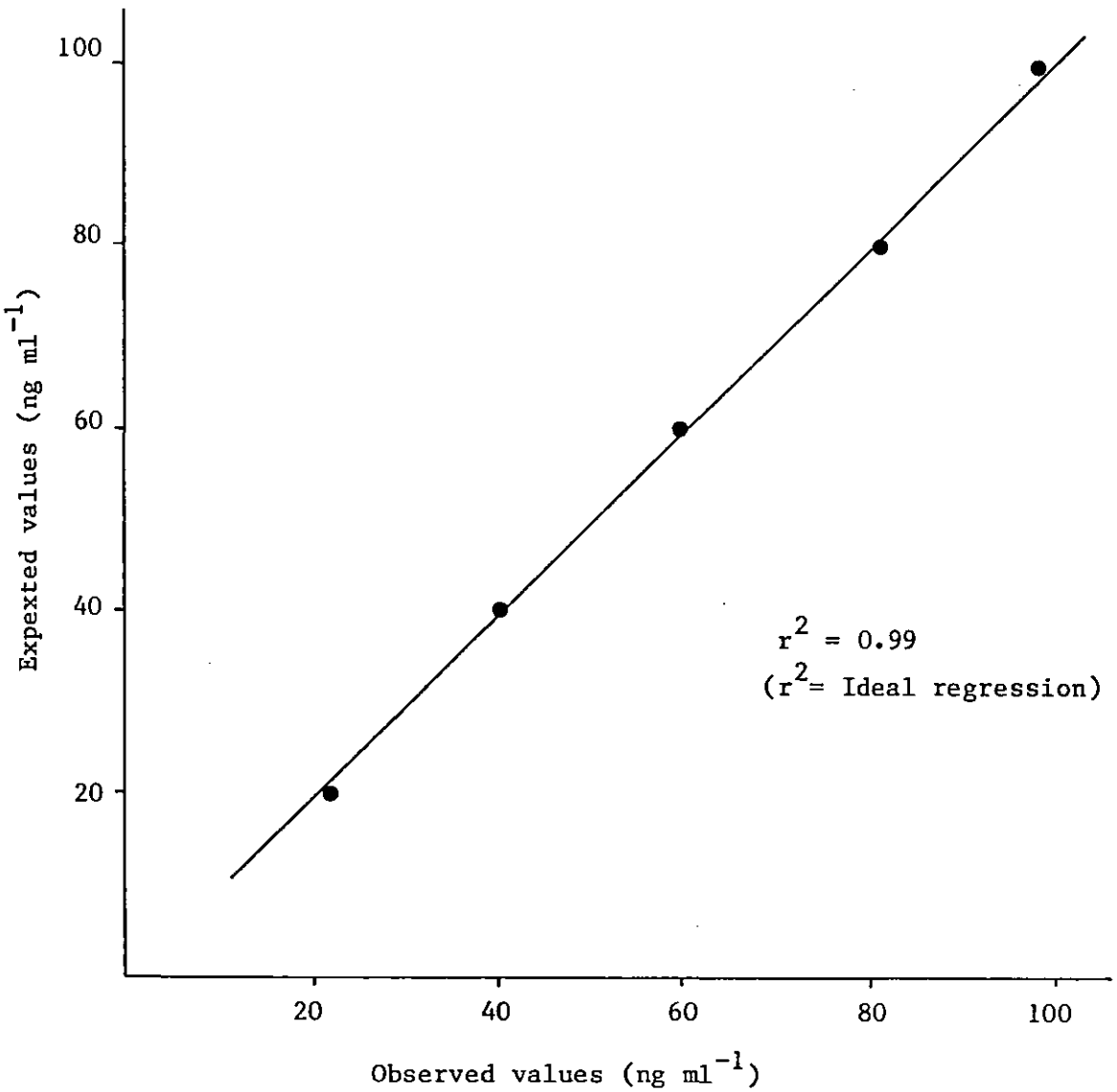


Figure 25 Regression curve for phenformin in plasma



lower limit encompassed zero, was calculated from the standard curve parameters and found to be 9.3 ng/ml.

The sample for blood glucose was collected using fluoride oxalate anticoagulant and the blood glucose was determined by the Department of Diagnostic Chemical Pathology using Trinder's colourimetric method. This method involved glucose oxidase and amino phenazone.

The blood lactic acid level was measured using Sigma Diagnostic reagents kit. Briefly, a 2 ml aliquot of blood is immediately deproteinised with perchloric acid and a sample of the clear supernatant is mixed with NAD, glycine buffer and lactic dehydrogenase and then transferred to a cuvette. A sample of perchloric acid is prepared in a like manner to serve as a blank. After 30 min incubation at 37°C, the absorbance of test is measured at 340 nm using the blank as a reference. This lactic acid measurement is based on the following reaction:

$$\text{Lactate} + \text{NAD} \longrightarrow \text{Pyruvate} + \text{NADH}$$

The reaction, catalysed by lactate dehydrogenase, is forced in the forward direction by trapping the pyruvate formed with hydrazine. Under these conditions, the increase in absorbance at 340 nm is due to generation of NADH, proportional to lactic acid concentration.

Pyruvic acid in blood was also determined by Sigma diagnostic reagent kit. Briefly, a 2 ml aliquot of blood is immediately deproteinised with perchloric acid and centrifuged. A sample of clear supernatant is treated with Trizma Base solution and NADH. The initial absorbance of the mixture is measured at 340 nm, using water as reference. Lactate dehydrogenase is added and after approximately 2-5 minutes, the final absorbance is read. The pyruvic acid concentration is calculated from the decrease in absorbance using a factor based on the molar extinction of NADH. This pyruvic acid measurement is based on the following reaction:

$$\text{Pyruvate} + \text{NADH} \longrightarrow \text{Lactate} + \text{NAD}$$

This reaction too is catalysed by lactate dehydrogenase and in the presence of excess NADH, virtually all of the pyruvate is converted to lactate. Therefore, the oxidation of NADH to NAD causes a decrease in the absorbance at 340 nm that is proportional to pyruvate acid concentration.

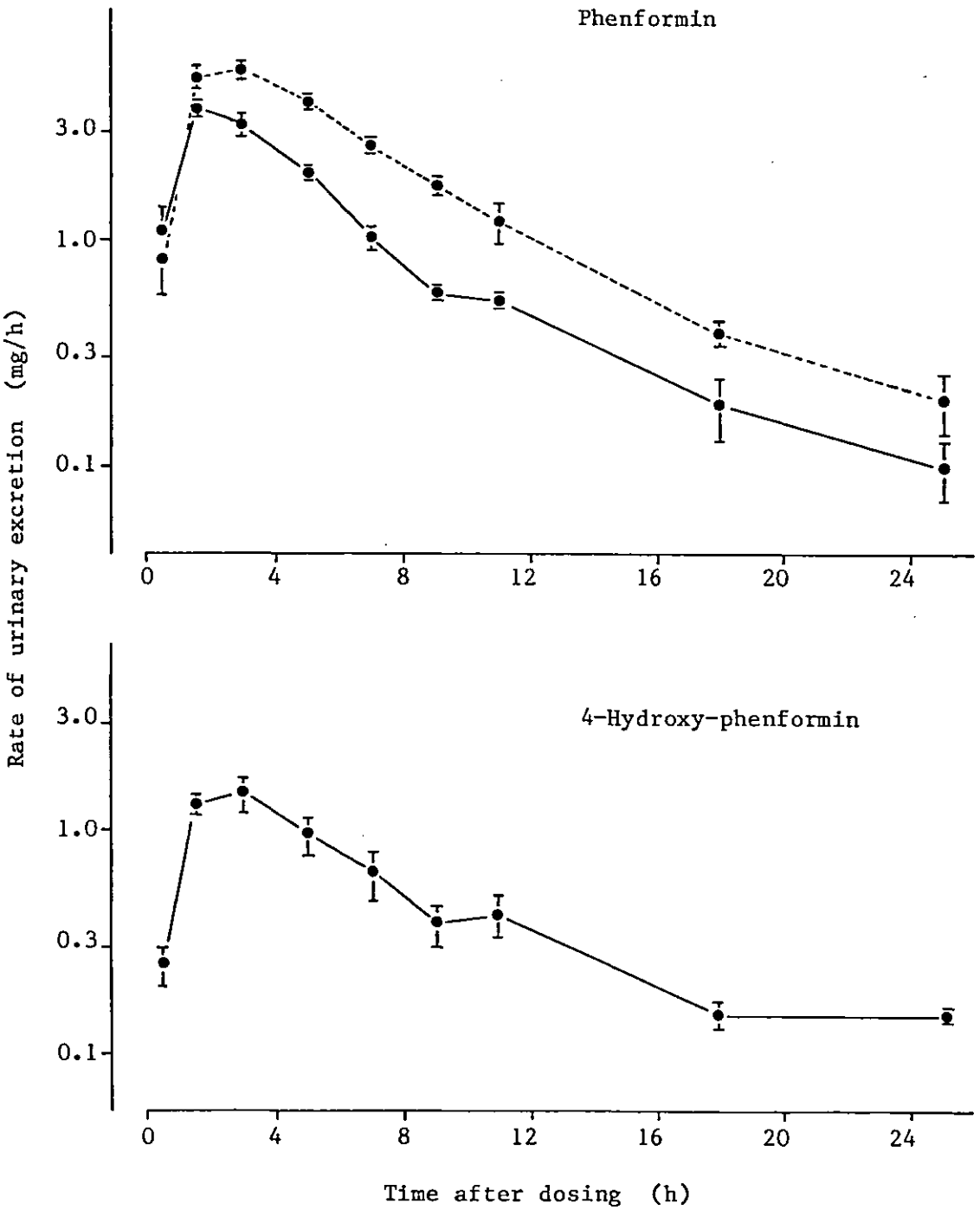
Results

The details of the 8 volunteers, together with their debrisoquine metabolic ratio are shown in Table 19. Their urinary excretions of phenformin and 4-hydroxy-phenformin are shown in Appendices VII and VIII and summarised in Fig. 26. The kinetic parameters of urinary elimination of

Table 19 Physical details, debrisoquine oxidation status and urinary phenformin kinetics of the eight volunteers.

	AW	EE	JR	AZ	LE	AR	SJ	CS
Age(years)/Sex	22/F	22/F	24/M	23/M	22/F	22/M	22/M	21/M
Weight (kg)	62	57	84	71	54	75	80	67
Height (cm)	155	165	183	165	160	183	178	168
Cigarettes (daily)	0	0	0	10	0	0	0	10
Debrisoquine metabolic ratio and phenotype	0.3 EM	0.03 EM	0.3 EM	0.1 EM	23.2 PM	26.8 PM	84.1 PM	59.2 PM
Urinary excretion data								
Phenformin								
Observed recovery (mg)	18.43	22.10	27.55	23.02	38.76	41.80	42.56	48.87
Recovery to infinity (mg)	18.53	22.29	28.15	23.38	39.53	42.44	43.05	50.51
K_{el} (h^{-1})	0.206	0.182	0.145	0.157	0.160	0.163	0.178	0.131
$t_{1/2}$ (h)	3.37	3.80	4.76	4.41	4.34	4.26	3.90	5.28
4-Hydroxy-phenformin								
Observed recovery (mg)	13.69	13.74	9.06	8.30	0.15	0.32	n.d.	n.d.
Recovery to infinity (mg)	14.56	14.28	9.92	9.06	-	-	-	-
K_{el} (h^{-1})	0.10	0.125	0.093	0.092	-	-	-	-
$t_{1/2}$	6.31	5.55	7.49	7.56	-	-	-	-
Phenformin metabolic ratio 0 - 8h	1.7	1.8	3.7	3.3	>100	>100	>100	>100
Phenformin metabolic ratio 0 - 26h	1.4	1.6	3.0	2.8	>100	>100	>100	>100

Figure 26 Mean (±s.e.mean) rate of urinary excretion of phenformin and 4-hydroxy-phenformin in 4 EM's (●—●) and 4 PM's (●····●)



phenformin and 4-hydroxy-phenformin are also shown in Table 19. The plasma levels of the drug and the blood concentrations of sugar, lactate, pyruvate and the lactate:pyruvate ratios are shown in Appendices IX-XIII respectively. The mean (± s.e.m.) plasma concentrations of phenformin for the two phenotyped groups against time are shown in Fig. 27, while the mean (± s.e.m.) concentrations of blood sugar, lactate:pyruvate ratio, lactate and pyruvate for the two phenotypes against time are shown in Figs. 28 and 29. Tables 20 and 21 indicate the various kinetic parameters for the two phenotyped groups derived for plasma phenformin concentrations and blood sugar, lactate and pyruvate concentrations respectively.

The results shown in Table 19 confirm that those who are poor metabolisers of debrisoquine are grossly impaired in their ability to hydroxylate phenformin. The kinetic parameters derived for urinary excretion are further confirmed in that the differences between the two phenotypes are not readily discernible from the urinary pharmacokinetic parameters such as elimination rate constant ($0.173 \pm 0.014\ h^{-1}$ in EMs v/s $0.158 \pm 0.010\ h^{-1}$ in PMs) or the elimination half-life (4.09 ± 0.31 h in EMs v/s 4.45 ± 0.30 h in PMs). The consistency between 0-8 hour and 0-26 hour phenformin ratios is also confirmed.

Figure 27 Mean (±s.e.mean) plasma phenformin concentrations in 4 EM's (■—■) and 4 PM's (●---●)

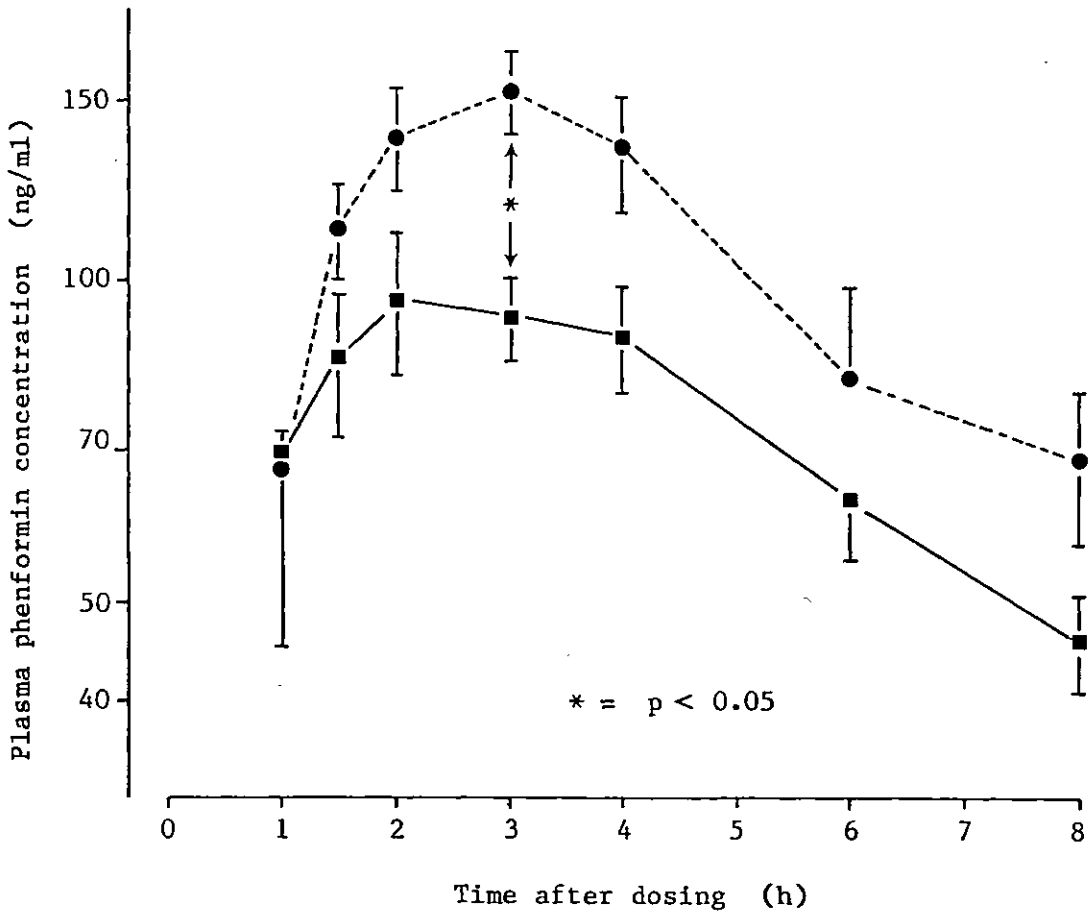


Figure 28 Blood concentrations of glucose and lactate/pyruvate ratio in 4 EM's (■—■) and 4 PM's (●---●) following 50mg oral phenformin (mean±s.e.mean)

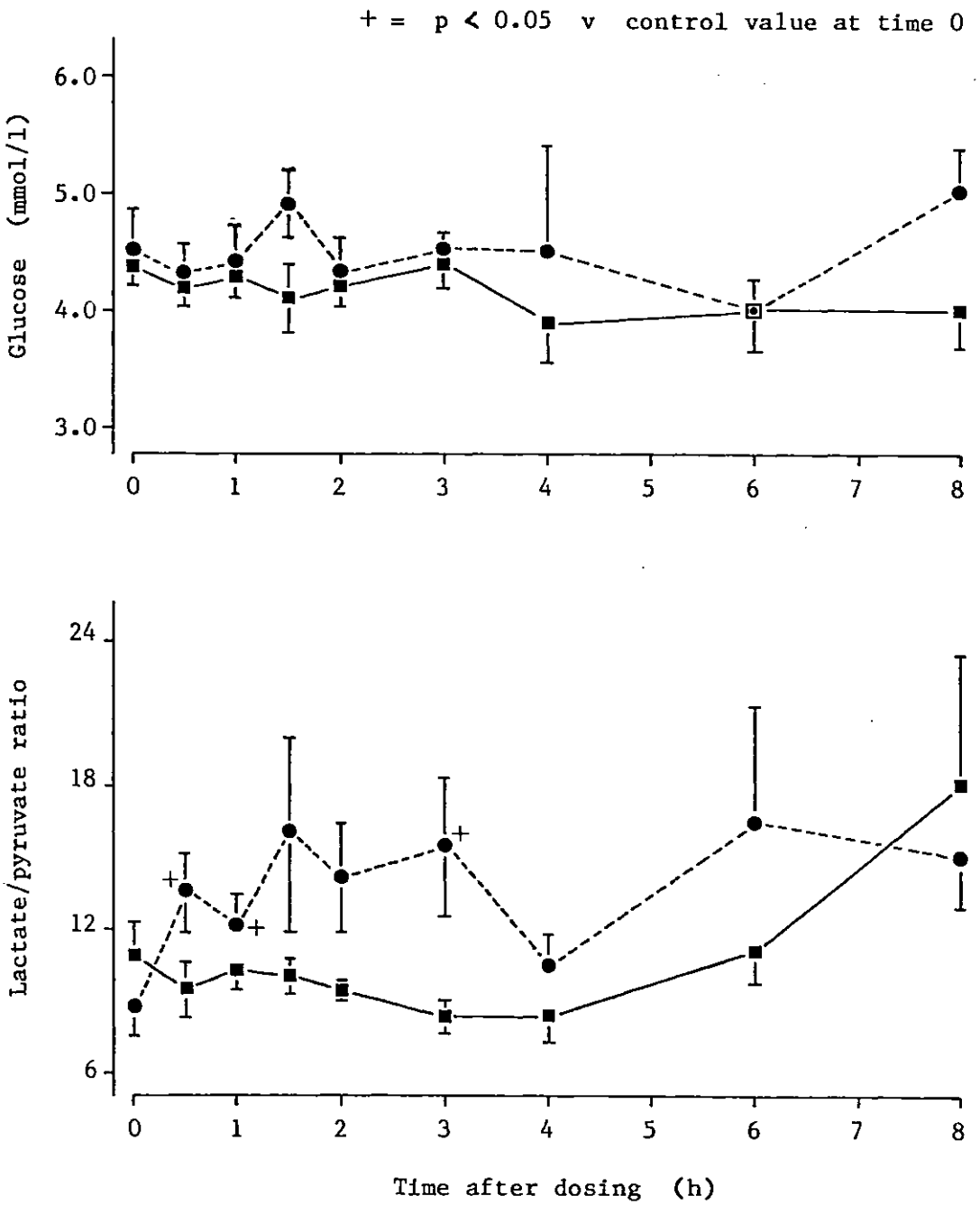


Figure 29 Blood concentrations of lactate and pyruvate in 4 EM's (■—■) and 4 PM's (●---●) following 50mg oral phenformin (mean±s.e.mean)

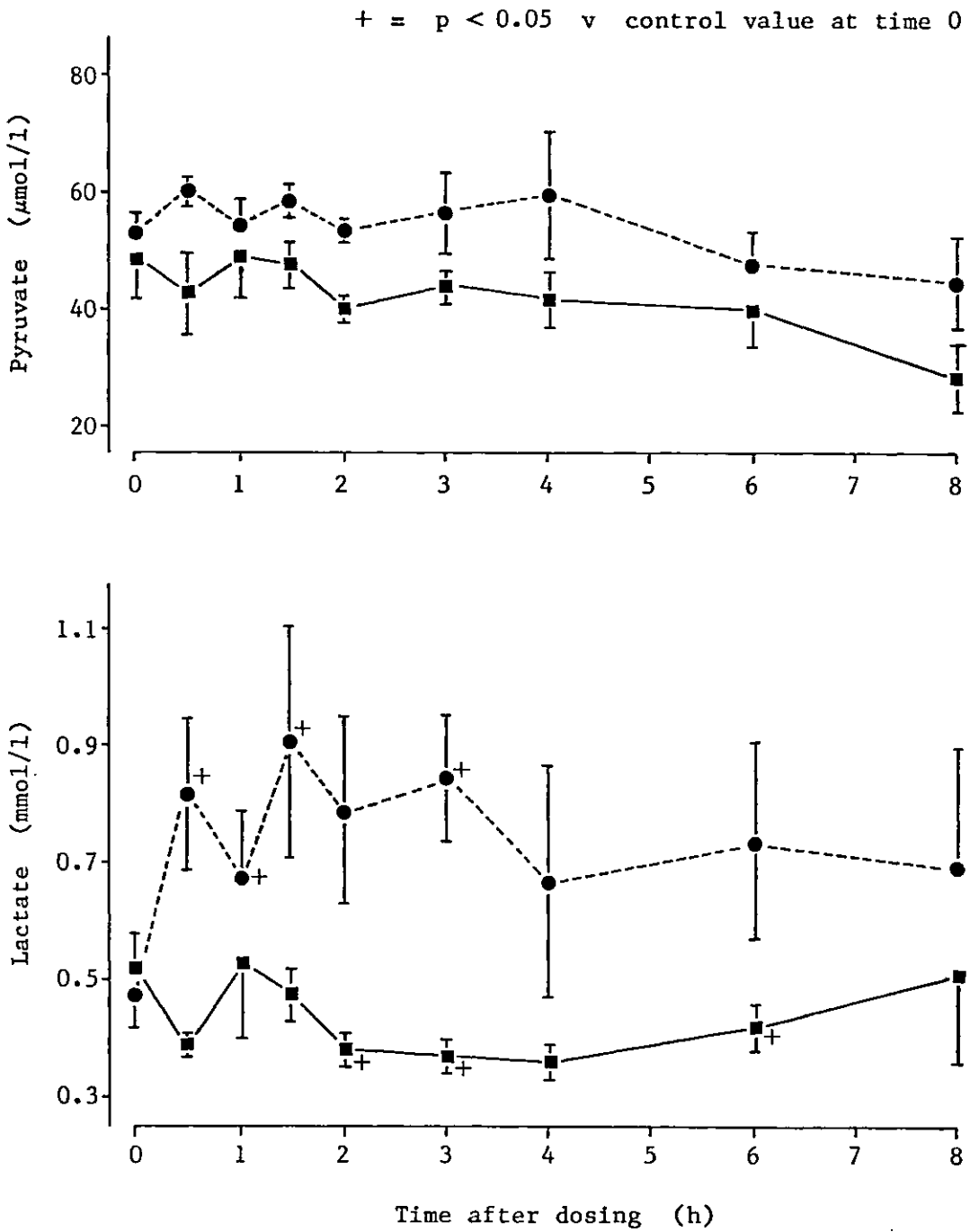


Table 20 Derived parameters of plasma phenformin kinetics in the two phenotypes

Volunteeer	C_{max} ng/ml	AUC_{0-8} ng.h/ml	$t_{1/2}$ h	Apparent total clearance ml/min/kg	Renal clearance ml/min/kg	Extra-renal clearance ml/min/kg
AW	140.5	668	3.3	9.6	6.0	3.6
EE	89.4	577	7.4	14.5	9.4	5.1
JR	82.0	474	5.3	11.1	8.7	2.4
AZ	87.3	476	4.4	11.5	8.8	2.7
Mean±sem for EM's	99.8 ±13.7	549 ±47	5.1 ±0.9	11.7 ±1.1	8.2 ±0.8	3.5 ±0.6
LE	184.0	1068	4.5	7.3	7.2	0.1
AR	136.2	678	4.0	11.1	11.0	0.1
SJ	160.7	636	2.5	10.7	10.7	0
CS	127.8	732	5.3	12.1	12.1	0
Mean±sem for PM's	152.2 ±12.7	779 ±99	4.1 ±0.6	10.3 ±1.1	10.3 ±1.1	0.05 ±0.03
*p	<0.01	<0.05	N.S.	N.S.	N.S.	<0.0001

* t-test

Table 21: Phenotypic differences in the metabolic response to 50 mg oral dose of phenformin

Volunteer	Change in AUC_{0-8} (mmol.h/L).		
	Blood sugar	Blood lactate	Blood pyruvate
AW	- 2.450	- 2.368	- 0.041
EE	- 6.325	- 0.915	- 0.140
JR	- 2.325	- 0.850	- 0.137
AZ	+ 1.250	- 1.593	+ 0.010
Mean ± s.e.m. for EMs	- 2.46 ± 1.55	- 1.43 ± 0.36	- 0.077 ± 0.037
LE	- 0.375	- 0.628	- 0.059
AR	+ 4.975	+ 0.835	- 0.088
SJ	- 9.275	+ 1.543	+ 0.100
CS	+ 2.600	+ 3.360	+ 0.027
Mean ± s.e.m. for PMs	- 0.52 ± 3.12	+ 1.59 ± 0.62	+ 0.003 ± 0.043
*p	N.S	< 0.05	N.S

* t-test

Figure 27 shows that the poor metabolisers of debrisoquine experience a significantly greater exposure to phenformin. This is conclusively apparent in the significant difference between the two phenotype groups in terms of the peak plasma levels of phenformin or area under the curve. Once again, it is evident that the differences between the two phenotype groups are not evident when pharmacokinetic parameters such as plasma half-life of the drug or the renal clearance of the drug are considered. Although the apparent total and renal clearances of plasma phenformin are not different between the two phenotypes, they differ significantly in terms of extra-renal clearance of the drug. The individuals of the poor metaboliser phenotype on the whole, consequently, experience a mean of 40% greater systemic exposure to phenformin.

As regards the metabolic response, no changes are observed in blood levels of pyruvate or blood glucose. However, the blood lactate concentration rose markedly in the poor metabolisers of the panel and was significantly greater than the control values at 0.5, 1, 1.5 and 3 hours, whereas a small but significant reduction was observed in the extensive metabolisers at 2, 3 and 6 hours. In absence of any significant changes in pyruvate levels, lactate:pyruvate ratios show changes compatible with changes in blood lactate concentration. When areas under the curve are considered,

it is clear that the major significant difference between the two phenotypes in terms of their metabolic response to phenformin consists of an increase in the poor metabolisers, and a decrease in the extensive metabolisers, of their blood lactate concentrations.

Discussion

The results of this study further underline the concurrence between alicyclic 4-hydroxylation of debrisoquine and the aromatic 4-hydroxylation of phenformin. The presystemic elimination of phenformin in extensive metaboliser phenotype is confirmed by coinciding of the maximum rates of urinary excretion of phenformin and 4-hydroxy-phenformin (1-4 hour period). This conclusion is further strengthened by a significant difference in the extra-renal plasma clearances of phenformin (in face of insignificant differences in renal or apparent total clearances) between the two phenotypes. As with debrisoquine, the individuals of poor metaboliser phenotype are unable to effect satisfactorily the oxidative clearance of phenformin and they have a systemic exposure of phenformin to the extent of 1.4-fold as judged by the area under the curve up to 8 hours. The peak levels of plasma phenformin concentrations are also greater in the poor metabolising individuals.

Schafer's (1976) unified theory to explain lactic acidogenic properties of phenformin would, therefore, result in greater degree of anaerobic glycolysis and disruption of mitochondrial oxidative electron transport chain in these individuals. This would result in a more intense hypoglycaemic and lactic acidogenic response in these individuals. However, it is probable that in normal healthy individuals, reflex mechanisms would tend to correct hypoglycaemic tendencies and significant hypoglycaemia may not occur. The observation of this study, in this respect, is consistent with the findings of previous investigations that phenformin does not lower blood sugar in healthy individuals. No significant changes in blood pyruvate levels will be expected in either phenotype because while in extensive metabolisers it will be channelled into tricarboxylic acid cycle, it will be diverted to the formation of lactic acid through the necessity to regenerate cytosolic NAD^+ in the poor metabolisers.

Since phenformin decreases intestinal absorption of glucose, an effect which is exerted pre-systemically, the rate of intestinal absorption of glucose will be diminished in both phenotypes. However, in the extensive metaboliser phenotype, phenformin is oxidised in liver and therefore the tricarboxylic acid cycle remains functional and glycolysis is not enhanced; it is possible that lactate is converted into pyruvate to fuel this cycle. This may explain the "lag" fall in lactate levels of the extensive

metabolising individuals (Fig. 29). In contrast, phenformin is not oxidised in the liver of poor metabolising phenotype and the tricarboxylic acid cycle is halted. Consequently, anaerobic glycolysis is enhanced and an earlier rise in blood lactate levels ensues.

It may be noted that Nattrass, Sizer and Alberti (1980) reported a significant correlation between the increase in blood lactate concentration and the plasma concentration of the drug after oral administration of 50 mg of phenformin to normal subjects. The results of this study suggest that the individuals of poor metabolising phenotype experience a systemic exposure to phenformin which is greater in intensity and duration, compared to their extensive metabolising counterparts. This results in profound differences in the toxic metabolic response, changes in blood lactate levels, between the two phenotypes. The observation of this study provides a justification to the use of phenotyped panel approach, not only to determine inter-individual differences in metabolism of drugs, but also to assess inter-individual differences in drug response. The results make it probable that the uncomplicated diabetic patients who develop lactic acidosis on chronic phenformin therapy may have impaired ability to effect oxidative clearance of phenformin and by inference, that of debrisoquine. This hypothesis is tested in the following section.

7.10 Debrisoquine oxidation status of patients experiencing phenformin-induced lactic acidosis

Introduction

The previous study suggested that individuals of poor phenformin oxidation status, and by inference, that of debrisoquine, may be at risk of developing lactic acidosis when phenformin is given chronically. Without resorting to the administration of a single dose of phenformin, could the diabetics at risk of this complication have been identified? It was decided to measure the debrisoquine oxidation capacity of 3 diabetics who had survived phenformin-induced lactic acidosis.

Methods

Through the courtesy of Committee on Safety of Medicines and Winthrop Laboratories, U.K., 3 patients who had survived well documented phenformin-induced lactic acidosis, on conventional phenformin 50 mg tds, were identified from 3 clinics. Three additional patients, who tolerated the drug well without any complications, were also included. This study was approved by the Ethics Committee of St. Mary's Hospital, London W2, and by the practitioners in charge of the patients. Each patient gave his/her informed consent.

These patients were phenotyped for their ability to effect debrisoquine hydroxylation. On the test morning, each took 10 mg debrisoquine orally, after voiding the bladder, at least one hour before a light breakfast. A bulk collection of urine was made over the following 8 hours. The patients refrained from alcohol during the test period and did not take any medications for a period extending from 10 hours before to 3 hours after swallowing debrisoquine. Within one week of the test, 20 ml of venous blood was drawn from each patient for biochemical measurement of liver function and serum creatinine. After measuring the volume of the 0-8 hour urine, an aliquot 20 ml was despatched to St. Mary's Hospital Medical School. The samples were analysed for the concentrations of debrisoquine and 4-hydroxy-debrisoquine by the gas chromatographic method as described by Idle et al, (1979).

Results

The details of the 6 patients, including the concurrent drug regimen and serum liver function tests and creatinine, are shown in Table 22 while the results of the debrisoquine phenotyping test can be seen in Table 23.

It can be seen that none of the patients had any evidence of serious hepatic dysfunction apart from mildly raised alkaline phosphatase levels in two. The serum creatinine level was slightly raised in one patient. The three patients who had developed lactic acidosis due to

Table 22: Details of the patients with and without phenformin-induced lactic acidosis

Patient	Age (yrs)	Sex	Wt (kg)	Serum Biochemistry*						Current drug treatment
				AST 7-40 U/L	Alk PO_4-ase 30-115 U/L	Bilirubin 5-17 umol/L	Albumin 35-51 g/L	Globulin 25-45 g/L	Creatinine 60-125 umol/L	
Lactic acidotic patients										
JMcS	67	F	55	13	191	13	41	35	91	Glibenclamide
TR	80	F	73	40	115	17	35	45	125	None
LS	66	F	39	28	62	2	44	27	112	Glibenclamide, Temazepam, Nifedipine
Non-lactic acidotic patients										
WC	57	M	95	31	111	7	46	34	102	Phenformin, Acebutalol, Sulindac, Amiloride
OI	77	M	65	25	111	5	39	26	133	Digoxin, Nitrates, Metoprolol, Frusemide, Chlorpropamide, Metformin
MM	65	M	59	21	143	8	45	29	78	Phenformin

* Parameter measured and its normal range.

Table 23: Debrisoquine oxidation status of the patients with and without lactic acidosis on chronic phenformin therapy.

Patient	% oral dose in 0 - 8 h urine excreted as		Total 0-8 h urinary recovery	Debrisoquine Metabolic Ratio
	Debrisoquine	4-hydroxy-debrisoquine		
Lactic acidotic patients				
JMcS	30.8	0.5	31.3	57.1
TR	31.3	5.1	36.4	6.2
LS	13.2	3.9	17.1	3.4
Non-lactic acidotic patients				
WC	31.1	15.2	46.3	2.0
01	19.3	42.2	61.5	0.5
MM	6.6	25.4	32.0	0.3

phenformin had ratios ranging from 3.4 to 57.1. In contrast, the 3 patients who tolerated the drug well had ratios ranging from 0.3 to 2. Thus, the six patients fell into two discrete groups; those with lactic acidosis displaying impaired debrisoquine oxidation status to a variable degree.

Discussion

Included amongst the factors that have previously been thought to predispose to phenformin-induced lactic acidosis are old age and hepato-renal dysfunction. However, an individual idiosyncrasy has also been strongly suggested since the above factors are conspicuously absent in many cases of phenformin-induced lactic acidosis (Sussman *et al*, 1970; Cohen, 1978).

The patients in this study are of comparable age and apart from mildly raised alkaline phosphatase levels in a total of two patients from both the groups, there is no evidence of hepatic dysfunction. These factors therefore cannot be of great significance in the precipitation of phenformin-induced lactic acidosis.

Even in normal individuals, about two-thirds of the absorbed phenformin is eliminated unchanged by renal excretion. It may be imagined that the presence of renal dysfunction would tend to delay the elimination of unchanged phenformin and therefore, that renal dysfunction may constitute a risk factor. However, one of the three

patients did not develop lactic acidosis on long-term phenformin therapy despite a raised serum creatinine level and this suggests that renal factors cannot be of significance in development of lactic acidosis due to phenformin. In a pharmacokinetic study involving eight diabetic patients with varied renal function, Biosisio et al (1981) found that phenformin half-lives were unrelated to the degree of renal impairment; whereas reduced renal clearance of inulin and creatinine was significantly correlated with the prolonged half-life of the 4-hydroxy metabolite of phenformin. These workers too concluded reduced (hepatic) hydroxylation of phenformin to be responsible for high plasma levels of the drug previously described in toxic patients. Clearly the body elimination of phenformin depends on hepatic metabolism and the elimination of metabolite on renal clearance. This is in contrast to metformin, a drug which is not metabolised, whose elimination is through renal clearance only, and hence renal dysfunction is a major risk factor in metformin-induced lactic acidosis.

The two groups of patients differ considerably in their genetically determined ability to effect oxidative clearance of certain drugs. It is clear that the patients who develop lactic acidosis due to phenformin displayed impaired debrisoquine oxidation when compared to those who tolerated the drug well. It seems, therefore, by inference that the patients at risk of this complication have impaired ability

to effect oxidation of phenformin. The factors responsible for this inability are resident in the liver and debrisoquine phenotyping of these patients could have indicated the risk. Clearly, the identification of the major risk factor, namely drug oxidation phenotype is vital since phenformin is a very effective hypoglycaemic (Bloom, 1969b; Patel and Stowers, 1964) agent and lactic acidosis has a very high mortality (50-70%) (Cohen, 1978). It is important to add at this stage that although genetically determined drug oxidation phenotype is a major determinant of drug toxicity, it is possible there may be additional, as yet unidentified, background factors that may play a minor role in predisposition to toxicity.

In view of the fact that amongst the patients who developed lactic acidosis, the lowest debrisoquine metabolic ratio was found to be 3.4 and the highest debrisoquine metabolic ratio amongst those who took the drug without any complication was 2, it is probable that the risk of developing lactic acidosis on conventional dose of phenformin commences at a ratio of about 3. Chronic alcoholism, through unrecognisable changes in the hepatic drug metabolising capacity, may tend to lower the value of debrisoquine metabolic ratio at which the risk begins. This, together with the possible presence of other minor factors, was also apparent in a report by Wiholm et al (1981).

In that study, 83% of the cases could have been identified as being at risk by consideration of the drug oxidation status and alcohol abuse. Interestingly, their patient with the highest metabolic ratio was also the one on the lowest dose of phenformin.

In the normal British population, the percentage of individuals with a debrisoquine metabolic ratio above 3 is about 15%. If one were to assume that diabetic patients had the same frequency distribution of debrisoquine metabolic ratios as normal population, approximately 15% of diabetics prescribed phenformin would be at risk of developing lactic acidosis. However, the currently available data on the incidence of phenformin-induced lactic acidosis do not bear this out. This suggests that either the diabetics have a significantly different frequency distribution of debrisoquine metabolic ratio or alternatively, the complication is grossly under-reported. It was therefore decided to study debrisoquine oxidation in a group of maturity-onset (non-insulin dependent, Type 2) diabetic patients.

7.11 Debrisoquine oxidation capacity of maturity-onset (Non-insulin dependent, Type 2) diabetic patients

Introduction

Previous studies have revealed that genetically determined polymorphic hydroxylation of phenformin accounts for inter-individual differences in its oxidation and that individuals with impaired drug oxidation are at risk of developing lactic acidosis. These individuals, when given phenformin, are unable to effect its oxidative clearance and therefore achieve higher peak plasma levels of phenformin and experience greater systemic exposure to this drug. Biosisio et al (1981) also showed that (hepatic) inability to form the 4-hydroxy-metabolite of phenformin is associated with high plasma levels of phenformin and consequently leads to the toxicity due to the drug. Renal dysfunction does not appear to be a significant factor predisposing to the development of high circulating plasma levels of phenformin (Biosisio et al, 1981).

The critical debrisoquine metabolic ratio, at which the risk of developing lactic acidosis from chronic phenformin therapy in diabetics commences, appears to be about 3. Approximately 15% of the normal British white caucasians have a debrisoquine metabolic ratio > 3. However, not 15% of the patients randomly treated with phenformin are reported to develop lactic acidosis. This raises the possibility

that either the epidemiological data on and the incidence of phenformin-induced lactic acidosis are inadequate through under-recognition and under-reporting or the diabetic state may be associated with extensive metaboliser phenotype.

Tokola *et al* (1975) have observed greater *in vitro* activities of drug metabolising enzyme systems in liver samples (obtained during abdominal surgery) from diabetic patients than in those without diabetes. The aetiology of non-insulin dependent or maturity-onset diabetes is a subject of debate but it has been concluded, on the basis of twin studies, that genetic factors are predominant (Barnett *et al*, 1981). In contrast to insulin dependent (juvenile-onset) diabetes, no genetic linkage with the HLA histocompatibility loci A and B has been found (Platz *et al*, 1982). No suitable candidate has been found for incrimination as the offending environmental agent. Bodanksy and his colleagues (1981) have already shown an association between Type 1 (insulin-dependent) diabetes and rapid acetylation status, although they concluded that the association is polygenically controlled.

To test the association of non-insulin dependent diabetes and oxidation phenotype, it was decided to study debrisoquine oxidation in these patients.

Methods

The study was approved by the Ethics Committee of St. Mary's Hospital, London W2 and by the Physician in charge of the diabetic clinic.

One hundred and twenty-eight non-insulin dependent diabetics, having given their informed consent, were included in the study. Each patient, after an overnight fast, voided the bladder in the morning and was phenotyped for debrisoquine oxidation as described by Mahgoub et al (1977). They all took light breakfast more than one hour after swallowing debrisoquine and did not consume any medication for a period extending from 10 hours before to 3 hours after swallowing debrisoquine. Alcohol was not permitted from the previous evening and during the 8 hours of the test period.

Each patient completed a questionnaire relating to their physical details as well as medical history and concurrent drug treatment.

After measuring the volume of the bulked 0-8 hour urine voided, an aliquot was frozen at -20^{o}C for later analysis for its debrisoquine and 4-hydroxy-debrisoquine contents by the electron capture gas chromatography method of Idle et al (1979). From this, the patient's debrisoquine metabolic ratio was derived by dividing the percentage oral dose excreted as unchanged debrisoquine in the 8 hours by the percentage oral dose excreted as 4-hydroxy-debrisoquine.

Results

A total of 128 non-insulin dependent diabetic patients were investigated in this study and they included 67 males and 61 females (Appendix XIV). Their mean (± S.D.) age,

height and weight were 63 years (± 10.3, range 34-83 years), 167.7 cm (± 9.0, range 145-188 cm) and 73.8 kg (± 12.1, range 43-108) respectively. Forty-five patients consumed tobacco in one of its various forms while only a few patients consumed alcohol, all of whom drank only socially. None of the patients had any clinical evidence of intestinal, hepatic or renal dysfunction. Thirty patients were hypertensive, while 19 patients had evidence of ischaemic heart disease. Twenty-eight patients were on no drug therapy at all, while in the remainder the major concurrent drug regimen consisted of:

Glibenclamide	(n=50)	K^+-sparing agents	(n=7)
Diuretics	(n=25)	Digoxin	(n=6)
Chlorpropamide	(n=24)	Analgesics	(n=6)
Beta-blockers	(n=19)	Tolbutamide	(n=6)
Anti-hypertensives	(n=10)	Thyroxine	(n=4)
Sedatives and hypnotics	(n= 9)	Anti-anginals	(n=4)
Anti-inflammatory agents	(n= 8)	Anti-depressants	(n=4)
		Disopyramide	(n=3)
		Allopurinol	(n=2)
		Phenformin	(n=2)
		Tamoxifen	(n=2)

The results of the debrisoquine phenotyping test and the individual concurrent drug regimen are shown in Appendix XIV.

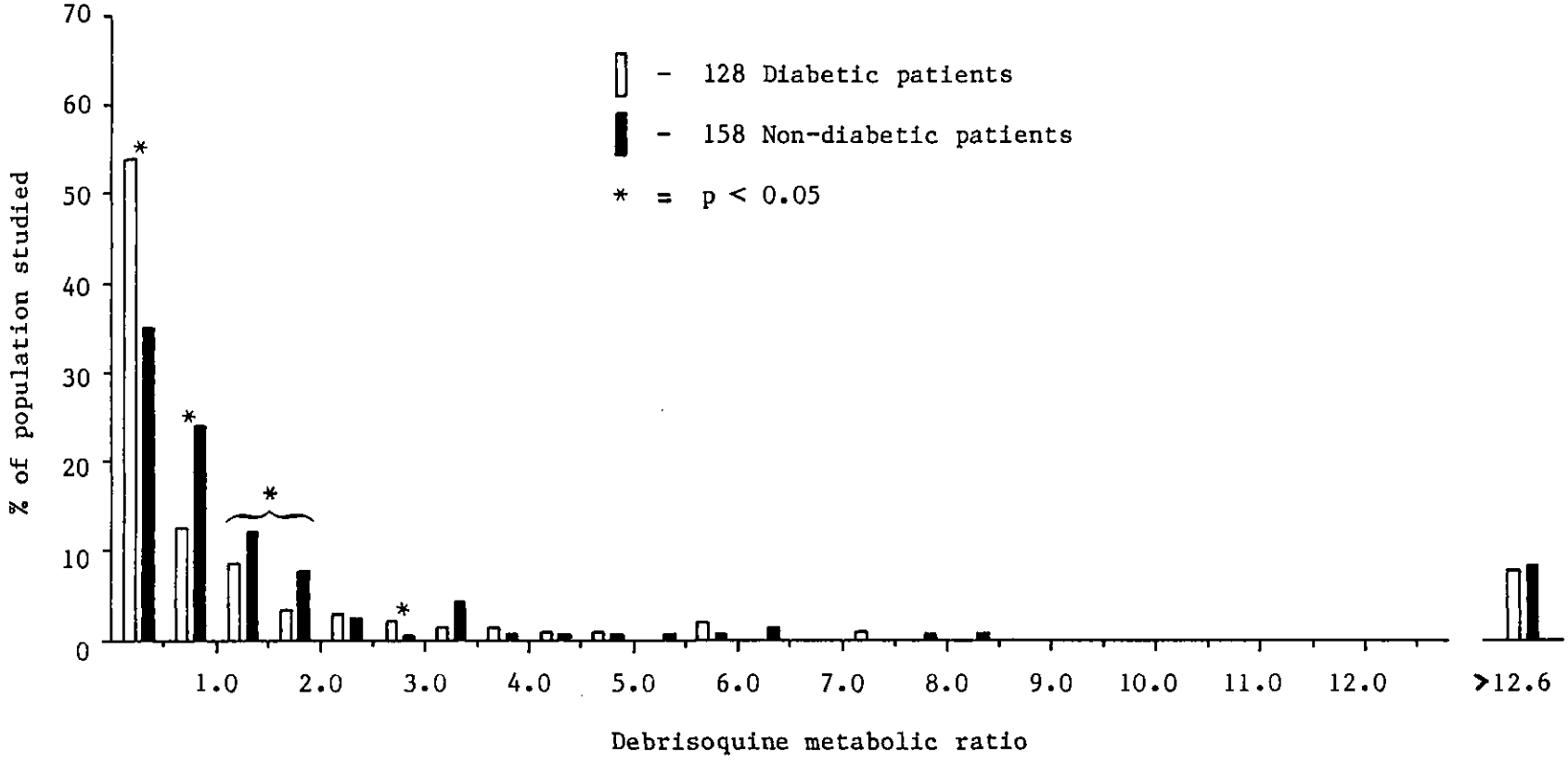
The 0-8 hour urinary recovery of debrisoquine + 4-hydroxy-debrisoquine, expressed as the percentage oral dose, ranged from 6.4 to 89,4% with a mean (± S.D.) of 33.8 (± 14.3%). The median debrisoquine metabolic ratio of diabetic patients is 0.5 in contrast to 0.8 in the 961 normal healthy population of the age range 18-50 and 0.8 in the 158 non-diabetic population with a mean (± S.D.) age of 62 years (±12, range 35-80 years - unpublished data). These 158 patients included individuals with ischaemic heart disease, hypertension, bronchitis, hernia and varicose veins and patients on anti-coagulants and patients in the geriatric wards and Senior Citizen's Homes.

The frequency distribution of the 128 diabetic patients and the 158 non-diabetic individuals is shown in Fig. 30. Table 24 shows the distribution of the 128 diabetic patients compared to the 158 non-diabetic patients in terms of their distribution within various ranges of debrisoquine metabolic ratios.

The median ratio of the diabetic population (0.5) is significantly ($p < 0.025$) lower than that of the non-diabetic population (0.8) when tested by Wilcoxon Rank Sum test. It is important to note that no significant differences are present between the two groups in the ratio range of 3.1-12.6 and also more than 12.6 (poor metabolisers).

It is also important to note that there is a significantly greater preponderance of individuals with a ratio < 0.5 and 2.6-3.0 among the diabetic patients and further, that these

Figure 30 Comparison of frequency distribution of metabolic ratio in diabetic and non-diabetic patients



individuals are drawn from the individuals with a ratio between 0.6-2.

No correlation was noted between the debrisoquine metabolic ratio and age, sex, weight, smoking habit, drug recovery or any of the concurrent drug treatment. This is consistent with previous findings where, in addition, no correlation has been shown with any of the routine biochemical liver function tests(vide infra, Section 8.5). Additionally a previous study (Foster et al, 1980) has shown that the pattern of liver disease in diabetics is similar to that in non-diabetics. This further excludes any hepatic disease as the cause of altered drug oxidation in diabetic patients.

Discussion

The results of the study lead to a number of interesting conclusions. It may be said at the outset that,in agreement with previous studies, no correlation can be detected between debrisoquine (and hence by inference, a number of other drugs including phenformin) oxidation status and age, sex, weight, smoking, concurrent drug medications or the recovery of the drug used as an oxidation probe.

There is no significant difference between the proportion of individuals with a ratio > 3 between the diabetic and non-diabetic populations of comparable age.

This confirms a previous suggestion that the incidence of phenformin-induced lactic acidosis is indeed high, approaching 15% and the lower values quoted in the literature reflect under-recognition and/or under-reporting (Cohen, 1978). This, in turn, jeopardises the validity of epidemiological data on phenformin-induced lactic acidosis and also the recommended guidelines, based on such data, on safe use of phenformin. This has been the experience in Sweden (Bergman, Boman and Wiholm, 1978) where guidelines on the safe use of phenformin were issued in 1977 but without any subsequent reduction in the frequency of lactic acidosis.

In the range of debrisoquine metabolic ratios 0.1-1.0, 1.1-2.0, 2.1-3.0, 3.1-12.6 and >12.6, again no significant differences are discernible between the diabetic and the non-diabetic groups. This suggests that the diabetic state per se is not associated with, or has any predeliction for, a particular oxidation phenotype. However, when only the individuals in the ratio range of 0.1-3.0 are sub-divided further into various sub-groups, an interesting pattern emerges (Table 24). Compared to the non-diabetics, the diabetic population contains significantly higher number in the ratio range 0.1-0.5 and 2.6-3.0 and significantly fewer in the ratio range 0.6-2.0. This suggests enhanced drug oxidising capacity in a substantial (about 27%) number of diabetics and diminished oxidising capacity in a small proportion (about 2%) of these individuals.

Table 24: Distribution of diabetic and non-diabetic patients into various ranges of metabolic ratios

Range of ratio	% of 128 Diabetics	% of 158 non-diabetics	Significance level (p*)
0.1 - 0.5	53.9	34.8	< 0.0005
0.6 - 1.0	12.5	24.1	< 0.01
0.1 - 1.0	66.4	58.9	< 0.3
1.1 - 1.5	8.6	12.0	< 0.3
1.6 - 2.0	3.9	7.6	< 0.2
1.1 - 2.0	12.5	19.6	< 0.06
2.1 - 2.5	3.1	2.6	< 0.8
2.6 - 3.0	2.4	0.6	< 0.025
2.1 - 3.0	5.5	3.2	< 0.1
3.1 - 12.6	7.8	10.1	< 0.4
> 12.6	7.8	8.2	< 0.8
Median Metabolic Ratio	0.5	0.8	**p < 0.025

* = by χ^2-test

** = by Wilcoxon Rank Sum test

This increase in drug metabolising capacity in diabetics is reflected in their significantly ($p < 0.025$) lower median debrisoquine metabolic ratio (0.5) compared to that in the non-diabetics (0.8) of comparable age. Qualitatively, this increase in drug metabolising capacity amongst certain diabetics is consistent with a previous study. Salmela, Sotaniemi and Pelkonen (1980) used antipyrine test as an *in vivo* index of drug metabolising capacity and the measurement of hepatic cytochrome P-450, determined from the biopsy samples, as the *in vitro* test. These workers showed that cytochrome P-450 levels were higher and antipyrine elimination faster in diabetics with normal liver than in those with fatty liver, parenchymal inflammatory changes or cirrhosis. They noted that overall, the diabetic population did not differ in their drug metabolising capacity and any concurrent drug treatment did not relate to drug metabolising capacity. In this particular context of overall drug metabolism, their findings are inconsistent with the findings of this study. This is probably explicable on the basis that antipyrine metabolism involves a number of metabolic pathways and hepatic contents of cytochrome P-450 do not necessarily reflect capacity to oxidise drugs (Davies *et al*, 1981). It is of interest to note that Thom *et al* (1981) noted shorter plasma half-lives of isoniazid in their eight healthy subjects studied during the post-glucose period

in comparison to the pre-glucose period, the mean (± S.E.M.) reduction being 24% (± 5.4%, $p < 0.01$). No apparent relationship between the reduction in isoniazid half-life attributable to glucose and either the subject's acetylator phenotype or the blood glucose or insulin response could be discerned.

This enhanced drug oxidation capacity in a substantial proportion of diabetics has an indirect adverse consequence on the individuals who have impaired drug oxidation capacity. It is easy to visualise that, in order to achieve desired therapeutic effects in the majority of diabetic patients, the recommended regimen for phenformin therapy will necessarily be set at a higher dose level. If, then, this recommended dose is prescribed to all the diabetics, therapeutic effects will be achieved in those appropriate patients with enhanced drug oxidation capacity (metabolic ratio < 2.0, who are not at risk in any case) but toxicity will be precipitated in those 15% with metabolic ratio > 3.0 (who are the at-risk group). Therefore, unless individual drug oxidation status is taken into consideration, recommendations based on hepatic, renal or myocardial status or concurrent drug therapy cannot be expected to lead to reduction in the frequency of phenformin-induced lactic acidosis.

The study described here emphasises a point that has been made previously in the context of drug metabolism. Unless individuals are investigated and considered singly, polymorphism could easily be overlooked. Similarly, if the diabetic and non-diabetic groups are examined in a single ratio range of 0.1-1.0, there is no statistical difference between the two populations. It is only by their further sub-division into the two ratio groups of 0.1-0.5 and 0.6-1.0 that the shift from the latter to the former group becomes evident. Also, if the whole population groups are compared by Wilcoxon Rank Sum test, the differences within a subgroup are not clearly apparent by this test, although the difference between the two populations as a whole may become evident.

7.12 Conclusions

The development of and the mechanisms of the hypoglycaemic effects of phenformin are reviewed. It appears that this therapeutic effect cannot be divorced from its propensity to raise blood lactic acid levels. Both these effects are concentration-dependent. Phenformin-induced lactic acidosis has a very high mortality and individual idiosyncrasy appears to be mainly responsible for the appearance of this complication. Available data suggest that hepatic and renal dysfunctions cannot be incriminated in the aetiology of phenformin-induced lactic acidosis. Phenformin is an effective hypoglycaemic agent and a need to its safer use in majority of the patients by identifying the at-risk minority is proposed.

It has been shown that phenformin is metabolised by oxidation to its single and exclusive metabolite, 4-hydroxyphenformin. Studies in phenotyped volunteers suggested that wide inter-individual variations in the ability to effect this oxidative clearance are present and this was confirmed in a population study; the variation being in the region of more than 90-fold. The ability or otherwise to clear phenformin by biotransformation is an individual characteristic and is genetically determined. The genetic control is exercised through a single pair of gene alleles and the mechanisms probably reside predominantly in the liver.

This results in a variable first-pass elimination of phenformin and hence, variable peak plasma concentrations of phenformin on a standard dose regimen.

It has further been shown that there is a high concurrence between aromatic hydroxylation of phenformin and alicyclic hydroxylation of debrisoquine and further, that these two reactions are controlled by a single pair of gene alleles at loci which are either identical or very closely linked. Approximately 8.9% of the population are poor metabolisers of debrisoquine while approximately 9.2% of the population are poor metabolisers of phenformin. If it turns out that these two gene loci are closely linked and not identical, only about 1.2-1.7% of the population can be expected to display discordance of the two polymorphisms. Consequently, phenformin metabolism could be accurately ascertained by studying a panel of few volunteers of known debrisoquine oxidation status, rather than involving a large population study which relies on a chance of including poor metabolisers. It may be recalled at this stage that the Committee on Problems of Drug Safety, Drug Research Board, U.S.A. in 1969 as referred to earlier, outlined some urgent needs regarding the prediction of the metabolism of another drug from the knowledge of the metabolism of one drug. The phenotyped panel approach to the study of oxidative clearance of phenformin is an example of what can be offered in response to this need. Furthermore,

it becomes possible to identify the population at risk of developing phenformin-induced lactic acidosis by prior phenotyping with a single 10 mg oral dose of debrisoquine - a dose low enough to have no haemodynamic consequences. It appears from the limited studies described that the risk of developing lactic acidosis on conventional doses of phenformin appears to commence at a debrisoquine metabolic ratio of 3 and becomes progressively greater as the value of this ratio rises in an individual.

The studies described show how phenotyped panel approach can be exploited not only to detect the likelihood of wide inter-individual variations in the ability to effect metabolic oxidation of phenformin but also to assess the drug response in individuals of widely differing ability to oxidise this drug.

The investigations on the drug metabolising capacity of non-insulin dependent diabetic patients have revealed that there is a sub-set of diabetics in whom this capacity is significantly enhanced. This probably is the effect of hyperglycaemic state *per se* and the data ruled out any phenotypic diathesis to the disease. The enhancement of drug metabolising capacity is, however, at the "wrong" end of the distribution in the sense that it is likely to result in a greater number of therapeutic failures rather than in increased frequency of drug toxicity due to phenformin. The phenotyped panel approach together with the study of debrisoquine oxidation status of patients who

are diabetic and also of those surviving lactic acidosis has allowed an insight into the probable frequency of this often fatal complication of phenformin therapy. The data again suggest that phenformin-induced lactic acidosis is greatly under-recognised and/or under-reported to drug safety evaluation bodies such as the Committee on Safety of Medicines. It is suggested that this, in turn, jeopardises the values of guidelines based on the currently available epidemiological data on phenformin-induced lactic acidosis. This has been the experience in Sweden where despite revised and stricter guidelines on phenformin usage, there has been no reduction in the frequency of phenformin-induced lactic acidosis. It is proposed, as a result, that only the titration of the phenformin dose regimen to individual index of debrisoquine (and by inference, phenformin) oxidation would lead to safer and more effective use.

C H A P T E R E I G H T

SCOPE OF DEBRISOQUINE OXIDATION POLYMORPHISM IN TOXICITY DUE TO OTHER DRUGS

8.1 Introduction

The studies in the previous chapter showed how inter-individual differences in drug oxidation within a population can be ascertained by studies on a phenotyped panel. Such studies allow elucidation of not only the differences in drug metabolic ability but also the differences in drug response. Importantly, the studies suggested that the differences are genetically determined and the control encompasses more than one drug. This raised the possibility that individuals at risk of toxicity from one drug may be identified by using another drug as the biochemical probe. Provided sufficient numbers are studied, it may become possible to assign a risk factor to each value of debrisoquine metabolic ratio in the context of a standard dose regimen. Once this is possible, it is relatively easy to tailor the dose regime to individual drug oxidation status. Alternatively, efforts and resources can be diverted to maintain a more effective surveillance for drug toxicity in particular individuals who are most at risk.

To assess the value of debrisoquine hydroxylation phenotype in the prevention of overt clinical toxicity, it was decided to investigate another drug which is not structurally related to either debrisoquine or phenformin but does undergo metabolic elimination by oxidation.

Apart from diabetes, hypertension and malignancies, ischaemic heart disease ranks amongst the most widely prevalent chronic killer diseases and the data currently available demonstrate the effectiveness of various calcium antagonists in the treatment of angina and arrhythmias. Currently, five calcium antagonists are approved for general use in the U.K. These are prenylamine, perhexiline, nifedipine, verapamil and lidoflazine. Of these, perhexiline maleate was found to be very effective with good patient tolerance but unfortunately, this drug is also the one which has been reported to cause various side-effects and is gradually going out of clinical favour. It was, therefore, decided to investigate the toxicological propensity of perhexiline in the context of debrisoquine oxidation phenotype.

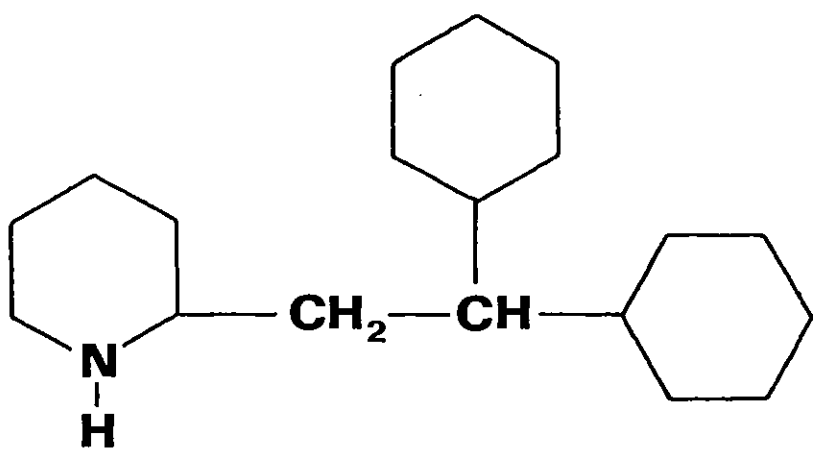
8.2 Pharmacology of perhexiline

Chemically, perhexiline is 2-(2',2'-dicyclohexyl)-ethylpiperdine (Fig. 31). It was first introduced in 1973 in France and was subsequently approved as an anti-anginal agent in the U.K. in 1975.

The exact mode of the action of perhexiline is not fully understood. It has been shown to have calcium antagonistic properties (Fleckenstein-Grun et al, 1978) and probably exerts some biochemical effects intracellularly to reduce the peak demand for oxygen (Pepine, Schang and Bemiller, 1973).

Electrophysiologically, the molecular mechanism of the action consists of a decrease of trans-membrane influx of calcium into the excited myocardial fibres. This decreases the calcium-dependent myofibrillar ATP-ase transformation of phosphate-bound energy into mechanical work. Within the therapeutic range of plasma levels, the bioelectric parameters such as resting membrane potential, upstroke velocity, over-shoot and duration of action potential are not significantly affected (Fleckenstein-Grun et al, 1978). The main effects are to depress the rate of sinus node discharge and the conduction velocity through the AV node. Ten Eick and Singer (1973) have shown that in canine heart, perhexiline reduces the automaticity of latent pacemakers. It also reduces the conduction of impulses in specialised fibres in the ventricles, though not in the atria.

Figure 31 Structure of perhexiline



Haemodynamically, perhexiline has a very weak negative chronotropic and mild vasodilatory effect (Stone et al, 1980). In terms of negative inotropic and negative dromotropic actions, it is even weaker. Its potency in these last two respects is only one hundredth when compared to nifedipine. Although the haemodynamic effects of the calcium channel blocking agents result from a complex interplay of direct and subsequent reflex effects, perhexiline does augment oxygen supply and lowers oxygen demand by reducing myocardial after-load. Pepine, Schang and Bemiller (1973) showed that perhexiline reduced exercise heart rate from (mean $\pm$ S.D.) 113 $\pm$ 5.7 to 104 $\pm$ 3.9 beats/minute, increased oxygen extraction from 60.3 $\pm$ 2.3% to 70.4 $\pm$ 2.7% and the lactate extraction changed from a mean of -4.5% to +6.5%.

Various clinical trials (Armstrong, 1973; Dettori et al, 1973; Cawein et al, 1973) have confirmed the anti-anginal effects of perhexiline in chronic angina pectoris. It reduces exercise-induced tachycardia and improves exercise tolerance without any significant effect on resting blood pressure or pulse (Morledge et al, 1978). The combined results of seven double-blind investigations, including between them a total of more than 300 patients with classic angina, showed that perhexiline produced a dose-related reduction in the frequency of anginal attacks and in the number of glyceryl trinitrate tablets consumed and an increase in the exercise tolerance (Stone et al, 1980).

In a study by Schimert (1978), the weekly consumption of glyceryl trinitrate decreased by 60% when compared to placebo. In that study (involving 19 patients), the weekly consumption of glyceryl trinitrate was (mean ± S.D.) 22.3 ± 9.2 before the treatment, 9.06 ± 7.4 during perhexiline treatment and 21.04 ± 8.7 whilst receiving placebo. The benefit was also reflected in the improvement of the electrocardiographic changes of ischaemia (Datey et al, 1973). The work done during the stress test increased by 31% from the control test after chronic perhexiline treatment at a dose of 100 mg bd (Reiterer, 1978) and by 77% with 200 mg bd (Morgans and Rees, 1973).

Calcium antagonists generally have been found to be as effective as long-acting nitrates and beta-blockers in the drug treatment of angina pectoris. However, perhexiline has been found to be more effective than propranolol and practolol (Garson, Gulin and Phear, 1973) and prenylamine (Cherchi et al, 1973). It often proves beneficial when beta-blockers have failed. In combination with beta-blockers, perhexiline has synergistic effects. The advantages of perhexiline over beta-blockers are particularly appreciated in cases where beta-blockers are fraught with hazards, as for example in cases of left ventricular dysfunction or chronic obstructive airways disease (Sasahara et al, 1978). Compared to other calcium antagonists, perhexiline has the advantage of being safe in combination with beta-blockers (Stone et al, 1980)

and also has anti-arrhythmic effects (vide infra). In contrast to nifedipine, perhexiline reduces peak pulse rate whilst unaltering peak arterial blood pressure during exercise (Stone et al, 1980). Various workers have demonstrated that perhexiline is also superior to prenylamine in reducing the workload of the heart, electrocardiographic changes of ischaemia and anginal episodes and increasing effort tolerance (Cherchi et al, 1973; Libretti et al, 1973).

In a few preliminary studies, perhexiline has been found to have anti-arrhythmic properties. Sukerman (1973) studied the anti-arrhythmic effect of perhexiline in 12 patients. He found a mean (± S.D.) reduction in the frequency of ventricular ectopics of 61 (± 42)% by 400 mg perhexiline daily. Bouvrain et al (1978) also studied 12 patients and noted a reduction in the frequency of ventricular e ctopics by 64% during treatment with perhexiline 150-400 mg daily. Pickering and Goulding (1978) showed that the drug was effective not only during rest but also during exercise. These workers, interestingly, noted large inter-individual variation in the anti-arrhythmic response. Further studies are of course required to confirm these as well as the role of perhexiline in the treatment of coronary artery spasm and hypertension.

Clearly it is difficult to overlook the important place of perhexiline in the management of ischaemic heart disease and some of its complications. The drug is sufficiently effective as to be recommended for use when other drug treatments have failed, despite the side-effects that accompany its usage. These side-effects are discussed next.

8.3 Human toxicology of perhexiline

The effectiveness of perhexiline resulted in its wide use initially. Earlier clinical studies have shown that the use of perhexiline is associated with adverse drug reactions. The commonest effect is mild elevation of serum enzymes (AST, ALT, alkaline phosphatase and LDH) as a consequence of probable mild hepatic damage (Newberne, 1973). These elevations are usually reversible after discontinuation of therapy. Often, they reverted to normal even on continued therapy. Dose-dependent diuresis, naturesis and chlorouresis have been observed, associated with a slight weight loss and decrease in creatinine clearance (Czerwinski et al, 1973). This latter probably reflects alteration in renal haemodynamics. Perhexiline has also been shown to accentuate the delayed and increased insulin secretion after a glucose load in diabetic as well as non-diabetic patients (Lucciani et al, 1978). A number of the patients complain of nausea and vomiting (Hoekenga et al, 1973; Garson, Gulin and Phear, 1973). Ataxia (Hoekenga et al, 1973) and diplopia (Ikram et al, 1973) have also been reported. Other minor effects consist of lethargy, insomnia, tremor and loss of libido (Pilcher et al, 1973; Gitlin and Nellen, 1973; Gitlin, 1973; Datey et al, 1973). These side-effects are, however, not too troublesome and in view of the beneficial anti-anginal effects, are easily tolerated by patients if they do not improve or disappear even on continued therapy (Pilcher et al, 1973; Gitlin and Nellen, 1973).

The major side-effects of perhexiline therapy consist of polyradiculitis, peripheral neuropathy (Abaza et al, 1973; Laplane et al, 1978), severe hepatic damage (Newberne, 1973; Lewis et al, 1979)including cirrhosis (Pessayre et al, 1979) in some cases, hypoglycaemia (Houdent, Wolf and Corriat, 1977), proximal myopathy (Tomlinson and Rosenthal, 1977) and papilloedema (Hutchinson, Williams and Cawler, 1978; Atkinson, McAreavey and Trope, 1980).

Whilst most of these effects are reversed on discontinuation of the drug, the return of the neurological and/or hepatic function to normal may, in some cases, be slow or incomplete.

Neuropathy associated with perhexiline was first reported by Abaza et al in 1973 and since then, many more cases have been reported, leading to a considerable fall in the use of perhexiline. By June 1982, the Committee on Safety of Medicines had received 104 reports from U.K. (Personal Communication). It is estimated that perhexiline-induced neuropathy with overt symptoms occurs in 0.1% of patients, while subclinical neuropathy in as many as 65% of such patients (Bates, 1981).

Laplane et al (1978) reviewed 35 cases of overt neuropathy. The median dose used was 300 mg daily (range 150-600 mg daily) and the onset of neuropathy was about 13 months after the start of treatment, with a minimum of 3 weeks and a maximum of 3 years. Interestingly, the patient who developed the neuropathy soonest was receiving the same daily dose as the one who developed it last.

The onset of neuropathy is characterised by paraesthesia of the extremities and by reduced strength, usually by both. Hands and feet were primarily affected with paraesthesia. Muscular strength was decreased in 75% of the cases. Proximal weakness was present in 63% and the tendon reflexes were abolished in legs (54%), arms (6%) or all the four limbs (29%). Objective signs of sensory loss were nearly always present. The electromyogram (EMG), as a rule, revealed a neurological process. Of the 35 patients, 4 did not have an EMG examination, whilst in another 4 cases, the EMG was normal. When abnormalities were present, they showed that conduction velocity was decreased in 26 of the remaining 27 cases. Distal latency was increased in the 14 of the 21 cases where it was measured. Nerve biopsy was performed in 14 patients and revealed demyelination in 12. In another 2 cases, no electron microscopy was performed and the nerve biopsy was considered normal on ordinary light microscopy. Muscle biopsy in 12 cases out of 16 showed evidence consistent with denervation. Electron microscopy revealed a decrease in the number of large myelinated fibres and the presence of polymorphous inclusion bodies in the Schwann cells, with associated segmental demyelination. Wallerian degeneration was observed less constantly. Liver function tests were normal in 8 of the 35 cases. These workers noted complete resolution of neuropathy after a variable follow up period of up to 18 months but the presentation of this data is

difficult to interpret since it is not indicated how many patients were lost during follow up.

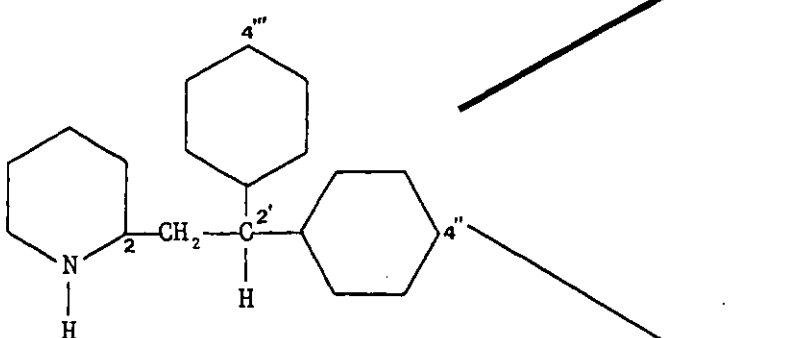
The reason why some patients develop serious reactions like neuropathy to perhexiline is not clear, although some evidence suggests that it may be the result of impaired oxidative metabolism of the drug in some individuals (Singlas, Goujet and Simon, 1978). L'Hermitte et al (1976) have postulated that perhexiline-induced neuropathy may represent an individual susceptibility, perhaps secondary to some latent inborn metabolic disorder. The metabolism of perhexiline is therefore discussed next.

8.4 The metabolism of perhexiline

The pharmacokinetics of perhexiline have not been studied extensively. The best study that is available is that of Wright et al (1973). The absorption of the drug appears to be rapid and almost complete after an oral dose within 6-12 hours. Maximum blood levels are obtained in about one hour and following a single dose, detectable levels are present for about 7 hours (Stone et al, 1980). It has a very large volume of distribution and it is about 90% bound to plasma proteins. Perhexiline is a lipophilic drug and its elimination depends on its metabolic oxidation to the more polar mono- and di-hydroxylated metabolites prior to their elimination (Fig. 32). Wright et al (1973) noted the plasma half-life of the drug to vary from 3-12 days between individuals. Consequently, this lipophilic drug accumulates unchanged after repeat doses. These pharmacokinetic features of perhexiline are summarised in Table 25.

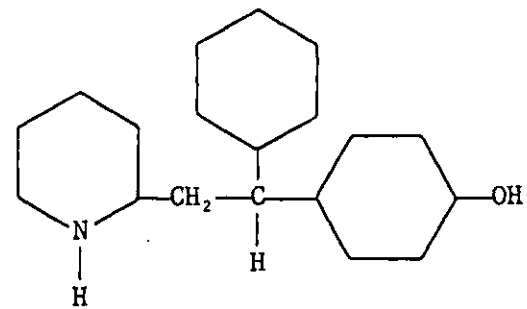
Large inter-individual variations in the plasma half-life of perhexiline have been observed and these have been interpreted as being due to inter-individual differences in the rate of metabolism of the drug. Subsequently, Singlas, Goujet and Simon (1978) compared the pharmacokinetics of perhexiline in anginal patients with (n=13) and without (n=14) the signs of peripheral neuropathy. Their findings are summarised in Table 26. Two of the 13 patients with

Figure 32 Metabolism of perhexiline by man



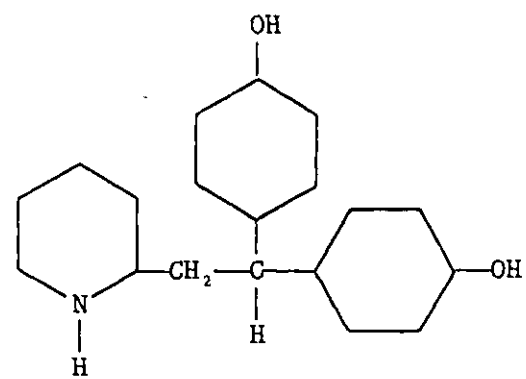
Perhexiline

2-(2',2'-dicyclohexyl)ethylpiperidine



4-Monohydroxy-perhexiline

2-[2'-cyclohexyl-2'-(4''-hydroxycyclohexyl)-ethyl]-piperidine



4,4'-Dihydroxy-perhexiline

2-[2',2'-bis(4''-hydroxycyclohexyl)-ethyl]piperidine

Table 25: *Pharmacokinetic features of perhexiline*

Highly lipophilic. Metabolised by oxidation. Optically active.	
Absorption	Virtually 100% within 6-12 hours.
Peak levels	In about 1 hour following single dose.
Detectable	Up to 7 hours following single dose.
Volume of distribution	Extremely large.*
Protein binding	90%.
Plasma elimination half-life	3 - 12 days.
Body-load following chronic dosing	About 50% as unchanged perhexiline.

* No firm estimate available

neuropathy had normal liver function tests, while 26 patients, except one in the neuropathy group, had normal renal function. The mean (± S.D.) daily dose of perhexiline was 304 (± 83) mg in the neuropathy group and 232 (± 87) mg in the non-neuropathy group. Allowing for weight loss in the toxic group, there was no significant difference in the dose regime between the two groups at the beginning of treatment. The onset of neuropathy was independent of the duration of treatment. These workers demonstrated that patients with neuropathy had higher plasma perhexiline levels and longer plasma half-lives (9-22 days) than patients without peripheral neuropathy. The reported plasma half-life for most normal volunteers and patients of these workers was 2-6 days. Furthermore, the mean ratio of plasma levels of parent drug to its major metabolite, a mono-hydroxy perhexiline (termed M_1), was some nine-times higher in the neuropathic patients than in the controls. Thus, patients with neuropathy had significantly lower levels of mono-hydroxy perhexiline (M_1), suggesting their impaired ability to transform perhexiline to its M_1 mono-hydroxylated metabolite. It was concluded that this toxic effect of perhexiline is associated with accumulation of the parent drug due to different volume of distribution or slow hepatic metabolism of the drug in the affected individuals. The metabolic differences, in turn, were attributed to either the genetic factors or hepatic damage that may be associated with perhexiline therapy.

Table 26: Perhexiline pharmacokinetics in patients with and without neuropathy

	Neuropathy group	Non-neuropathy group
n	13	14
Dose at start of treatment (mg/kg)	4.6 ± 0.3	3.8 ± 0.4
Dose at the end of treatment (mg/kg)	5.6 ± 0.4	3.8 ± 0.4
Mean duration of treatment (months) (range)	20 (4 - 36)	12.4 (3 - 24)
Abnormal liver function tests in:	11	None
Abnormal creatinine clearance in:	1	None
Onset of neuropathy (months)	4 - 36 independent of duration of treatment	-
Plasma perhexiline mg/L	3.78 ± 0.43	1.07 ± 0.18
Plasma M_1 metabolite of perhexiline mg/L	1.62 ± 0.23	3.83 ± 0.47
Plasma ratio of levels of perhexiline/M_1	2.82 ± 0.4	0.30 ± 0.04
Plasma perhexiline half-life (days)	9 - 22	(Reported as 2 - 6 for most individuals)

(After Singlas, Goujet and Simon, 1978)

In view of the known implications of the genetic control of oxidative polymorphism, encompassing a wide variety of drugs, it was thought that perhexiline-neuropathy could represent the toxic sequelae of impaired drug oxidation status. It was, therefore, decided to investigate the debrisoquine oxidation status of patients who had developed perhexiline-neuropathy and compare them to a population of patients who had taken perhexiline without developing this complication and also to a population of patients who had ischaemic heart disease but had never received perhexiline.

8.5 Debrisoquine oxidation status of patients with perhexiline-neuropathy

Introduction

Perhexiline-neuropathy appears to be a sequelae of individual susceptibility. It is associated with accumulating levels of perhexiline in the affected individuals and this accumulation results from the inability of these individuals to hydroxylate the drug to facilitate its renal elimination. It is thought that this inability may have genetic basis with a latent inborn susceptibility, secondary to a metabolic disorder (L'Hermitte *et al*, 1976).

Wide inter-individual variation in the ability to hydroxylate perhexiline has been reported (Wright *et al*, 1973) and individuals with perhexiline-induced neuropathy have a longer plasma half-life of perhexiline, compared to their non-afflicted counterparts (Singlas, Goujet and Simon, 1978).

Studies with genetic polymorphism in drug oxidation have revealed that it is possible to predict the individual oxidation capacity with regard to a number of drugs from the hydroxylation of debrisoquine. Although the work on *in vivo* evidence regarding concurrence of debrisoquine and perhexiline oxidations is in progress, there is strong *in vitro* evidence to confirm this co-inheritance (Kupfer *et al*, 1982).

The working hypothesis was that perhexiline hydroxylation is controlled by the same pair of gene alleles as that which controls debrisoquine hydroxylation. With this hypothesis in mind, it was decided to investigate the debrisoquine oxidation status of individuals who had developed perhexiline-neuropathy and compare them to ischaemic heart disease patients who did not develop the neuropathy on perhexiline treatment and also to those who were "perhexiline-naive".

Methods

The study was approved by the Ethical Committee of St. Mary's Hospital and the Physicians in charge of the patients. Each participating patient gave his/her fully informed consent.

Seventy-two patients were included in the study. Of these, 38 had never received perhexiline while of the remainder who had received perhexiline, 20 had developed perhexiline-neuropathy, confirmed by nerve conduction studies in 18 and by definite clinical evidence in the other two. Fourteen patients who received perhexiline without the neuropathy served as controls.

The patients with perhexiline-neuropathy were identified from various cardiac outpatients, through the courtesy of the Committee on Safety of Medicines, Merrell Pharmaceuticals Ltd (the makers of perhexiline in the U.K.), literature search and personal approaches.

These 72 patients were phenotyped for their debrisoquine oxidation status according to Mahgoub et al (1977) as described previously.

A number of these patients were on other drugs and a drug free period extending from 10 hours before to 3 hours after swallowing debrisoquine was allowed and alcohol was not permitted during and 10 hours preceding the test.

In order to assess their hepatic function, routine biochemical liver function tests were carried out on all the patients included in this study. Most of these tests were performed within one week of the debrisoquine phenotyping test.

Results

The details of the 34 patients and their approximate weekly intake of perhexiline, together with the results of the liver function tests and the debrisoquine metabolic ratios are shown in Table 27. The drug therapy of these patients at the time of phenotyping are shown in Table 28.

The 20 patients who developed neuropathy included 18 males and 2 females, while 10 males and 4 females formed the 14 control patients who did not develop neuropathy on perhexiline. There are no significant differences between these two groups with regard to their age, weight, alcohol consumption or perhexiline dosage. Ten of the 20 patients with neuropathy and 2 of the 14 non-neuropathy patients consumed tobacco in one of its various forms. In the 0-8 hour period, the mean ($\pm$ S.D.) urinary recovery of

Table 27 Details and results of the two groups of 34 patients who received long-term perhexiline

Details						§ Liver function tests						
Patient	Age(years)/Sex	Weight (kg)	Daily smoking*	Alcohol consumption*	Approximate weekly consumption of perhexiline (g/week)	Bilirubin µmol/l (5-17)	AST units/l (7-40)	Alk. phosphatase units/l (30-115)	Albumin g/l (35-51)	Globulin g/l (25-45)	ALT units/l (7-40)	Debrisoquine Metabolic ratio
NEUROPATHIC PATIENTS												
HP	57/F	70	0	0	3.0	5	-	78	35	31	43	0.5
AG	52/M	75	10c	++	2.8	7	-	86	40	32	18	0.8
ASs	62/M	54	1cg	0	1.3	6	-	72	39	33	12	0.9
EJ	58/M	57	1cg	+	2.2	8	-	113	41	26	18	1.6
PE	68/M	80	8c	++	2.2	11	31	69	43	31	-	2.6
TB	54/M	65	0	+	2.1	10	67	234	24	43	-	2.6
RPn	62/M	72	0	+	2.1	<17	55	25	40	25	-	4.5
BG	55/M	76	40c	0	2.1	<17	<40	<115	>35	<45	-	5.7
RH	68/M	73	Pipe	+	2.1	12	23	54	40	32	-	8.5
WL	69/M	89	Pipe	0	2.4	8	45	9	38	31	62	10
GA	60/M	76	0	0	2.0	8	46	112	45	27	-	19
DW	73/F	45	0	0	1.4	4	106	67	22	28	-	25
RBn	60/M	79	0	+	2.3	8	123	10	40	32	176	27
RS	56/M	86	0	+	2.0	14	18	160	51	20	-	28
JC	66/M	77	Pipe	+	1.4	<17	34	<115	>35	<45	61	30
RC	63/M	68	0	+	1.4	6	-	85	29	31	16	32
KA	68/M	62	0	+	1.8	11	152	110	36	34	-	35
GM	68/M	65	6c	+	1.4	8	15	50	>35	<45	-	69
NT	60/M	76	0	+	1.4	<17	<40	<115	>35	<45	-	77
RBr	67/M	76	5c	+	2.3	9	25	94	47	25	-	>100
Mean	62.3	71.1			1.99	10.2	54.7	88.8	38	33.1		
±SD	±5.8	±10.7			±0.47	±4.2	±40.8	±51.5	±6.1	±7.6		
NON-NEUROPATHIC PATIENTS												
WT	66/M	70	0	++	3.1	4	33	110	43	29	-	0.4
JO	57/M	78	0	+	3.8	<17	23	23	29	34	-	0.4
HW	76/F	57	0	0	1.4	12	17	92	42	29	11	0.5
RM	55/M	69	Pipe	+	1.5	<17	<40	<115	>35	<45	-	0.5
KB	62/M	58	0	0	2.1	9	64	87	39	29	-	0.6
MCy	50/M	91	0	+	2.1	6	36	57	44	28	-	0.6
BH	67/M	75	6c	+	2.1	11	23	79	40	24	22	0.6
RPk	58/M	65	0	+	3.7	3	15	191	44	23	-	0.7
MCn	71/M	74	0	+	2.1	9	23	85	43	32	-	1.0
VB	71/F	53	0	+	2.1	14	28	92	41	27	-	1.1
AC	74/M	71	0	0	2.8	12	21	110	42	32	-	1.1
ASr	71/F	57	0	0	1.4	7	-	74	38	30	21	1.6
AL	60/M	52	0	0	3.8	<10	16	25	41	27	-	1.7
AK	73/F	67	C	++	2.1	8	112	93	40	26	-	4.6
Mean	65.1	66.9			2.44	9.9	34.7	88.1	40.1	29.6		
±SD	±8.1	±10.9			±0.86	±4.3	±26.7	±41	±4.0	±5.4		

* c = cigarette cg = cigar 0 = none

\+ = only occasionally ++ = mild to moderate

\- = Not determined § = normal range in brackets

Table 28 Details of concurrent drug therapy of 34 perhexiline-treated patients at the time of phenotyping

Patient (metabolic ratio)		Drug therapy
NEUROPATHIC PATIENTS		
HP	(0.5)	Frusemide, Nitrates, Potassium supplements
AG	(0.8)	Propranolol, Nifedipine
ASs	(0.9)	Propranolol, Nifedipine, Digoxin
EJ	(1.6)	Metoprolol, Frusemide, Potassium supplements
PE	(2.6)	Nitrates
TB	(2.6)	Warfarin, Salbutamol, Thiazide, Spironolactone
RPn	(4.5)	Nifedipine
BG	(5.7)	Atenolol, Nifedipine, Nitrates, Warfarin, Dipyridamole
RH	(8.5)	Propranolol
WL	(10)	Oxprenolol, Thiazide, Potassium supplements
GA	(19)	Atenolol, Perhexiline, Nitrates
DW	(25)	Propranolol
RBn	(27)	Acebutolol, Thiazide, Potassium supplements
ES	(28)	(None)
JC	(30)	Oxprenolol, Metformin, Chlorpropamide
RC	(32)	Metoprolol, Thiazide, Amiloride
KA	(35)	Oxprenolol, Perhexiline
GM	(69)	Metoprolol
NT	(77)	Propranolol, Nifedipine, Thiazide, Amiloride
RBr	(>100)	Dipyridamole, Theophylline, Chlordiazepoxide, Cinnarizine
NON-NEUROPATHIC PATIENTS		
WT	(0.4)	(None)
JO	(0.4)	Nitates, Frusemide, Spironolactone
HW	(0.5)	Atenolol, Nifedipine, Frusemide, Potassium supplements
RM	(0.5)	Aspirin, Allopurinol, Dipyridamole
KB	(0.6)	Digoxin, Perhexiline, Nitrates, Thiazide, Potassium supplements
MCy	(0.6)	Metoprolol, Nitrates, Triamterene
BH	(0.6)	Nitrates
RPk	(0.7)	Bumetanide, Potassium supplements, Phenytoin, Phenobarbitone, Salbutamol, Naproxen
MCn	(1.0)	Propranolol, Thiazide, Potassium supplements
VB	(1.1)	Propranolol, Nitrates, Thiazide, Potassium supplements
AC	(1.1)	Propranolol, Nifedipine, Allopurinol
ASr	(1.6)	Triamterene, Oxazepam
AL	(1.7)	Perhexiline
AK	(4.6)	Metoprolol, Perhexiline, Nitrates

debrisoquine plus 4-hydroxy-debrisoquine (expressed as the percentage of oral dose of debrisoquine) were 29.8 ± 13.1% (range 14-67%) in the neuropathic group and 26.1 ± 10.7% (range 12-53%) in the non-neuropathic group. These 34 patients fall into two fairly defined groups with respect to their debrisoquine oxidation status and the development of neuropathy.

The patients with neuropathy had debrisoquine metabolic ratio in the range 0.5 - >100, with a median value of 14.4, while in those without neuropathy, the corresponding values were 0.4-4.6 and 0.65. The distribution of the debrisoquine metabolic ratios for the two groups are graphically shown in Fig. 33. The median metabolic ratio of the neuropathic group is significantly greater than that of the non-neuropathic group as determined by the Wilcoxon Rank Sum test. It is important to note that there is a considerably higher proportion of patients (50%) with a ratio > 12.6 and smaller proportion of patients (15%) with a ratio <1 in the neuropathy group. This excess of individuals with high metabolic ratios is significantly greater (x^2 = 37.9, $p < 0.0001$) in the neuropathy group than would be expected in normal healthy population or in a random sample of patients with ischaemic heart disease *per se* (*vide infra*). Similarly, there were significantly fewer (x^2 = 7.6, $p < 0.025$) individuals with metabolic ratio <1 in the neuropathic group (Table 29).

Figure 33 Metabolic ratios of perhexiline-treated patients with and without neuropathy

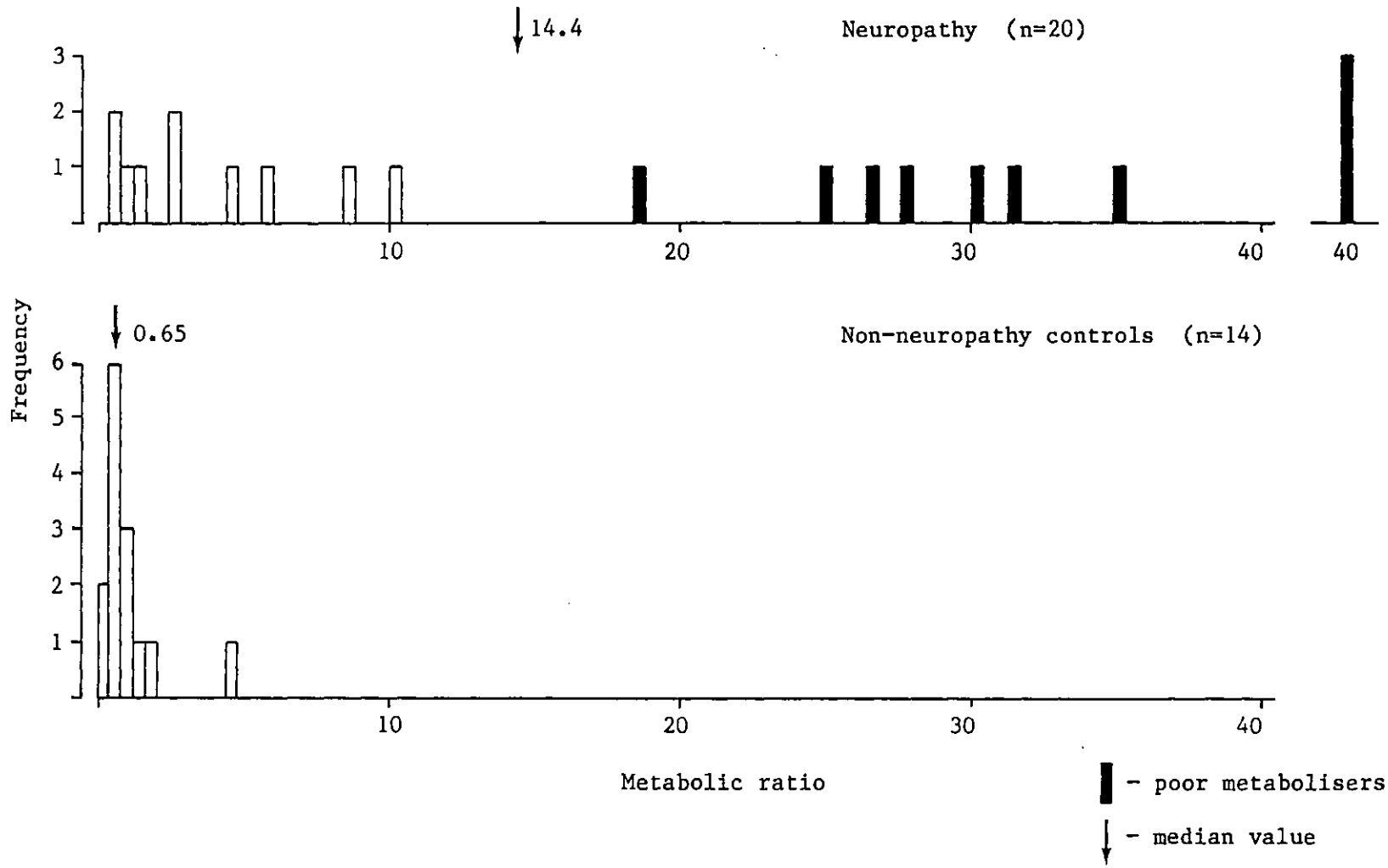


Table 30 shows the details of the 38 patients (35 males + 3 females) with ischaemic heart disease who were perhexiline-naive and the result of the liver function tests and the debrisoquine metabolic ratios. The details of their drug therapy at the time of phenotyping test are shown in Table 31. This group of patients did not differ with respect to their age, weight, alcohol or tobacco consumption and their liver function tests from either of the two perhexiline-treated groups. The mean (± S.D.) 0-8 hour urinary recovery of debrisoquine plus 4-hydroxy-debrisoquine expressed as a percentage of the oral dose in these 38 patients was 33.9 ± 9.3% (range 11-62%), which is comparable to the perhexiline -treated group. These patients had debrisoquine metabolic ratios in the range of 0.1-39 with a median value of 0.55. The distribution of their metabolic ratios in the ranges < 1 (66%), 1.1-12.6 (26%) and > 12.6 (8%) is virtually identical to that expected in normal random population, i.e. 55%, 36% and 9% respectively. These data on the distribution of metabolic ratios of normal population, perhexiline-naive patients and patients on perhexiline are summarised in Table 29.

Discussion

It has been suggested that the metabolic impairment seen in the neuropathic patients may be the consequence of hepatic dysfunction produced by perhexiline therapy. All except

Table 29: Comparison of the two perhexiline-treated groups of patients with perhexiline-naive anginal patients and normal healthy population

Metabolic Ratio			< 1.0	1.1 - 12.6	> 12.6	Median
Normal Population n = 258			55%	36%	9%	0.8
Ischaemic Heart Disease	NOT treated with perhexiline n = 38		66% *p = N.S.	26% *p = N.S.	8% *p = N.S.	0.55 N.S. ***
	Treated with Perhexiline	Developed Neuropathy n = 20	15% *p <0.025	35% *p = N.S.	50% *p <0.0001	14.4 p<0.01 **
		Did not develop Neuropathy n = 14	64% *p = N.S	36% *p = N.S.	0% *p = N.S.	0.65

* χ^2 - test comparing test subgroup with normal population subgroup
** Wilcoxon Rank Sum test comparing neuropathy with non-neuropathy groups
*** Wilcoxon Rank Sum test comparing ischaemic heart disease group with normal population

N.S. = Not Significant

Table 30 Details and results of 38 patients who were never prescribed perhexiline

Patient	Details				§ Liver Function Tests					
	Age(years)/Sex	Weight (kg)	Daily smoking*	Alcohol consumption*	Bilirubin µmol/l (5-17)	AST units/l (7-40)	Alk. phosphatase units/l (30-115)	Albumin g/l (35-51)	Globulin g/l (25-45)	Debrisoquine Metabolic ratio
FT	68/M	67	0	+	15	105	74	45	28	0.1
GF	58/M	107	0	+	7	22	42	44	23	0.1
BB	74/M	71	0	++	9	26	140	42	27	0.1
GT	70/M	65	0	0	8	19	102	45	29	0.2
KG	59/M	89	0	++	8	77	297	41	27	0.2
JH	59/M	92	5c	+	5	20	100	43	25	0.2
WB	53/M	76	2cg	++	12	27	221	42	37	0.2
JG	58/M	69	0	+	7	20	58	41	23	0.2
PC	39/M	83	0	++	7	36	101	48	26	0.2
DH	54/M	61	0	+	7	26	110	42	29	0.3
JN	60/M	68	0	+	14	36	78	43	23	0.3
WS	71/M	60	7c	+	8	29	98	41	24	0.3
EM	48/M	80	0	0	7	29	108	45	23	0.3
EH	68/F	47	0	+	7	20	112	46	30	0.4
FB	50/M	86	0	+	9	32	99	49	24	0.4
MD	51/M	54	6c	0	4	23	98	40	20	0.5
OI	77/M	65	0	0	5	25	111	39	26	0.5
JV	38/M	76	0	+	4	26	57	44	32	0.5
EH	71/M	81	10c	+	8	19	92	41	28	0.5
AM	32/M	69	0	+	9	25	83	48	18	0.6
JM	45/M	73	0	+	7	22	95	44	24	0.6
PB	41/M	86	15c	0	4	23	140	45	29	0.6
VV	46/M	65	0	0	15	32	95	48	34	0.7
MH	53/M	69	1cg	+	9	32	56	43	23	1.0
HR	79/M	52	0	+	6	22	151	46	31	1.0
DM	51/M	83	3c	0	3	32	104	44	27	1.1
WW	66/M	67	0	++	7	21	82	43	18	1.4
JD	55/M	82	0	+	11	45	101	45	27	1.7
JL	69/M	87	14c	+	5	28	139	44	29	1.9
AL	70/M	69	0	0	7	33	114	43	25	2.3
GM	67/M	57	15c	+	5	21	126	41	33	2.6
SG	47/M	83	0	+	3	15	76	46	26	2.8
MS	62/M	73	15c	++	6	19	89	45	29	4.0
KM	52/F	71	10c	+	5	22	78	44	26	4.5
LS	70/F	55	0	+	3	21	130	44	26	5.6
RB	62/M	74	5c	+	7	38	65	44	28	15
KL	59/M	67	0	+	13	27	97	41	24	26
JW	59/M	80	0	+	5	19	74	40	23	39
Mean ±SD	58.2 ±11.5	72.6 ±12.3			7.4 ±3.2	29.3 ±16.4	102 ±47.7	43.7 ±2.4	26.4 ±4.0	

* c = cigarette cg = cigar 0 = none

\+ = only occasionally ++ = mild to moderate

\- = Not determined § = normal range in brackets

Table 31 Details of concurrent drug therapy of the 38 patients who had never received perhexiline

Patient (metabolic ratio)		Drug therapy
FT	(0.1)	Nitrates, Thiazide, Frusemide, Potassium supplements
GF	(0.1)	Thyroxine, Dipyridamole
BE	(0.1)	(None)
GT	(0.2)	Nitrates, Thiazide, Potassium supplements
KG	(0.2)	Digoxin, Metoprolol, Warfarin
JH	(0.2)	(None)
WB	(0.2)	Digoxin, Frusemide, Spironolactone, Tetracycline
JG	(0.2)	(None)
PC	(0.2)	(None)
DH	(0.3)	Digoxin, Labetalol, Thiazide, Amiloride
JN	(0.3)	Digoxin
WS	(0.3)	Propranolol, Thiazide, Potassium supplements
EM	(0.3)	Metoprolol, Nitrates, Warfarin
EM	(0.4)	Metoprolol
FB	(0.4)	Digoxin, Frusemide, Spironolactone, Prazosin
MD	(0.5)	(None)
OI	(0.5)	Digoxin, Metoprolol, Nitrates, Frusemide, Metformin, Chlorpropamide, Potassium
JV	(0.5)	Propranolol
EH	(0.5)	Metoprolol, Nitrates
AM	(0.6)	Propranolol
JM	(0.6)	Warfarin
PB	(0.6)	Propranolol
VV	(0.7)	Digoxin, Nitrates, Bumetanide, Potassium supplements, Clofibrate
MH	(1.0)	Nitrates
HR	(1.0)	Digoxin, Metoprolol, Nitrates, Bumetanide, Potassium supplements
DM	(1.1)	Digoxin, Propranolol, Nitrates, Thiazide, Amiloride, Warfarin
WW	(1.4)	Metoprolol, Nitrates, Amiloride
JD	(1.7)	(None)
JL	(1.9)	Nifedipine, Thiazide
AL	(2.3)	Digoxin, Nitrates, Thiazide, Frusemide, Amiloride, Potssium supplements
GM	(2.6)	Digoxin, Nitrates, Thiazide
SG	(2.8)	Propranolol, Nitrates, Frusemide, Dipyridamole, Potassium supplements
MS	(4.0)	(None)
KM	(4.5)	Labetalol
LS	(5.6)	Propranolol
RB	(15)	Atenolol, Nitrates, Chlorthalidone
KL	(26)	(None)
JW	(39)	Propranolol, Prenylamine, Thioridazine

four of the perhexiline-treated patients had discontinued perhexiline therapy for more than four weeks prior to being phenotyped. Furthermore, there is no significant difference between the three groups of patients studied with respect to their biochemical liver function tests measured. The present evidence on the effect of hepatic dysfunction on drug oxidation is difficult to generalise since the findings are either inconsistent or often contradictory (Williams and Mamelok, 1980). The rate of metabolism of bishydroxycoumarin (Brodie, Burns and Weiner, 1959), antipyrine (Nelson, 1964), phenylbutazone (Weiner, Chenkin and Burns, 1954) pentobarbitone (von Oldershausen, Held and Remmer, 1970), tolbutamide (Nelson, 1954) and aminopyrine (Brodie, Burns and Weiner, 1959) was not significantly changed by hepatic dysfunction, while other investigations showed prolonged half-lives of phenylbutazone (Levi, Sherlock and Walker, 1968) and meprobamate (Held and von Oldershausen, 1969) against a similar clinical background. It has even been concluded in a leading Editorial (Anonymous, 1974) that drugs are metabolised surprisingly normally by patients with even the advanced chronic liver disease. Consistent with this view, no correlation between the measured debrisoquine metabolic ratios and any of the liver function parameters could be shown in the 72 patients included in this study. It seems therefore, most unlikely that the observed metabolic differences between the two perhexiline-treated groups are secondary to any hepatic factors. Likewise,

no correlation could be shown between the drug oxidation status and either the smoking or the alcohol habits of the patients.

It could be argued that certain concurrent drug therapy may influence the metabolic fate of debrisoquine and therefore, artefactually raise the debrisoquine metabolic ratio. Evidence against such an argument is afforded by the frequency distribution of our 38 patients with ischaemic heart disease who did not receive perhexiline. It is obvious that despite being on a variety of drug regimens, these patients had a distribution virtually identical to that seen in a normal random population. The drugs consumed by these patients, at the time of debrisoquine phenotyping test, consisted of beta-blockers (n=18), diuretics (n=15), nitrates (n=14), digoxin (n=11), potassium sparing agents (n=6), warfarin (n=4), dipyridamole (n=2), other calcium antagonists (n=2), oral hypoglycaemic agents (n=1), prazosin (n=1), thioridazine (n=1), thyroxine (n=1) and clofibrate (n=1). Tables 28 and 31 show that no single drug in use was associated with propensity to any particular oxidation status and the pattern of drug therapy in 38 patients with ischaemic heart disease alone was similar to that in the perhexiline-treated groups. Therefore, it is obvious that the marked differences observed in the metabolic ratios of the two perhexiline-treated groups are not due to the effects of any concurrent drug therapy.

It is also worth noting that ischaemic heart disease per se does not exhibit any phenotypic diathesis. This has considerable clinical implications. The following is the percentage breakdown of individuals in the three groups of patients studied in terms of ranges of metabolic ratios.

Metabolic Ratio	I.H.D.	Neuropathy patients	Non-neuropathy patients
< 1	65.8%	15%	64.3%
1.1-4.6	23.7%	20%	35.7%
> 4.7	10.5%	65%	0%

Based on this, it could be inferred that $\frac{15 \times 100}{15 + 64.3} = 19\%$ of people below the ratio of 1, $\frac{20 \times 100}{20 + 35.7} = 36\%$ of people with a ratio between 1.1-4.6 and $\frac{65 \times 100}{65 + 0} = 100\%$ of patients above the ratio of 4.7 would develop neuropathy.

Applying these figures to random population with ischaemic heart disease as a whole, it can be seen that 12.5% + 8.5% + 10.5% = 31.5% of individuals are at risk of developing perhexiline-induced neuropathy. The figure quoted by Sebille and Rozensztajn (1978) is about 66%. In their study, patients were followed prospectively with repeated electromyograms and 66% of their patients had developed subclinical evidence of neuropathy. The differences in figures could be due to different criteria employed by Sebille and Rozensztajn (1978). Whichever figure is accepted, it is nonetheless clear that individuals most at risk could easily be identified.

These results show a clear association between the susceptibility to perhexiline-induced neuropathy and diminished drug oxidation status as defined by the ability to hydroxylate debrisoquine. The findings clearly suggest that the individuals of poor metabolic phenotype are at considerably increased risk of developing neuropathy from chronic perhexiline therapy. This risk is strikingly reduced in individuals of the extensive metaboliser phenotype with a ratio of < 1. This association makes it plausible to suggest that in individuals with impaired drug oxidation capacity, perhexiline would tend to accumulate from the consequence of defective metabolic hydroxylation. The accumulation of the parent drug to excessive levels would then lead to toxic sequelae. Although the exact mechanism of neuropathy remains speculative, evidence suggests some drug-induced disturbance of lipid metabolism (Pollet et al, 1977).

It is important to point out that 3 of the 20 patients developed neuropathy despite their metabolic ratios being <1. No other obvious causes were recognised in these three patients. It seems, therefore, that other, as yet unidentified, factors may also contribute to the neuro-toxic sequelae of perhexiline in a minority of cases (Dawes and Moulder, 1982; Timbrell et al, 1981; Welsh and Batchelor, 1981). Nonetheless, it is clear that the individual's drug oxidation status emerges as a major pharmacokinetic determinant of drug toxicity.

The result of this study suggests that an individual's drug oxidation status can be of predictive value in determining that individual's perhexiline dosage and by implication, controlling its neuro-toxicity. Interestingly, significant clinical associations have already been shown to exist between impaired debrisoquine oxidation status and susceptibility to debrisoquine-induced postural hypotension (Idle et al, 1978), phenacetin-induced methaemoglobinaemia (Kong et al, 1982), nortriptyline-induced vertigo, dizziness and confusional state (Bertilsson et al, 1981), sparteine-induced visual disturbances (Eichelbaum et al, 1979, 1982) beta-blocker-induced bradycardia with syncope (Alvan et al, 1982; Shah et al, 1982) and captopril-induced agramulocytosis (Oates et al, 1982). Bertilsson et al (1981) were able to reintroduce nortriptyline at a much lower dose, without complications, in the clinical management of patient with high debrisoquine metabolic ratio. The finding of this study would suggest that similarly, perhexiline can be used successfully in the treatment of angina pectoris. However, the dose would have to be tailored to an individual's drug oxidation status or if the conventional dose is to be used, a high debrisoquine metabolic ratio should alert the clinician to an increased surveillance for toxicity in such individuals before the overt development of disabling clinical neuropathy.

In the following section, the clinical application of these conclusions is demonstrated with respect to three patients who were studied prospectively.

8.6 The predictive value of debrisoquine oxidation status in detection of subclinical perhexiline-neuropathy

Introduction

The retrospective study of patients with perhexiline-induced neuropathy described has shown that the majority of these patients have impaired ability to effect oxidative clearance of debrisoquine and by inference, perhexiline. As a consequence, chronic therapy with perhexiline leads to accumulation of the drug in the body and neurotoxic sequelae follow. It was also shown that the risk of developing neuropathy on perhexiline rises progressively with increasing metabolic ratio; roughly, the probability of developing neuropathy appears to be in the magnitude of 19% at a ratio < 1, 36% in the ratio range of 1.1-4.6 and virtually 100% when the ratio exceeds 4.7.

In order to test the predictive value of debrisoquine metabolic ratio, it was decided to treat three suitable patients with perhexiline without prior knowledge of their drug oxidation phenotype and prospectively follow their progress as regular outpatients for a period of 5-10 months before being phenotyped. All the patients were fully informed of the risks versus benefits of perhexiline therapy, tests involved and the need for careful follow ups. They gave their fully informed consents.

The patients were three males under the age of 65 years and had definite evidence of ischaemic heart disease. Their angina was refractory to the treatment with beta-blockers and long-acting nitrates. None had neurological symptoms or history of diabetes and there were no abnormal cardiovascular, respiratory, hepato-renal or neurological signs. They all had normal liver function tests, renal function tests and blood sugar. One patient had refused coronary arteriogram while in the other two, coronary lesions did not warrant surgical intervention.

Therefore, they were prescribed perhexiline 100 mg bd and following favourable response after one month, the dose was increased to 100 mg tds in each. Mild and transient abnormalities of liver function tests (elevation of AST) were noted during the follow up visits in two of the three patients. None of the patients complained of any neurological symptoms and reported excellent anti-anginal response. After a period, during the follow up visits, patients were phenotyped for their debrisoquine oxidation status and electromyograms were requested with appropriate urgency to check their neurological status objectively.

In one of these patients, the determination of debrisoquine hydroxylation status during the follow up alerted to the probability of neuropathic sequelae and called for appropriate urgent investigation (EMG). This resulted in the confirmation in him of subclinical perhexiline-neuropathy which may have otherwise progressed to overt clinical stage. His case history is described below in detail.

Case History

The patient was a 60-year old man who was on treatment with cimetidine 400 mg nocte for duodenal ulcer. In 1978, he developed extensive acute myocardial insufficiency as evidenced by severe ST-T segment changes. Following recovery, he continued to experience 12-15 episodes of angina daily, even at rest. Treatment with beta-blockers and nifedipine had not reduced the frequency of angina to any great extent. There were no abnormal cardiac, hepato-renal or neurological signs and serum biochemical values for liver function, renal function and blood sugar were all normal. Coronary angiography showed moderately good left ventricular function with no major coronary artery lesion.

Nifedipine was replaced with perhexiline 100 mg bd and four weeks later, encouraged by good therapeutic response, without any serum changes of hepatic dysfunction, the dose was increased to 100 mg tds. On regular follow ups, the patient reported considerable amelioration of his angina and had no abnormal neurological symptoms or signs.

During his follow up,he developed a transient elevation of AST to 75 U/l (normal 7-40 U/l), which dropped to 46 U/l despite continuation of treatment. At the end of 33 weeks of treatment with perhexiline, the patient was phenotyped for debrisoquine oxidation when he was being treated with atenolol, perhexiline, cimetidine and isosorbide dinitrate. His debrisoquine metabolic ratio was found to be 18.1 and this confirmed his status of poor metaboliser phenotype (Price-Evans et al, 1980).

In view of the fact that the patient was on perhexiline and cimetidine at the time of phenotyping, he was re-tested again on two separate occasions when each one of the above medications was stopped. The results of this phenotyping test are shown in Table 32.

The results of the previous study had already suggested that this patient was at 100% risk of developing neuropathy and therefore, despite the absence of any neurological symptoms or signs, an urgent electromyogram was performed 72 hours later. This showed a definite evidence of subclinical demyelinating, predominantly sensory, neuropathy.

As a result, perhexiline was stopped and the patient was commenced on prenylamine 60 mg tds which did not prove to be as effective as perhexiline. Following discontinuation of perhexiline, blood samples were taken from this patient at timed intervals to measure the plasma levels of perhexiline. These levels are shown in Table 33. The plasma elimination half-life of perhexiline in this patient was estimated as being 9.5 days.

Sixteen weeks after stopping perhexiline, a repeat electromyogram showed considerable resolution of his neuropathic changes; the improvement was particularly marked in the sensory component.

The electromyographic data of this patient are shown in Table 34. The data from similar studies of the other two patients, both of whom had debrisoquine metabolic ratios < 1,

Table 32: Consistency of oxidation status during various drug treatment periods

Drug therapy when phenotyped	% oral dose eliminated as		Metabolic Ratio
	Debrisoquine	4-OH-debrisoquine	
Perhexiline, Cimetidine, Atenolol, Nitrates.	30.8	1.7	18.1
Prenylamine, Cimetidine, Atenolol, Nitrates.	24.7	0.9	27.4
Prenylamine, Atenolol, Nitrates.	29.0	1.2	24.2

Table 33: Plasma levels of perhexiline following last dose

Hours after last dose	Drug level mg/L
12	0.89
60	0.76
168	0.56
Estimated plasma perhexiline half-life	9.5 days

Table 34 Details of the three patients together with the results of electromyography

Patient	GA		MC		KB	
Age(years)/Sex	60/M		50/M		62/M	
Current drug therapy when phenotyped	Atenolol, Cimetidine Perhexiline Nitrates		Metoprolol, Nitrates Triamterene Perhexiline		Digoxin, Nitrates Thiazide, Amiloride Perhexiline	
Debrisoquine metabolic ratio (and % recovery)	18.1 (32.5%)		0.6 (25.3%)		0.6 (27.7%)	
ALT (u/l) during perhexiline treatment	Normal —> 75 —> 46		Normal (<40)		Normal —> 64	
Perhexiline treatment mg daily dose	300	16 weeks after stopping perhexiline	300	300	300	300
weeks duration	33		22	41	29	48
Sensory conduction						
1. Right median nerve						
Latency - msec	4.8	3.5	2.5	2.4	3.6	3.0
Amplitude - μV	3	5	22	10	16	10
2. Right ulnar nerve						
Latency - msec	No conduction at all	3.4	2.5	2.3	3.4	2.8
Amplitude - μV		2.5	15	10	13	8
Motor conduction Velocity m/sec	50	51	Not determined	Not determined	Not determined	55
Electromyographic conclusion	Demyelinating neuropathy, predominantly sensory	Neuropathic changes, particularly sensory, resolving	No evidence of neuropathy	No evidence of neuropathy	No evidence of neuropathy	No evidence of neuropathy

are also included for comparison. It is obvious that neither of them developed any neuropathic changes, despite receiving identical daily dosage for longer periods.

The finding of subclinical neuropathy in the patient with impaired debrisoquine oxidation status, compared to the other two patients with extensive metabolic activity who were not affected, strengthens the predictive value of debrisoquine hydroxylation phenotype as suggested by the previous retrospective study. Furthermore, it was also confirmed that the presence of perhexiline, prenylamine or cimetidine as part of the concurrent drug treatment did not influence the debrisoquine oxidation status. It may also be noted that the biochemical liver tests (AST) of the affected patient were only mildly transiently elevated, thus excluding a primary liver injury as the cause of impaired drug oxidation. This is further supported by the fact that one of the unaffected patients had serum AST of 64U/l and yet, displayed extensive metabolism of debrisoquine. The predictive value of debrisoquine oxidation status, as shown retrospectively and prospectively, strongly identifies the unspecified latent inborn metabolic disorder, postulated by L'Hermitte *et al* (1976) in the aetiology of perhexiline-neuropathy, with genetically controlled polymorphism in drug oxidation of a variety of drugs. The patient described here clearly demonstrates how clinically overt toxic effects can be prevented by appropriate interventions based on the knowledge of individual's drug oxidation status at some

convenient time during the patient's follow up. It could be asked if the tragic consequences of perhexiline therapy could have been averted in the patient that was described at the beginning of this thesis by such a test. This is examined in the next Chapter.

C H A P T E R N I N E

THE DEBRISOQUINE OXIDATION STATUS OF THE WATCH-REPAIRER WITH PERHEXILINE-INDUCED NEUROPATHY

The case history presented at the beginning of this thesis already described the tragic socio-economic and morbid consequences that afflicted the watch-repairer following chronic therapy with perhexiline.

He was phenotyped for his debrisoquine oxidation status; at the time of the phenotyping test he was on atenolol, nifedipine, long-acting nitrates, warfarin and dipyridamole. His liver function tests were all normal. In the 0-8 hour period, he eliminated 21.6% of the 10 mg oral dose as unchanged debrisoquine and 3.9% of the oral dose of debrisoquine as 4-hydroxy-debrisoquine. This yielded a metabolic ratio of 5.7, confirming that he had significant impairment in his ability to effect metabolic drug oxidation.

Clearly, this patient was predictably certain to develop the tragic consequences of perhexiline therapy; an earlier knowledge of his debrisoquine oxidation status would have averted the tragic iatrogenic consequences that followed.

CHAPTER TEN

CONCLUSIONS

It is obvious that the explosive advance in therapeutic agents available is not without its adverse consequences. These consequences are followed by considerable socio-economic as well as morbid and mortal sequelae.

Very often, the adverse reactions are preventable through simple considerations of general drug pharmacokinetics and its disposition in relation to patient's own hepato-renal functions and physical details. Nonetheless, a large number of reactions arise without apparently obvious causes and show individual, ethnic as well as geographical susceptibilities. An unspecified idiosyncrasy, probably genetically controlled, has been invoked as possible explanation on a number of occasions.

In 1969, the Committee on Problems of Drug Safety, Drug Research Board, U.S.A. reported certain urgent needs in relation to safe use of drugs.

The drugs administered to individuals are metabolised by a variety of metabolic routes and inter-individual differences in the rates of the metabolic elimination of many of these drugs have been evident.

In search of the cause for these variations, a strong genetic component was discerned. This led to the uncovering of polymorphism in metabolic reactions involving acetylation and hydrolysis. The impact of these discoveries was soon felt. The use of drugs such as hydralazine which, despite being effective anti-hypertensive agents, was threatened

and its use declined very sharply until therapy with it was more rationalised in terms of individual acetylation phenotype. Furthermore, the acetylations of a number of drugs were found to be controlled by the same genetic factors. This finding has been shown to have considerable clinical implications as regards drug toxicity and therapeutic failures. Acetylation polymorphism, nonetheless, did not 'catch on' in view of the limited number of drugs undergoing acetylation.

Oxidation, however, is by far the widely used reaction in metabolic elimination of a vast majority of drugs and scattered reports in literature had already alluded to the probable existence of polymorphism in drug oxidation. It is also significant that the majority of clinical trials have reported patient non-compliance and drop-outs. Rather than to pursue the factors which accounted for this patient withdrawal, they were usually conveniently ignored and recommendations were generally made from the data derived from those who completed the trial protocols. Such recommendations, and the revised guidelines on "safer" use of drugs even after a few years of experience, have failed to reduce the frequency and the impact of adverse drug reactions.

A systematic search for oxidation polymorphism resulted in the characterisation of some properties conferred by a single pair of gene alleles which control 4-hydroxylation of debrisoquine.

Further studies have confirmed the inter-ethnic differences in the frequency of poor oxidisers of debrisoquine and also that the same pair of gene alleles controlled oxidations of a number of drugs through its diverse oxidative pathways such as aromatic hydroxylation, sulphur oxidation, nitrogen dehydrogenation and oxidative de-alkylation. The control is subject to a degree of stereo-selectivity as well.

The work presented in this thesis has shown the value of a debrisoquine-phenotyped panel approach in gaining very valuable and important information regarding inter-individual differences, at either extreme, in oxidation of as well as response to phenformin. Aromatic 4-hydroxylation of phenformin is shown to be controlled by genetic factors which are similar, if not identical, to those controlling alicyclic 4-hydroxylation of debrisoquine. In accordance with this finding, it was found that individuals with impaired ability to effect oxidation of debrisoquine were susceptible to phenformin-induced lactic acidosis, a complication which is related to plasma levels of phenformin.

This polymorphism in drug oxidation has a number of important consequences when standard dose regimes are used. Individuals with extensive metabolic ability will be exposed to lower levels of parent drug, while metabolites will accumulate rapidly. Therefore, metabolite-related toxicity or parent drug-related therapeutic failures could

result. The reverse will be the case in individuals of impaired metabolic ability who will tend to accumulate the less polar parent compounds. The toxic sequelae attributed to the parent drug will then follow. This has been shown to be the case with perhexiline-neuropathy as well as with phenformin-induced lactic acidosis. Significant associations have already been described between impaired debrisoquine oxidation status and

a. debrisoquine-induced postural hypotension
b. phenacetin-induced methaemoglobinaemia
c. nortriptyline-induced vertigo, dizziness and confusional state
d. sparteine-induced visual disturbances
e. beta-blockers-induced dizziness and syncope and
f. captopril-induced agranulocytosis.

Obviously, phenotyping for drug oxidation status would allow at-risk populations to be identified. Although a frequency of less than 1 in 10 may not appear great, it must be appreciated that in the U.K. there are approximately 5 million such individuals and every General Practitioner with a list of 3,000 patients would have to cope with about 300 individuals at risk of adverse drug reactions. Clearly, since the debrisoquine oxidation status of an individual is a

consistent characteristic and it allows the prediction of the oxidative clearance of a number of drugs, it is important that patients are, at some time, phenotyped for debrisoquine oxidation status. This should contribute significantly to rational medical practice. Either the dose can be titrated to individual drug oxidation ratio or resources and efforts aimed at toxicity surveillance can be more effectively diverted to those most at risk. As shown by one of the patients treated prospectively with perhexiline, it may become possible to diagnose toxic effects at an occult, subclinical and reversible stage. The immense socio-economic and financial benefits of introducing phenotyping for oxidation in the rational drug therapy programme are obviously clear.

Cardiovascular diseases rank amongst the top three in being the major cause of morbidity and mortality in man. It is, therefore, not surprising that a wide variety of cardioactive medications are available to treat ischaemic heart disease, arrhythmias, hypertension and congestive cardiac failure. It is equally not surprising that cardiovascular drugs feature prominently in all epidemiological studies on adverse drug reactions.

Beta-blockers are widely prescribed and a large number of them are metabolised by oxidation. Wide inter-individual variations in their plasma levels are reported and poor hydroxylators of debrisoquine have been shown to attain much

higher than normal plasma levels of a number of beta-blockers (Alvan et al, 1982; Dayer et al, 1982; Lennard et al, 1982). The susceptibility of poor metabolisers to excessive beta-blockade has already been shown (Shah et al, 1982; Lennard et al, 1982). The beta-blockers have been shown to be of value in post-myocardial infarction period (BHAT, 1981; Pedersen, 1981; Wilhelmsson et al, 1974; Hjalmarson et al, 1981) and a number of individuals were deprived of these drugs in each of these trials because of excessive beta-blockade (or sequelae) on fixed-dose protocols. This is clearly irrational when all that need be done was to titrate the dose to the individual oxidation status. Similarly, it has been shown that the metabolism of encainide, an effective anti-arrhythmic agent is also controlled by the alleles controlling debrisoquine 4-hydroxylation (Woosley et al, 1981). It is significant that encainide induced ventricular tachyrrhythmias in 11% of 90 patients receiving the drug (Winkle et al, 1981). It is possible that this was due to high plasma levels of encainide. It may be worth phenotyping these individuals for debrisoquine oxidation capacity. Clearly, if they are poor metabolisers of drugs generally, it will be illogical to abandon a drug benefitting 89% of individuals receiving it. Many such examples can be quoted where clear definition of the patients' genetically determined oxidation status is required before valuable drugs, expensively researched and developed and benefitting a

vast majority fall foul of clinicians or are considered for withdrawal from market. Amongst the many existing cardiovascular drugs, a few such examples are prenylamine (induces tachyrrhythmias, Meanock and Noble, 1981), verapamil (induces heart blocks of varying degrees, Stone et al, 1980), quinidine (induces syncope, Davies, Leak and Oram, 1965), mexiletine (induces tachyrrhythmias, Cocco et al, 1980), disopyramide (induces tachyrrhythmias, Meltzer et al, 1978), captopril (induces agranulocytosis, Amann et al, 1980) and urapidil (induces hypotension, Leibetseder, 1981).

It is proposed that the principles of investigative approaches outlined in this thesis, when applied to these drugs as well as the ones developed in the future, could yield valuable information of considerable clinical significance. No doubt the probability is that similar lines of investigations could be exploited equally beneficially in the clinical assessment of many drugs in common use, e.g. anti-neoplastic, analgesic, hypnotic, sedative and anti-depressant drugs.

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a) The journal abbreviations used are in the style of Index Medicus where appropriate.

b) For brevity, the references are listed without the titles of papers but in the Vancouver style.

c) Books and the Chapters in books are indicated simply as follows:

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APPENDICES

Appendix I Urinary excretion of phenformin (mg) and 4-hydroxy phenformin (mg) in 4 EM's and 3 PM's following a single oral dose of 50mg phenformin

Period (hour)	AZ (EM)		JR (EM)		LW (EM)		JI (EM)	
	Phen	4-OH-Phen	Phen	4-OH-Phen	Phen	4-OH-Phen	Phen	4-OH-Phen
0 - 1	1.35	0.30	0.34	-	0.82	0.28	0.93	0.24
1 - 2	5.80	1.53	2.28	0.53	3.11	1.05	3.80	0.67
2 - 3	4.96	1.39	2.28	0.53	2.48	0.96	2.46	0.695
3 - 4	2.90	0.82	4.06	1.13	2.27	0.98	2.46	0.695
4 - 5	2.47	0.63	1.45	0.62	1.79	0.86	3.65	0.96
5 - 6	1.92	0.62	1.59	0.39	1.49	0.72	0.97	0.17
6 - 7	1.27	0.52	1.29	0.30	1.29	0.72	0.97	0.17
7 - 8	1.14	0.54	0.98	0.29	0.78	0.45	0.91	0.31
8 - 9	0.625	0.35	0.91	0.23	0.63	0.315	0.91	0.31
9 - 10	0.625	0.35	0.91	0.23	0.63	0.315	0.91	0.31
10 - 12	0.92	0.56	1.46	0.39	0.81	0.515	1.82	0.64
12 - 24	1.21	1.24	5.28	2.85	2.34	2.585	6.13	1.19
24 - 26	0.10	0.24	0.03	0.02	0.09	0.18	0.31	0.14

Period (hour)	RS (PM)		JO'G (PM)		JD'S (PM)	
	Phen	4-OH-Phen	Phen	4-OH-Phen	Phen	4-OH-Phen
0 - 1	0.88	-	0.80	0.044	1.00	-
1 - 2	4.70	-	3.63	0.176	4.74	0.041
2 - 3	3.48	-	3.30	0.105	3.28	0.034
3 - 4	3.86	-	3.76	0.105	4.41	0.0435
4 - 5	3.51	-	3.21	0.175	4.41	0.0435
5 - 6	2.67	-	2.24	0.112	1.69	0.011
6 - 7	2.34	-	2.34	0.129	2.18	0.021
7 - 8	1.88	-	1.42	0.080	1.16	0.0045
8 - 9	1.41	-	1.53	0.055	0.92	-
9 - 10	1.22	-	1.53	0.055	0.92	-
10 - 12	1.23	-	2.36	0.083	1.69	-
12 - 24	6.65	-	6.46	0.562	3.44	-
24 - 26	-	-	0.63	0.019	0.24	-

Appendix II Physical details and habits of the 195 subjects

Subject	Age (Yrs)	Sex	Body Height (cm)	Body Weight (kg)	Alcohol Consumption	Smoking Habits
1	22	F	173	61	+	0
2	20	F	170	59	0	0
3	21	F	170	57	+	0
4	19	F	163	51	+	0
5	19	F	156	47	+	0
6	20	F	163	58	+	0
7	20	F	165	55	+	+
8	32	F	163	56	+	+
9	22	F	175	64	+	0
10	24	F	165	56	+	+
11	23	M	170	70	+	0
12	23	F	168	61	+	0
13	21	M	175	70	+	0
14	20	F	163	57	+	+
15	20	F	180	61	+	+
16	21	F	173	64	+	+
17	29	F	168	61	+	+
18	23	F	168	62	+	0
19	22	F	160	62	+	++
20	22	F	173	57	+	0
21	22	F	178	57	+	0
22	21	F	160	46	+	0
23	22	F	158	64	+	0
24	20	F	165	50	+	0
25	21	F	158	51	+	0
26	20	F	173	61	+	0
27	21	F	163	48	+	+
28	21	F	168	64	+	+
29	21	F	168	58	+	0
30	27	M	175	64	+	0
31	26	M	183	85	++	0
32	21	M	188	74	++	0
33	20	F	173	56	+	0
34	27	M	163	84	+	0
35	22	M	158	72	+	0
36	23	F	170	55	+	0
37	23	F	168	61	+	0
38	22	M	163	80	+	0
39	28	M	178	73	+	0
40	20	F	168	57	+	0
41	22	F	165	64	+	0
42	29	M	180	82	+	+
43	22	M	193	76	+	0
44	52	M	165	72	+	0
45	34	M	178	86	0	0
46	27	F	165	59	+	0
47	31	M	180	74	+	0
48	24	F	165	56	+	0
49	24	F	155	51	+	+
50	21	M	180	61	+	0
51	24	M	178	55	+	0
52	30	M	180	57	+	+
53	22	F	155	61	0	0
54	31	F	173	62	0	++
55	24	F	158	56	+	0
56	39	M	160	86	+	++
57	29	F	165	51	+	++
58	21	F	163	50	+	0
59	24	F	165	61	+	++
60	30	M	183	67	+	0
61	27	F	165	61	+	0
62	28	F	168	67	+	0
63	23	F	168	65	+	+
64	27	M	168	61	+	0
65	20	F	163	48	+	+
66	27	F	178	64	++	++
67	19	F	173	56	+	0
68	20	F	170	55	+	0
69	20	F	173	62	+	0
70	19	F	163	59	+	0
71	21	F	155	52	+	0
72	20	F	170	52	+	0
73	19	F	173	64	+	0
74	19	F	163	49	+	0
75	19	F	168	56	+	0
76	19	F	155	41	+	0
77	19	F	168	56	+	0
78	19	F	160	61	+	0
79	19	F	168	65	+	++
80	19	F	160	54	+	++
81	23	F	165	61	0	0
82	26	F	168	60	+	0
83	20	F	163	51	+	+
84	19	F	165	55	+	++
85	20	F	163	57	+	0
86	19	M	183	70	++	0
87	20	F	168	54	+	+
88	22	F	170	61	+	+
89	28	F	173	55	+	++
90	28	M	170	71	+	++
91	22	F	168	57	+	0
92	19	F	168	53	+	0
93	20	M	165	58	+	0
94	24	F	178	64	+	0
95	20	F	163	63	0	0
96	22	M	188	99	++	0
97	23	M	178	73	++	+
98	26	M	198	146	+	++
99	21	F	165	55	0	0
100	19	F	170	67	+	0
101	35	F	168	51	+	++
102	43	F	163	57	+	++
103	21	F	165	59	+	++
104	21	F	165	59	+	++
105	18	F	152	48	+	+
106	22	F	163	51	+	+
107	21	F	165	61	+	0
108	21	F	160	54	+	0
109	21	F	163	57	+	0
110	19	F	165	48	+	0
111	38	F	163	57	+	0
112	21	F	165	64	+	0
113	21	F	165	61	+	++
114	21	F	168	64	+	0
115	26	M	183	65	+	0
116	21	F	160	48	+	0
117	46	M	168	59	+	0
118	21	F	173	47	+	+
119	23	F	165	61	+	++
120	21	M	185	83	++	0
121	21	F	178	70	+	0
122	21	F	163	61	+	0
123	21	F	168	76	+	0
124	21	F	173	64	+	0
125	27	M	172	72	++	++
126	24	F	168	57	+	+
127	37	F	165	59	+	0
128	23	M	168	55	+	0
129	23	M	175	64	+	0
130	21	F	165	54	+	+
131	20	F	168	67	+	++
132	21	F	170	61	+	0
133	23	F	175	67	+	+
134	19	M	178	80	+	0
135	20	M	175	79	+	0
136	20	M	173	67	+	0
137	20	M	180	68	+	0
138	19	M	175	54	0	0
139	20	F	163	55	+	0
140	27	F	178	55	+	0

Appendix II cont.

Subject	Age (Yrs)	Sex	Body Height (cm)	Body Weight (kg)	Alcohol Consumption	Smoking Habits
141	20	M	180	64	+	0
142	19	F	170	59	0	0
143	19	M	180	93	+	0
144	20	F	165	60	0	0
145	19	F	160	55	+	0
146	19	F	168	60	+	+
147	25	F	163	52	+	0
148	20	F	163	55	+	0
149	19	M	185	70	+	0
150	22	F	170	62	+	0
151	19	F	168	55	+	0
152	19	F	163	59	+	+
153	19	M	175	83	+	0
154	19	M	175	67	+	0
155	19	M	188	87	+	+
156	19	M	180	82	+	0
157	20	M	173	76	+	+
158	20	F	173	60	+	0
159	20	M	173	64	+	0
160	20	F	170	54	+	0
161	25	F	168	58	+	0
162	20	M	174	61	+	0
163	19	M	170	74	+	0
164	19	F	165	53	+	0
165	20	M	170	53	+	0
166	19	M	178	74	+	0
167	20	M	183	91	+	0
168	20	M	168	67	+	0
169	19	F	168	53	+	0
170	20	M	180	86	+	+

Subject	Age (Yrs)	Sex	Body Height (cm)	Body Weight (kg)	Alcohol Consumption	Smoking Habits
171	19	M	178	74	+	0
172	20	M	183	69	+	0
173	19	M	191	83	+	0
174	19	M	180	76	+	0
175	20	M	168	65	+	0
176	20	M	180	80	0	0
177	21	M	183	76	+	++
178	19	M	183	69	+	0
179	19	M	170	61	+	0
180	21	M	178	76	+	0
181	20	M	178	89	+	0
182	19	M	188	83	+	0
183	20	M	180	73	+	0
184	20	M	173	67	+	++
185	19	F	160	52	+	0
186	19	M	180	70	+	0
187	19	F	168	52	+	0
188	19	F	168	55	+	0
189	20	F	152	48	0	0
190	19	F	165	55	+	0
191	20	M	173	67	+	0
192	19	M	193	89	+	0
193	24	F	178	48	0	0
194	19	M	175	70	+	0
195	20	M	180	73	+	0

Code

Alcohol consumption
0 = None
+ = Occasional
++ = Moderate

Smoking habits
0 = None
+ = 1-15 cigs./day
++ = >15 cigs./day

Appendix III Urinary recoveries of phenformin and 4-hydroxy-phenformin and phenformin ratio (PR)

Subject	Phenformin (%/8h)	4-OH-Phen (%/8h)	PR	Subject	Phenformin (%/8h)	4-OH-Phen (%/8h)	PR
1	26.2	9.6	2.7	71	33.2	5.0	6.6
2	44.6	2.8	15.9	72	22.4	6.2	3.6
3	39.6	6.0	6.6	73	46.6	2.8	16.6
4	28.2	11.6	2.4	74	32.4	3.0	10.8
5	27.2	9.2	3.0	75	22.6	4.2	5.4
6	18.2	4.4	4.1	76	28.8	3.6	8.0
7	34.0	7.0	4.8	77	32.4	1.2	27.0
8	48.6	1.6	30.4	78	44.6	15.8	2.8
9	14.6	9.4	1.6	79	49.0	6.2	7.9
10	18.2	4.2	4.3	80	22.2	10.4	2.1
11	22.8	11.4	2.0	81	24.4	6.8	3.6
12	24.2	1.8	13.4	82	41.6	6.6	6.3
13	23.8	4.4	5.4	83	22.4	3.4	6.6
14	27.8	5.6	5.0	84	28.2	2.4	11.8
15	41.6	1.8	23.1	85	21.2	10.4	2.0
16	18.6	12.2	1.5	86	35.4	7.2	4.9
17	12.8	1.2	10.7	87	30.2	6.8	4.4
18	42.2	7.6	5.6	88	22.2	2.4	9.3
19	27.2	8.2	3.3	89	36.6	0.8	45.8
20	26.6	7.8	3.4	90	29.8	9.2	3.2
21	14.0	7.4	1.9	91	36.8	9.8	3.7
22	23.0	6.0	3.8	92	56.0	0.4	140
23	36.2	0.6	60.3	93	35.2	5.8	6.1
24	31.0	3.8	8.2	94	38.2	2.6	14.7
25	31.2	4.0	7.8	95	28.2	14.8	1.9
26	29.0	4.4	6.6	96	39.6	9.2	4.3
27	17.8	2.0	8.9	97	30.8	8.8	3.5
28	16.6	8.0	2.1	98	40.2	2.8	14.4
29	16.4	9.6	1.7	99	26.6	9.6	2.8
30	31.6	10.2	3.1	100	22.8	7.6	3.0
31	38.6	3.6	10.7	101	23.4	10.0	2.3
32	43.8	1.0	43.8	102	42.2	10.2	4.1
33	19.6	20.6	1.0	103	36.6	9.8	3.7
34	45.2	14.0	3.2	104	32.6	8.6	4.0
35	24.0	26.8	0.9	105	30.2	7.4	4.1
36	36.0	17.0	2.1	106	25.2	11.8	2.1
37	24.8	12.4	2.0	107	29.0	14.4	2.0
38	24.8	2.4	10.3	108	30.4	31.6	1.0
39	39.8	16.8	2.4	109	42.2	1.0	42.2
40	16.2	0.6	27.0	110	41.0	7.0	5.9
41	61.0	5.0	12.2	111	51.8	1.6	32.4
42	32.2	7.6	4.2	112	36.2	12.2	3.0
43	62.6	7.0	8.9	113	28.2	5.8	4.9
44	24.8	5.8	4.3	114	29.2	2.8	10.4
45	16.8	4.4	3.8	115	20.6	11.0	1.9
46	31.8	7.0	4.5	116	30.8	8.4	3.7
47	19.0	3.0	6.3	117	11.6	5.4	2.1
48	28.8	11.2	2.6	118	28.2	12.0	2.4
49	12.8	7.2	1.8	119	43.6	3.0	14.5
50	33.4	12.2	2.7	120	44.8	4.2	10.7
51	21.4	12.2	1.8	121	26.4	5.4	4.9
52	25.6	11.2	2.3	122	20.4	11.8	1.7
53	43.2	0.8	54.0	123	24.6	12.4	2.0
54	15.4	4.6	3.3	124	44.8	8.2	5.5
55	55.2	8.4	6.6	125	33.8	5.8	5.8
56	8.6	1.2	7.2	126	25.2	4.0	6.3
57	26.8	3.8	7.1	127	18.8	9.8	1.9
58	42.6	4.8	8.9	128	48.6	1.8	27.0
59	19.2	5.2	3.7	129	35.6	1.2	29.7
60	33.6	9.2	3.7	130	29.2	7.0	4.2
61	22.6	6.8	3.3	131	24.2	13.0	1.9
62	48.6	4.8	10.1	132	29.2	0.8	36.5
63	29.0	2.2	13.2	133	24.2	5.8	4.2
64	45.2	1.8	25.1	134	36.0	10.2	3.5
65	27.2	4.2	6.5	135	68.6	8.2	8.4
66	38.0	4.2	9.0	136	45.4	9.6	4.7
67	25.4	10.2	2.5	137	51.8	15.2	3.4
68	21.8	8.4	2.6	138	44.2	2.6	17.0
69	26.4	9.8	2.7	139	28.0	4.6	6.1
70	52.0	7.8	6.7	140	26.4	3.2	8.3

Appendix III cont.

Subject	Phenformin (%/8h)	4-OH-Phen (%/8h)	PR
141	35.2	13.8	2.6
142	47.2	5.6	8.4
143	44.0	6.2	7.1
144	28.0	5.6	5.0
145	27.0	18.6	1.5
146	42.0	1.0	42.0
147	76.0	11.8	6.4
148	33.2	9.2	3.6
149	23.4	11.8	2.0
150	55.0	5.2	10.6
151	27.4	3.8	7.2
152	46.2	9.4	4.9
153	43.0	8.6	5.0
154	46.8	11.8	4.0
155	46.8	16.8	2.8
156	40.6	11.4	3.6
157	41.6	6.8	6.1
158	38.4	9.8	3.9
159	36.2	12.4	2.9
160	37.6	13.0	2.9
161	32.2	10.0	3.2
162	30.2	10.8	2.8
163	37.8	11.2	3.4
164	39.0	7.4	5.3
165	12.8	2.0	6.4
166	36.8	0.2	184
167	26.4	7.4	3.6
168	39.4	12.4	3.2
169	52.2	1.4	37.3
170	42.6	15.6	2.7
171	27.4	18.6	1.5
172	46.2	8.2	5.6
173	52.4	8.0	6.6
174	60.6	29.0	2.1
175	46.8	2.6	18.0
176	55.2	9.8	5.6
177	35.2	18.6	1.9
178	30.6	11.2	2.7
179	42.0	28.6	1.5
180	27.2	16.4	1.7
181	73.4	15.2	4.8
182	50.8	6.4	7.9
183	70.8	3.6	19.7
184	25.6	15.4	1.7
185	40.2	10.2	3.9
186	28.8	12.6	2.3
187	34.0	17.4	2.0
188	48.4	7.8	6.2
189	36.0	6.4	5.6
190	43.2	14.2	3.0
191	38.2	14.6	2.6
192	39.6	12.0	3.3
193	51.4	10.2	5.0
194	38.0	7.2	5.3
195	38.8	11.6	3.3

$$PR = \frac{\text{Recovery of Phenformin}}{\text{Recovery of 4-Hydroxy-phenformin}}$$

Appendix IV Volunteers who had taken medications (other than contraceptive steroids) within one week preceding completion of phenformin test for population study

Volunteer number	Medication(s)	Phenformin metabolic ratio
11	'Beconase' nasal spray	2.0
54	'Actifed' syrup	3.3
61	Sodium cromogycate	3.3
89	Imipramine 75mg t.d.s.	45.8
114	Flupenthixol 0.5mg b.d.	10.4
122	Colofac	1.7
135	'Beconase' nasal spray	8.4
139	Metronidazole 200mg t.d.s.	6.1
172	Temazepam 10mg	5.6
184	Salbutamol and cromglycate sprays	1.7
Median		4.5

Appendix V The physical and clinical details, together with phenformin oxidation status, of 27 probands and their 87 family members

No.	Relation	Age (yrs)	Medical history	Drug treatment	0-8 h Phenformin Recovery (%)	Ratio
2	Proband	20	-	c/pill	47.4	15.9
	Father	48	-	-	36.6	3.5
	Mother	44	-	-	39.0	10.5
9	Proband	22	-	c/pill	24.0	1.6
	Father	57	-	-	25.8	4.6
	Mother	53	Diverticulosis	-	19.4	3.6
	Sister	26	Acne	Oxytetracycline	28.2	2.7
16	Proband	21	-	-	30.8	1.5
	Father	50	Crohn's disease	Azathioprine	25.2	4.3
	Mother	50	-	-	20.6	5.1
20	Proband	22	-	c/pill	34.4	3.4
	Father	51	-	-	68.2	2.2
	Mother	58	-	-	49.2	3.1
	Sister	14	Congenital heart disease	-	57.6	2.3
23	Proband	22	-	-	36.8	60.3
	Father	52	-	-	30.0	9.7
	Mother	49	Hypertension	Atenolol,Metoclopramide,Bumetanide	32.2	6.3
	Sister	20	-	c/pill	35.4	28.5
	Sister	16	-	c/pill	32.0	25.7
27	Poband	21	-	-	19.8	8.9
	Father	52	-	-	24.4	6.2
	Mother	56	-	-	31.4	3.9
	Brother	20	-	-	38.8	8.2
30	Proband	27	-	-	41.8	3.1
	Father	60	-	-	30.6	2.6
	Mother	63	-	-	25.2	2.4
	Sister	33	-	-	31.6	4.5
	Sister	31	-	-	31.8	4.5
31	Proband	26	-	-	42.2	10.7
	Father	62	Angina	Nifedipine,Oxprenolol,Diazepam	31.8	10.4
	Mother	54	Hypertension	Metoprolol,Thiazide,Diazepam	26.2	4.5
	Brother	28	-	-	35.8	4.0
	Sister	21	-	-	51.6	10.7
32	Proband	21	-	-	44.8	43.8
	Father	45	-	-	26.8	13.9
	Mother	52	-	-	57.2	12.0
	Sister	19	-	c/pill	42.2	6.5
	Brother	16	-	-	60.4	5.7
	Sister	11	-	-	54.8	6.6
33	Proband	20	-	c/pill	40.2	1.0
	Father	49	Hypertension	-	34.4	1.7
	Mother	41	-	-	36.2	2.1
	Sister	19	-	-	28.2	3.7
	Brother	15	-	-	31.2	2.9
	Brother	10	-	-	36.2	3.3
39	Proband	28	-	-	56.6	2.4
	Father	63	-	-	41.6	3.3
	Mother	57	-	-	74.0	15.8
	Sister	33	-	-	30.0	6.9
64	Proband	27	-	-	47.0	25.1
	Father	49	-	-	25.2	125
	Mother	46	-	-	47.4	6.2
	Identical twin	27	-	-	24.5	36.1
	Sister	26	Crohn's disease	Azathioprine	43.4	6.5

Appendix V cont.

No.	Relation	Age (yrs)	Medical history	Drug treatment	0-8 h Phenformin Recovery (%)	Ratio
73	Proband	19	-	c/pill	49.4	16.6
	Father	51	-	-	28.4	2.1
	Mother	47	-	-	54.4	6.2
	Brother	18	-	-	57.4	7.0
77	Proband	19	-	c/pill	33.6	27.0
	Father	50	-	-	27.8	138
	Mother	51	-	-	23.8	4.2
	Sister	22	-	c/pill	43.8	6.1
78	Proband	19	-	c/pill	61.4	2.8
	Father	52	-	-	37.0	25.4
	Mother	50	-	-	28.8	4.1
	Sister	26	-	-	41.8	4.7
82	Proband	26	-	c/pill	48.2	6.3
	Father	58	-	-	41.0	5.8
	Mother	58	Diabetes	Insulin	40.2	3.3
	Brother	31	-	-	69.0	5.4
85	Proband	20	-	-	31.6	2.0
	Father	54	-	-	48.2	3.2
	Mother	49	-	-	30.2	5.3
	Brother	18	-	-	41.6	6.7
91	Proband	22	-	c/pill	46.6	3.7
	Father	56	-	-	25.6	6.1
	Mother	54	Hypertension	Oxprenolol	17.0	5.5
	Sister	26	-	c/pill	31.0	5.5
92	Proband	19	-	-	56.4	140
	Mother	47	Carcinoma-breast	-	30.2	13.8
	Brother	17	-	-	30.2	2.7
	Sister	11	-	-	13.8	8.9
108	Proband	21	-	-	62.0	1.0
	Mother	50	Arthritis	Ibuprofen	41.6	8.5
	Brother	19	-	-	32.6	3.4
109	Proband	21	-	-	43.2	42.2
	Father	46	-	-	26.6	32.3
	Mother	41	-	-	29.2	72.0
	Brother	18	-	-	51.5	33.3
111	Proband	38	-	-	53.4	32.4
	Husband	38	-	-	31.0	7.2
	Son	17	-	-	42.8	6.9
119	Proband	23	-	c/pill	46.6	14.5
	Father	50	-	-	49.4	9.7
	Mother	48	-	-	52.6	8.1
	Sister	16	-	c/pill	42.0	3.7
120	Proband	21	-	-	49.0	10.7
	Father	48	Angina	Atenolol,Warfarin	54.2	8.7
	Mother	46	-	-	37.6	2.6
	Sister	18	-	-	18.6	6.2
128	Proband	23	-	-	50.4	27.0
	Father	55	-	-	41.8	4.7
	Mother	54	Migraine	Trimipramine,Pizotifen,Diazepam	47.8	9.4
	Brother	20	-	-	43.6	6.5
	Brother	16	-	-	81.0	33.3
129	Proband	23	-	-	36.8	29.7
	Father	61	Bladder papilloma	Triazolam,Nitrazepam	38.0	5.8
	Mother	50	-	Triazolam	23.2	57.0
	Sister	14	-	-	29.0	5.9
	Sister	11	-	-	24.8	61.0
135	Proband	20	Hay fever	Beconase nasal spray	76.8	8.4
	Father	52	Epilepsy	Phenytoin,Mysoline,Carbamazepine	27.8	0.6
	Mother	49	-	-	22.8	7.8
	Brother	16	-	-	41.0	6.6

Appendix VI Recoveries and oxidation status of 101 volunteers tested separately on 50mg phenformin and 10mg debrisoquine.

Volunteer Number	Phenformin Recovery (%)	Phenformin Ratio (PR)	Debrisoquine Recovery (%)	Debrisoquine Ratio (MR)
2	47.4	15.9	26.7	1.5
3	45.6	6.6	35.1	0.4
4	39.8	2.4	30.2	0.1
5	36.4	3.0	28.3	0.3
6	22.6	4.1	23.6	0.7
8	50.2	30.4	33.5	32.5
9	24.0	1.6	33.6	0.4
10	22.4	4.3	37.0	0.4
14	33.4	5.0	40.7	0.5
15	43.4	23.1	43.0	31.1
16	30.8	1.5	33.6	0.3
20	34.4	3.4	22.3	0.3
21	21.4	1.9	30.3	0.4
23	36.8	60.3	32.7	46.6
27	19.8	8.9	29.8	0.9
28	24.6	2.1	31.2	0.4
29	26.0	1.7	26.6	0.3
30	41.8	3.1	36.4	0.3
31	42.2	10.7	27.1	2.0
32	44.8	43.8	25.2	36.5
33	40.2	1.0	33.4	0.2
34	59.2	3.2	18.0	0.1
39	56.6	2.4	23.2	0.7
40	16.8	27.0	28.0	32.5
53	44.0	54.0	21.2	23.5
60	42.8	3.7	29.2	0.5
62	53.4	10.1	28.4	1.1
63	31.2	13.2	22.1	4.8
64	47.0	25.1	22.6	21.6
70	59.8	6.7	13.8	0.4
73	49.4	16.6	39.8	1.2
76	32.4	8.0	23.7	0.6
77	33.6	27.0	34.4	3.2
78	60.4	2.8	33.8	0.6
81	31.2	3.6	41.8	0.3
82	48.2	6.3	14.2	0.4
85	31.6	2.0	8.3	0.3
91	46.6	3.7	32.1	0.5
102	52.4	4.1	41.9	0.3
108	62.0	1.0	19.5	0.6
109	43.2	42.2	34.6	25.0
110	48.0	5.9	24.1	0.6
111	53.4	32.4	37.0	41.3
114	32.0	10.4	31.5	2.5
119	46.6	14.5	27.1	10.2
120	49.0	10.7	32.5	1.2
128	50.4	27.0	25.2	2.3
129	36.8	29.7	29.2	21.0
134	46.2	3.5	21.8	1.1
135	76.8	8.4	7.7	0.6
136	55.0	4.7	19.5	0.5
138	46.8	17.0	28.4	7.6
139	32.6	6.1	10.0	1.7
140	29.6	8.3	23.8	2.2
141	49.0	2.6	38.7	0.6
142	52.8	8.4	32.7	0.8
143	50.2	7.1	21.3	3.0
144	33.6	5.0	22.7	0.7
145	45.6	1.5	26.7	0.3
146	43.0	42.0	40.0	38.3
148	42.4	3.6	27.2	0.9
149	35.2	2.0	20.8	1.5
150	60.2	10.6	28.3	2.6
151	31.2	7.2	24.8	2.4
152	55.6	4.9	18.0	1.6
153	51.6	5.0	10.7	0.6
154	58.6	4.0	14.0	1.2
155	63.6	2.8	15.7	1.1
157	48.4	6.1	12.9	0.5
158	48.2	3.9	14.8	0.3
159	48.6	2.9	17.5	1.0
160	50.6	2.9	15.2	0.6
161	42.2	3.2	18.9	1.7
162	41.0	2.8	19.6	0.8
163	49.0	3.4	20.0	0.5
164	46.4	5.3	20.3	1.1
165	14.8	6.4	22.8	1.3
166	37.0	184	22.6	133.3
167	33.8	3.6	15.0	0.6
168	51.8	3.2	5.8	0.5
169	53.6	37.3	18.9	38.2
170	58.2	2.7	20.3	1.0
171	46.0	1.5	18.2	0.4
172	54.4	5.6	14.2	1.0
173	60.4	6.6	27.4	1.8
174	89.6	2.1	15.1	0.4
175	49.4	18.0	16.7	12.2
177	53.8	1.9	15.9	0.3
178	41.8	2.7	19.4	0.5
179	70.6	1.5	13.7	0.5
181	88.6	4.8	22.8	0.6
182	57.2	7.9	30.5	4.9
183	74.4	19.7	20.1	5.6
185	50.4	3.9	23.9	0.6
187	51.4	2.0	28.2	0.6
188	56.2	6.2	21.3	1.5
189	42.4	5.6	28.1	1.6
190	57.4	3.0	24.5	0.5
192	51.6	3.3	13.6	0.7
193	61.6	5.0	24.8	1.6
194	45.2	5.3	37.1	0.9

	Phenformin	Debrisoquine
Median recovery	46.6	24.5
Mean±s.d.	45.9±13.6	24.9±8.4

Appendices VII & VIII Time v/s urinary excretion of phenformin and its metabolite following a single oral dose of 50mg phenformin to 4 EM's and 4 PM's

VII EM's

	Phenformin (mg)					4-OH-Phenformin (mg)				
Volunteer	AW	EE	JR	AZ	Mean±SD	AW	EE	JR	AZ	Mean±SD
Period (h)										
0 - 1	0.58	0.67	1.96	1.30	1.13±0.65	0.20	0.13	0.34	0.31	0.25±0.10
1 - 2	3.64	3.54	4.85	3.72	3.94±0.61	1.40	1.61	1.15	0.98	1.29±0.28
2 - 4	4.45	7.80	7.68	6.96	6.72±1.56	3.36	4.12	2.08	2.03	2.90±1.02
4 - 6	4.08	4.45	3.63	4.46	4.15±0.39	2.04	2.82	1.22	1.43	1.88±0.72
6 - 8	2.13	2.12	2.68	1.48	2.10±0.49	1.94	1.52	0.86	0.68	1.25±0.58
8 - 10	1.17	0.99	1.12	1.48	1.19±0.21	1.19	0.86	0.40	0.68	0.78±0.33
10 - 12	1.08	0.89	1.11	1.31	1.10±0.17	1.30	0.97	0.40	0.57	0.81±0.40
12 - 24	1.23	1.52	4.25	2.00	2.25±1.37	1.97	1.46	2.38	1.24	1.76±0.51
24 - 26	0.07	0.12	0.25	0.32	0.19±0.12	0.29	0.25	0.23	0.38	0.29±0.07
Total 0 - 8	14.88	18.58	20.82	17.91	18.05±2.45	8.94	10.20	5.65	5.43	7.56±2.38
Total 0 - 26	18.43	22.10	27.55	23.02	22.78±3.75	13.69	13.74	9.06	8.30	11.20±2.92

VIII PM's

	Phenformin (mg)					4-OH-Phenformin (mg)				
Volunteer	LE	AR	SJ	CS	Mean±SD	LE	AR	SJ	CS	Mean
Period (h)										
0 - 1	1.30	0.68	0.16	1.26	0.85±0.54	0.06	n.d.	n.d.	n.d.	0.015
1 - 2	4.71	5.60	4.41	7.06	5.45±1.19	0.03	0.12	n.d.	n.d.	0.038
2 - 4	8.00	12.69	12.98	12.87	11.64±2.43	0.06	0.20	n.d.	n.d.	0.065
4 - 6	6.70	8.93	9.60	8.41	8.41±1.24	n.d.	n.d.	n.d.	n.d.	-
6 - 8	4.29	5.61	5.64	6.00	5.39±0.75	n.d.	n.d.	n.d.	n.d.	-
8 - 10	4.04	2.51	3.94	3.79	3.57±0.71	n.d.	n.d.	n.d.	n.d.	-
10 - 12	3.84	1.33	2.12	2.61	2.48±1.05	n.d.	n.d.	n.d.	n.d.	-
12 - 24	5.66	4.10	3.44	6.14	4.84±1.27	n.d.	n.d.	n.d.	n.d.	-
24 - 26	0.22	0.35	0.27	0.73	0.39±0.23	n.d.	n.d.	n.d.	n.d.	-
Total 0 - 8	25.00	33.51	32.79	35.60	31.73±4.64	0.15	0.32	n.d.	n.d.	0.118
Total 0 - 26	38.76	41.80	42.56	48.87	43.00±4.25	0.15	0.32	n.d.	n.d.	0.118

Appendix IX Plasma phenformin concentrations (ng/ml) v/s time in the two phenotypes

Volunteer	Hours after a single oral dose of 50mg phenformin								
	0	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0
AW	n.d.	n.d.	78.8	123.4	140.5	116.0	117.1	63.9	41.6
EE	n.d.	n.d.	60.7	75.6	80.9	89.4	88.4	82.6	60.7
JR	n.d.	n.d.	68.2	71.3	77.7	76.7	82.0	51.1	43.7
AZ	n.d.	24.6	67.1	68.2	85.2	87.3	68.2	52.2	38.4
Mean±sem for EM's	-	6.2 ±	68.7 ±3.8	84.6 ±13.0	96.1 ±14.9	92.4 ±8.4	88.9 ±10.3	62.5 ±7.3	46.0 ±5.0
LE	n.d.	n.d.	127.7	140.5	177.7	184.0	183.0	121.3	91.0
AR	n.d.	n.d.	44.8	118.1	105.4	136.2	111.7	78.8	56.5
SJ	n.d.	32.0	30.0	84.1	136.2	160.7	119.2	37.0	47.0
CS	n.d.	11.4	64.9	105.8	127.8	122.8	116.9	88.7	69.7
Mean±sem for PM's	-	10.9 ±7.6	66.9 ±21.5	112.2 ±11.8	136.8 ±15.1	150.9 ±13.6	132.7 ±16.8	81.5 ±17.4	68.1 ±11.3

n.d. = not detected

Appendices X & XI Blood sugar and lactate concentrations (mmol/l) v/s time in the two phenotypes

X Blood sugar

Volunteer	Hours after a single oral dose of 50mg phenformin								
	0	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0
AW	4.2	3.8	3.8	3.9	4.0	4.9	4.0	3.8	3.1
EE	4.6	4.5	4.6	4.5	4.5	4.5	2.9	3.1	4.1
JR	4.8	4.4	4.6	4.5	4.5	4.3	4.3	4.7	4.6
AZ	4.0	4.0	4.2	3.3	3.8	3.9	4.5	4.4	4.2
Mean±sem for EM's	4.4 ±0.2	4.2 ±0.2	4.3 ±0.2	4.1 ±0.3	4.2 ±0.2	4.4 ±0.2	3.9 ±0.3	4.0 ±0.3	4.0 ±0.3
LE	4.0	4.1	4.1	4.1	4.3	4.7	3.5	3.4	4.5
AR	3.8	3.3	3.7	5.4	3.5	4.3	4.0	4.6	6.1
SJ	5.5	5.4	5.2	5.1	5.0	4.2	3.3	4.1	4.4
CS	4.6	4.5	4.5	4.9	4.4	4.8	7.2	3.7	5.0
Mean±sem for PM's	4.5 ±0.2	4.3 ±0.2	4.4 ±0.2	4.9 ±0.2	4.3 ±0.2	4.5 ±0.2	4.5 ±0.5	4.0 ±0.2	5.0 ±0.2

XI Blood lactate

Volunteer	Hours after a single dose of 50mg phenformin								
	0	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0
AW	0.55	0.36	0.40	0.42	0.36	0.38	0.43	0.43	0.39
EE	0.68	0.40	0.91	0.56	0.47	0.43	0.33	0.51	0.58
JR	0.44	0.43	0.40	0.40	0.36	0.29	0.36	0.36	0.90
AZ	0.34	0.36	0.40	0.51	0.33	0.36	0.30	0.36	0.18
Mean±sem for EM's	0.50 ±0.08	0.39 ±0.02	0.53 ±0.13	0.47 ±0.04	0.38 ±0.03	0.37 ±0.03	0.36 ±0.03	0.42 ±0.04	0.51 ±0.16
LE	0.45	0.71	0.60	0.90	1.23	0.72	0.40	0.47	0.37
AR	0.36	0.52	0.43	0.52	0.47	0.59	0.56	0.55	0.55
SJ	0.48	1.03	0.72	0.76	0.65	1.01	0.43	0.72	0.58
CS	0.66	1.03	0.97	1.46	0.79	1.06	1.27	1.22	1.29
Mean±sem for PM's	0.49 ±0.07	0.82 ±0.13	0.68 ±0.12	0.91 ±0.20	0.79 ±0.16	0.85 ±0.12	0.67 ±0.21	0.74 ±0.17	0.70 ±0.21

Appendices XII & XIII Blood pyruvate concentrations (μmol/l) and lactate/pyruvate ratios v/s time in the two phenotypes

XII Blood pyruvate

Volunteer	Hours after a single oral dose of 50mg phenformin								
	0	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0
AW	45	37	44	37	40	48	51	46	43
EE	60	52	70	52	47	47	44	53	31
JR	60	56	44	49	36	43	47	35	27
AZ	32	28	38	53	37	36	27	26	26
Mean±sem for EM's	49 ±7	43 ±7	49 ±7	48 ±4	40 ±3	44 ±3	42 ±6	40 ±6	32 ±4
LE	57	54	52	54	60	58	46	45	41
AR	58	57	47	64	48	48	48	43	36
AJ	46	67	56	62	54	77	54	66	34
CS	56	62	64	54	54	44	92	39	70
Mean±sem for PM's	54 ±3	60 ±3	55 ±4	59 ±3	54 ±3	57 ±8	60 ±11	48 ±6	45 ±9

XIII Blood lactate/pyruvate ratio

Volunteer	Hours after a single oral dose of 50mg phenformin								
	0	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0
AW	12.2	9.7	9.1	11.4	9.0	7.9	8.4	9.3	9.1
EE	11.3	7.7	13.0	10.8	10.0	9.1	7.5	9.6	18.7
JR	7.3	7.7	9.1	8.2	10.0	6.7	7.7	10.3	33.3
AZ	10.6	12.8	10.5	9.6	8.9	10.0	11.1	13.8	6.9
Mean±sem for EM's	10.4 ±1.1	9.5 ±1.2	10.4 ±0.9	10.0 ±0.7	9.5 ±0.3	8.4 ±0.7	8.7 ±0.8	10.8 ±1.1	17.0 ±6.0
LE	7.9	13.1	11.5	16.7	20.5	12.4	8.7	10.4	9.0
AR	6.2	9.1	9.1	8.1	9.8	12.3	11.7	12.8	15.3
SJ	10.4	15.4	12.9	12.3	12.0	13.1	8.0	10.9	17.1
CS	11.8	16.6	15.2	27.0	14.6	24.1	13.8	31.3	18.4
Mean±sem for PM's	9.1 ±1.3	13.6 ±1.7	12.2 ±1.3	16.0 ±4.1	14.2 ±2.3	15.5 ±2.9	10.6 ±1.4	16.4 ±5.0	15.0 ±2.1

Appendix XIV Details and debrisoquine oxidation status of 128 maturity-onset diabetic patients

Patient	Age (y)	Sex	Weight (kg)	Height (cm)	Smoker	Drug therapy*	Debrisoquine Recovery (%)	Debrisoquine Ratio
1	68	M	91	179	-	-	39.4	0.6
2	66	F	83	163	-	-	24.2	0.4
3	69	F	73	160	+	G,D,BB,Digoxin	22.7	0.9
4	59	M	64	180	+	-	42.1	0.3
5	65	M	89	165	-	G,Ketoprofen	24.8	0.2
6	53	F	67	152	+	Thyroxine	31.1	0.2
7	71	M	65	161	+	D,BB	22.7	0.3
8	45	F	61	155	-	-	41.9	1.2
9	79	F	54	155	-	G,Indomethacin	33.5	0.7
10	66	F	88	165	+	D	21.8	0.3
11	77	M	75	157	-	Inositol,Analgesics	31.5	0.2
12	56	M	70	174	+	Tolbutamide	49.0	0.1
13	63	F	91	165	-	D,Fluphenazine,Nortriptyline,Disopyramide	37.5	14.0
14	63	M	79	166	-	D,Hydralazine,Allopurinol	42.6	2.4
15	51	M	74	173	+	-	27.9	0.3
16	59	F	91	168	-	G,BB,D	24.4	4.3
17	63	M	71	170	-	BB,Spironolactone	37.7	1.1
18	51	M	77	163	+	-	44.7	5.7
19	69	F	89	156	-	-	27.4	0.3
20	71	F	70	152	+	D	30.0	0.6
21	62	F	70	151	+	-	28.3	0.2
22	69	M	76	185	-	C,BB,Digoxin	33.1	0.1
23	54	F	67	160	-	Indomethacin	43.8	0.5
24	76	F	65	155	-	-	29.4	4.0
25	56	F	50	161	-	Dothiepin,Clorazepate,Imipramine	41.9	14.5
26	71	F	71	168	-	Inositol,Analgesics	28.3	1.2
27	66	F	60	165	-	Methyldopa	18.9	2.6
28	87	F	64	168	-	G	20.1	2.1
29	46	M	80	170	+	-	39.9	0.3
30	56	F	91	160	-	Amitriptyline,Perphenazine	52.1	0.3
31	60	F	76	168	-	G,Diazepam	77.7	0.2
32	56	F	73	160	+	Amitriptyline,Perphenazine	58.6	6.0
33	56	F	57	168	+	-	31.6	0.3
34	48	M	77	175	-	-	24.6	6.0
35	62	F	55	165	-	Thyroxine	48.2	0.2
36	57	M	93	187	-	-	47.3	0.8
37	72	F	65	160	-	G	21.7	0.5
38	63	M	75	178	-	G,Analgesics	85.2	1.0
39	75	F	59	163	-	D,Tolbutamide,Guanethidine	37.3	0.1
40	54	M	79	175	-	Nitrates,Nifedipine	36.0	2.9
41	66	F	62	165	-	G	26.9	0.4
42	78	F	58	157	+	G	36.5	0.3
43	61	M	86	182	+	G	17.9	0.3
44	43	M	78	173	-	-	48.5	0.2
45	72	M	69	169	+	C	21.7	1.0
46	50	F	89	168	-	G,BB,D,Prazosin,Clonidine,Amiloride	36.5	51.1
47	75	F	58	178	-	Tolbutamide	19.2	0.4
48	59	F	92	160	-	G	89.4	117.8
49	67	M	75	178	+	G,Indomethacin,Prednisolone	62.5	38.1
50	71	F	80	165	+	G,Hydralazine,Prazosin	83.4	0.8
51	68	F	71	157	-	C,BB,D,Tamoxifen	32.1	0.9
52	51	M	77	171	+	-	32.6	0.5
53	58	M	91	185	-	Digoxin	22.3	0.2
54	73	F	78	168	-	G,D,Nitrates	32.2	0.4
55	52	M	83	178	-	C	35.6	0.3
56	43	M	65	168	+	G	49.6	0.2
57	65	M	61	178	-	C,Diazepam	20.0	1.7
58	83	M	94	175	-	G,BB,D,Amylobarbitone	22.1	0.5
59	73	M	70	174	+	G,Phyllocontin	26.5	0.1
60	57	M	83	173	-	-	19.6	0.8
61	80	F	63	157	-	BB,Nitrazepam	40.5	0.4
62	78	F	65	175	-	G,Thyroxine	27.5	0.2
63	59	M	99	183	-	G,D,Digoxin,Quinidine	34.8	1.3
64	75	F	73	157	-	C,D,BB,Ibuprofen,Amiloride,Analgesics	19.5	1.2
65	61	F	53	157	-	G,BB,Tamoxifen,Analgesics	35.2	0.3
66	70	M	51	168	-	-	7.6	0.4
67	74	M	63	164	-	-	34.6	4.7
68	68	M	75	174	+	G	32.6	0.7
69	54	M	72	173	-	C	56.5	0.4
70	46	M	76	168	-	G	28.4	0.3

* G = Glibenclamide C = Chlorpropamide D = Diuretics BB = β-Blockers

Appendix XIV cont.

Patient	Age (y)	Sex	Weight (kg)	Height (cm)	Smoker	Drug therapy*	Debrisoquine Recovery (%)	Debrisoquine Ratio
71	51	M	86	165	+	C,D,Spironolactone	21.6	0.4
72	67	M	85	178	+	G	43.2	1.5
73	50	M	108	178	+	-	28.4	0.2
74	55	F	59	166	-	G	20.5	0.4
75	72	F	67	165	-	C,D,Digoxin,Methyldopa	23.4	0.3
76	54	F	89	155	+	D,Phenformin,Ketoprofen	40.6	1.9
77	58	F	62	157	+	G	26.1	0.2
78	67	F	88	170	-	G,Thyroxine,Methyldopa	20.5	0.4
79	48	M	92	170	+	-	29.9	0.5
80	34	M	73	180	+	C	36.3	0.5
81	82	F	60	158	-	-	11.0	2.1
82	69	F	73	160	-	G,D,Spironolactone	22.5	0.4
83	60	M	66	163	-	G	37.8	0.4
84	68	F	60	160	+	BB,D,Tolbutamide	16.8	0.3
85	64	M	86	173	-	-	36.6	0.6
86	65	M	59	165	-	Phenformin	32.0	0.3
87	43	F	64	155	-	G	37.3	0.4
88	48	M	60	177	-	G	21.3	1.2
89	71	F	92	164	-	C	32.0	0.4
90	58	M	74	165	-	G	29.4	0.3
91	67	F	88	165	-	C	47.1	15.8
92	43	M	90	188	+	C	43.6	3.5
93	64	F	58	145	-	-	36.7	1.5
94	70	M	57	170	+	G	24.7	0.5
95	73	M	62	164	-	C,BB,D,Hydralazine	26.0	1.2
96	60	M	79	179	+	G	36.5	0.4
97	82	M	81	173	-	-	51.0	2.1
98	64	F	70	163	-	-	23.8	0.4
99	62	M	85	180	+	BB,D,Tolbutamide,Prazosin	12.5	0.6
100	51	M	79	165	-	G	29.3	0.3
101	49	M	82	170	+	C	33.1	0.5
102	73	F	86	157	-	G	6.4	3.3
103	62	M	83	168	-	-	36.4	0.4
104	59	M	84	185	+	G,Nifedipine,Trimeprazine,Disopyramide	12.7	7.5
105	72	F	80	165	-	C	34.9	1.1
106	67	M	64	177	+	C	33.9	0.2
107	76	F	67	159	-	C,BB,Hydralazine	38.4	0.8
108	66	M	68	179	-	G	34.7	0.9
109	75	M	76	178	-	C,Spironolactone	23.0	16.7
110	75	M	83	178	-	G	66.0	49.8
111	76	F	54	157	-	C,D	57.0	0.2
112	58	F	66	160	-	G	24.2	3.7
113	51	M	70	180	+	G	37.4	0.4
114	74	F	73	163	+	G	46.7	1.9
115	62	F	73	157	+	G	30.9	1.5
116	53	F	82	152	-	G,Benoxaprofen,Analgesics	45.9	24.5
117	77	M	68	180	-	C	24.8	0.2
118	58	M	82	178	-	C	38.2	0.5
119	50	F	43	160	+	G	29.6	0.3
120	63	M	76	166	-	G,BB,Allopurinol	47.0	1.6
121	57	M	86	163	+	G,BB,D	48.0	0.2
122	72	M	60	165	-	G,D,Amiloride,Nitrates,Disopyramide	19.0	3.0
123	69	M	87	168	-	C,BB,D,Digoxin	29.3	0.7
124	69	F	67	163	+	C,BB,Ibuprofen	19.8	32.0
125	44	M	95	183	+	G	25.1	0.5
126	68	M	67	178	-	-	18.9	1.9
127	70	F	67	168	-	-	26.6	0.5
128	61	F	75	171	+	Tolbutamide	30.7	0.5

* G = Glibenclamide C = Chlorpropamide D = Diuretics BB = β-Blockers

ADDENDUM AND CORRIGENDA

Section 6.5c (p 113)

Some mucodyne sulphoxides are unstable if exposed to room temperatures for long periods. Therefore, rapid deep-freezing or analysis of the urine sample after the completion of the test is essential. The individual consistency of mucodyne sulphoxidation has been confirmed by repeat biological tests (Waring RH et al., Biochem Pharmacol 1982; 31: 3151-3154) as well as by technical repeat tests on the same sample (RH Waring - personal communication).

Section 7.6 (p 182)

Price Evans and his colleagues (1960) have elegantly outlined the methods by which data from population and family studies can be treated to confirm genetic polymorphism. Based on the allele frequencies deduced from population study, the frequencies of various phenotypic mating combinations among the parents and the frequencies of their various phenotypic offsprings in pedigree studies can be calculated. If the genetic hypothesis is true, there should be no significant difference between the expected and the observed numbers. This is indeed the case with phenformin oxidation data (Tables A and B, overleaf) if poor oxidation phenotype is assumed to be an autosomal recessive trait. The whole analysis proves unsatisfactory if poor oxidation phenotype is assumed to be an autosomal dominant characteristic. Additionally, the identical twins in family 64 are both of the same phenotype and in the family with the critical poor x poor mating, there are no offsprings of extensive oxidation phenotype.

Section 7.6 (p 187)

To identify the heterozygote genotypes from the pedigree studies, an antimode of 20 has been employed. Their phenformin oxidation ratios range from 2.8 to 13.9 with a median ratio of 6.3 . These values are only the estimates because the identification of these 16 individuals is biassed by the consistent need to include a poor oxidiser in the family - the above values take no account of the families with only the heterozygote and the homozygote genotypes among them.

Section 7.11 (p 234)

Any possibility of artefacts in the frequency distributions due to the administration of concurrent drug treatments is excluded by the fact that the median metabolic ratio (0.5) of the 128 diabetic group as a whole is identical to those of the 28 diabetics on no treatment at all (0.5) and of the remaining 100 patients on various drugs (0.5).

Section 7.11 (p 236)

The χ^2-test was performed on data of actual expected and observed numbers of patients and not on percentages. The altered drug oxidising capacity among the diabetic patients of the EM phenotype can also be demonstrated simply by t-test on their normalised(by logarithmic transformation) data. The mean±S.D. (-0.22 ± 0.43 n=118) of the diabetic group is significantly ($p<0.0125$) different from the mean±S.D. (-0.09±0.44 n=145) of the age-matched non-diabetic group.

Section 7.9 (p 208)

In assessing the plasma phenformin pharmacokinetic parameters, the limitations of blood sampling upto only 8 hours post-dosing must be remembered. Furthermore, volunteers of the EM phenotype in that study have significantly ($p<0.01$ by t-test) lower urinary observed recovery of phenformin-related products than those of the PM phenotype.This may be due to the fact that EM volunteers produce 4-hydroxy-phenformin which by virtue of its greater polarity may have a wider volume of

Table A Genetic inference from mating frequencies

Putative Genotypic matings and frequencies			Phenotypic Mating Type	Expected Phenotypic Mating Frequency	Phenotypic Matings		$\frac{(O-E)^2}{E}$
					Expected Number out of 25 matings	Observed Number out of 25 matings	
♂ ♀	♂ ♀		♂ ♀				
$q^2 \times q^2$	0.09 x 0.09	0.0081	PM x PM	0.0081	0.20	1	3.200
$q^2 \times 2pq$	0.09 x 0.42	0.0378	PM x EM	0.0819	2.05	3	0.440
$q^2 \times p^2$	0.09 x 0.49	0.0441					
$2pq \times q^2$	0.42 x 0.09	0.0378	EM x PM	0.0819	2.05	2	0.001
$p^2 \times q^2$	0.49 x 0.09	0.441					
$p^2 \times p^2$	0.49 x 0.49	0.2401	EM x EM	0.8281	20.70	19	0.140
$2pq \times p^2$	0.42 x 0.49	0.2058					
$p^2 \times 2pq$	0.49 x 0.42	0.2058					
$2pq \times 2pq$	0.42 x 0.42	0.1764					
						$\chi^2 =$	3.781

Table B Genetic inference from offspring frequencies

Putative Genotypic Mating	Phenotypic Mating	Total Number of offspring	Frequencies of expected Putative genotypes of offspring			Expected and Observed numbers of offspring of different phenotype				χ^2 §
						PM		EM		
♂ ♀	♂ ♀		q^2	pq	p^2	Exp	Obs	Exp	Obs	
$q^2 \times q^2$	PM x PM	2	0.0081	0	0	2	2	0	0	-
$q^2 \times 2pq$	PM x EM	6*	0.0189	0.0189	0	1.4	2*	4.6	4	0.0093
$q^2 \times p^2$			0	0.0441	0					
$2pq \times q^2$	EM x PM	4	0.0189	0.0189	0	0.9	2	3.1	2	0.5161
$p^2 \times q^2$			0	0.0441	0					
$p^2 \times p^2$	EM x EM	44	0	0	0.2041	2.4	6	41.6	38	4.3157
$2pq \times p^2$			0	0.1029	0.1029					
$p^2 \times 2pq$			0	0.1029	0.1029					
$2pq \times 2pq$			0.0441	0.0882	0.0441					
								Total		= 4.8411

EM = Extensive Oxidiser of Phenformin

PM = Poor Oxidiser of Phenformin

25 analysable pedigrees included

* identical twins ascertained via proband 64 counted as one offspring

p = Allele for extensive oxidation

q = Allele for poor oxidation

§ With Yates's correction

Analysis satisfactory only if PM phenotype is autosomal recessive trait

distribution than phenformin. Consequently, the difference between the two phenotypes in phenformin oxidation capacity would be greater than that discerned from urine testing alone. However, any explanation offered requires caution since no differences regarding recovery of drug-related products are evident between the two phenotypes in the initial panel (Section 7.3) as well as the subsequent population (Section 7.5) studies.

Section 7.7 (p 194)
Based on the results of only 2 - 3 volunteers, the suggestion has been made that debrisoquine and phenformin oxidations may be controlled by closely linked and not identical genetic loci. However, the proof of this contention will require further detailed work, including complex pedigree studies, in order to identify the 4 different haplotypes.

Section 8.5 (p 275)
It must be appreciated that the risk of developing perhexiline neuropathy, inferred from the oxidation status of these 34 patients, is probably an overestimate since the patients may have been subject to a selection bias when being reported to the Committee on Safety of Medicines.

Section 8.6 (p 287)
The watch-repairer developed perhexiline neuropathy despite being of extensive metaboliser phenotype. His ratio of 5.7 ,denoting some impairment in drug oxidation capacity, may suggest a heterozygote genotype but only the pedigree study can clarify his genotype.

GENETIC POLYMORPHISM OF PHENFORMIN 4-HYDROXYLATION

N. S. OATES, PH.D.,
R. R. SHAH, M.R.C.P.,
J. R. IDLE, PH.D.
and
R. L. SMITH, PH.D., D.SC.
London, England

Department of Pharmacology, St. Mary's Hospital Medical School

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Genetic polymorphism of phenformin 4-hydroxylation

The ability to oxidize a single 50-mg dose of phenformin to its 4-hydroxy metabolite was determined in 195 individuals. Variations in the urinary ratio of phenformin/4-hydroxyphenformin ranged from 1 to 184. Family studies were consistent with the hypothesis that this variability resulted from a single gene mode of inheritance in which impaired hydroxylation of phenformin appears as an autosomal recessive trait. Both genotype frequencies and the degree of dominance of the extensive metabolizer phenotype over the recessive showed a remarkable resemblance to those described for debrisoquine 4-hydroxylation, which was confirmed by the high degree of correlation ($r_s = 0.785$, $P < 0.0001$) between the phenformin ratio and the debrisoquine metabolic ratio. Such close agreement between the metabolism of these drugs may indicate that the same genetic control is in operation. Such genetic polymorphism of phenformin hydroxylation may have important implications for therapeutic response and for the possibility of toxic effects in a few individuals.

N. S. Oates, Ph.D., R. R. Shah, M.R.C.P., J. R. Idle, Ph.D., and R. L. Smith, Ph.D., D.Sc., *London, England*
Department of Pharmacology, St. Mary's Hospital Medical School

It is becoming increasingly clear that there is marked interindividual variability in man's response to drugs and that such variability arises, to a large extent, from genetic differences in both kinetic and dynamic factors. Genetic polymorphisms of certain drug metabolic pathways, one of the major determinants of drug disposition, have already been described and include isoniazid acetylation,[8] debrisoquine 4-hydroxylation,[15] and sparteine dehydrogenation.[4] Each of these results from a single gene mode of inheritance that can usually be recognized by polymodal distribution of the particular property within the population and from appropriate family studies.[6]

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Reprint requests to: Dr. N. S. Oates, Department of Pharmacology, St. Mary's Hospital Medical School, London, W2 1PG, U. K.

The large interindividual differences in the rate of drug metabolism due to these polymorphisms may, in some cases, result in such widely differing responses that, while one individual may display the expected response to a drug, another who received the same dose may suffer an adverse reaction.[21] For example, subjects given debrisoquine who are poor hydroxylators of this drug are more prone to postural hypotension than are those who hydroxylate the drug extensively.[12]

Phenformin (β-phenethylbiguanide) may be used safely in most people who suffer from maturity onset diabetes, but in certain susceptible patients it may induce adverse reactions of varying degrees of severity. The development of the often fatal condition lactic acidosis[3] in a small proportion of diabetics treated with phenformin has led to its withdrawal in many countries and limitation of its use in others. Metabolism of phenformin in man is confined to the formation

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Table I. *Clinical details of the subjects*

Subject No.	*Age (yr)*	*Sex*	*Body weight (kg)*	*Alcohol* consumption*	*Smoking† habits*	*Subject No.*	*Age (yr)*	*Sex*	*Body weight (kg)*	*Alcohol* consumption*	*Smoking† habits*
1	22	F	61	+	0	54	31	F	62	+	++
2	20	F	59	0	0	55	24	F	56	+	0
3	21	F	57	+	0	56	39	M	86	+	++
4	19	F	51	+	0	57	29	F	51	+	++
5	19	F	47	+	0	58	21	F	50	+	0
6	20	F	58	+	0	59	24	F	61	+	++
7	20	F	55	+	+	60	30	M	67	+	0
8	32	F	56	+	+	61	27	F	61	+	0
9	22	F	64	+	0	62	28	F	67	+	0
10	24	F	56	+	+	63	23	F	65	+	+
11	23	M	70	+	0	64	27	M	61	+	0
12	23	F	61	+	0	65	20	F	48	+	0
13	21	M	70	+	0	66	27	M	64	++	++
14	20	F	57	+	+	67	19	F	56	+	0
15	20	F	61	+	+	68	20	F	55	+	0
16	21	F	64	+	+	69	20	F	62	+	0
17	29	F	61	+	+	70	19	F	59	+	0
18	23	F	62	+	0	71	21	F	52	+	0
19	22	F	62	+	++	72	20	F	52	+	0
20	22	F	57	+	0	73	19	F	64	+	0
21	22	F	57	+	0	74	19	F	49	+	0
22	21	F	46	+	0	75	19	F	56	+	0
23	22	F	64	+	0	76	19	F	41	+	0
24	20	F	50	+	0	77	19	F	56	+	0
25	21	F	51	+	0	78	19	F	61	+	0
26	20	F	61	+	0	79	19	F	65	+	++
27	21	F	48	+	+	80	19	F	54	+	++
28	21	F	64	+	+	81	23	F	61	0	0
29	21	F	58	+	0	82	26	F	60	+	0
30	27	M	64	+	0	83	20	F	51	+	+
31	26	M	74	++	0	84	19	F	55	+	++
32	21	M	74	++	0	85	20	F	57	+	0
33	20	F	56	+	0	86	19	M	70	++	0
34	27	M	84	+	0	87	20	F	54	+	+
35	22	M	72	+	0	88	22	F	61	+	+
36	23	F	55	+	0	89	28	F	55	+	++
37	23	F	61	+	0	90	28	M	71	+	++
38	22	M	80	+	0	91	22	F	57	+	0
39	28	M	73	+	0	92	19	F	53	+	0
40	20	F	57	+	0	93	2	M	58	+	0
41	22	F	64	+	0	94	24	F	64	+	0
42	29	M	82	+	+	95	20	F	63	0	0
43	22	M	76	+	0	96	22	M	99	++	0
44	52	M	73	+	0	97	23	M	73	++	+
45	34	M	86	0	0	98	26	M	146	+	++
46	27	F	59	+	0	99	21	F	55	0	0
47	31	M	74	+	0	100	19	F	67	0	0
48	24	F	56	+	0	101	35	F	51	+	++
49	24	F	51	+	+	102	43	F	57	+	++
50	21	M	61	+	0	103	21	F	59	+	++
51	24	M	55	+	0	104	21	F	59	+	++
52	30	M	57	+	+	105	18	F	48	+	+
53	22	F	61	0	0	106	22	F	51	+	+

*0 = none; + = occasional; ++ = moderate.

†0 = none; + = 1-15 cigs/day; ++ = >15 cigs/day.

Table I—*cont'd*

Subject No.	*Age (yr)*	*Sex*	*Body weight (kg)*	*Alcohol* consumption*	*Smoking† habits*	*Subject No.*	*Age (yr)*	*Sex*	*Body weight (kg)*	*Alcohol* consumption*	*Smoking† habits*
107	21	F	61	+	0	152	19	F	59	+	+
108	21	F	54	+	0	153	19	M	83	+	0
109	21	F	57	+	0	154	19	M	67	+	0
110	19	F	48	+	0	155	19	M	87	++	+
111	38	F	57	+	0	156	19	M	82	+	0
112	21	F	64	+	0	157	20	M	76	+	+
113	21	F	61	+	++	158	20	F	60	+	+
114	21	F	64	+	0	159	20	M	64	+	0
115	26	M	65	+	0	160	20	F	54	+	0
116	21	F	48	+	0	161	25	F	50	+	0
117	46	M	59	+	0	162	20	M	61	+	0
118	21	F	47	+	+	163	19	M	74	+	0
119	23	F	61	+	++	164	19	F	64	++	0
120	21	M	83	++	0	165	20	M	53	+	0
121	21	F	70	+	0	166	19	M	53	+	0
122	21	F	61	+	0	167	20	M	91	+	0
123	21	F	76	+	0	168	20	M	67	+	0
124	21	F	64	+	0	169	19	F	53	+	0
125	27	M	72	++	++	170	20	M	86	+	+
126	24	F	57	+	+	171	19	M	74	+	0
127	37	F	59	+	0	172	20	M	69	+	0
128	23	M	55	+	0	173	19	M	83	+	0
129	23	M	64	+	0	174	19	M	76	+	0
130	21	F	54	+	0	175	20	M	65	+	0
131	20	F	67	+	++	176	20	M	80	0	0
132	21	F	61	+	+	177	21	M	76	+	++
133	23	F	67	+	+	178	19	M	69	+	0
134	19	M	80	+	0	179	19	M	61	+	0
135	20	M	79	+	0	180	21	M	76	+	0
136	20	M	67	+	0	181	20	M	89	+	0
137	20	M	68	+	0	182	19	M	83	+	0
138	19	M	54	0	0	183	20	M	73	+	0
139	20	F	55	+	0	184	20	M	67	+	++
140	20	M	64	+	0	185	19	F	52	+	0
141	20	M	64	+	0	186	19	F	70	+	0
142	19	F	59	0	0	187	19	F	52	+	0
143	19	M	93	+	0	188	19	F	55	+	0
144	20	F	60	0	0	189	20	F	48	0	0
145	19	F	55	+	0	190	19	F	55	+	0
146	19	F	60	+	+	191	20	M	67	+	0
147	25	F	52	+	0	192	19	M	89	+	0
148	20	F	55	+	0	193	24	F	48	0	0
149	19	M	70	+	0	194	19	M	70	+	0
150	22	F	62	+	0	195	20	M	73	+	0
151	19	F	55	+	0						

of a single oxidized product, 4-hydroxyphenformin,[1] which, together with unchanged drug, is cleared from the body virtually exclusively by the kidneys. Our study was undertaken to investigate whether or not phenformin hydroxylation is polymorphic in a British white population and, if so, how such a polymorphism might explain variable responses to the drug[17] and the development of lactic acidosis.

Methods

Our subjects were 72 men and 123 women, all of whom were Caucasians in good health and who were recruited from the staff and students

Table II. *Urinary recoveries of phenformin and 4-hydroxyphenformin and PR*

Subject No.	*Phenformin (%/8 hr)*	*4-OH-Phen (%/8 hr)*	*PR*	*Subject No.*	*Phenformin (%/8 hr)*	*4-OH-Phen (%/8 hr)*	*PR*
1	26.2	9.6	2.7	57	26.8	3.8	7.1
2	44.6	2.8	15.9	58	42.6	4.8	8.9
3	39.6	6.0	6.6	59	19.2	5.2	3.7
4	28.2	11.6	2.4	60	33.6	9.2	3.7
5	27.2	9.2	3.0	61	22.6	6.8	3.3
6	18.2	4.4	4.1	62	48.6	4.8	10.1
7	34.0	7.0	4.8	63	29.0	2.2	13.2
8	48.6	1.6	30.4	64	45.2	1.8	25.1
9	14.6	9.4	1.6	65	27.2	4.2	6.5
10	18.2	4.2	4.3	66	38.0	4.2	9.0
11	22.8	11.4	2.0	67	25.4	10.2	2.5
12	24.2	1.8	13.4	68	21.8	8.4	2.6
13	23.8	4.4	5.4	69	26.4	9.8	2.7
14	27.8	5.6	5.0	70	52.0	7.8	6.7
15	41.6	1.8	23.1	71	33.2	5.0	6.6
16	18.6	12.2	1.5	72	22.4	6.2	3.6
17	12.8	1.2	10.7	73	46.6	2.8	16.6
18	42.2	7.6	5.6	74	32.4	3.0	10.8
19	27.2	8.2	3.3	75	22.6	4.2	5.4
20	26.6	7.8	3.4	76	28.8	3.6	8.0
21	14.0	7.4	1.9	77	32.4	1.2	27.0
22	23.0	6.0	3.8	78	44.6	15.8	2.8
23	36.2	0.6	60.3	79	49.0	6.2	7.9
24	31.0	3.8	8.2	80	22.2	10.4	2.1
25	31.2	4.0	7.8	81	24.4	6.8	3.6
26	29.0	4.4	6.6	82	41.6	6.6	6.3
27	17.8	2.0	8.9	83	22.4	3.4	6.6
28	16.6	8.0	2.1	84	28.2	2.4	11.8
29	16.4	9.6	1.7	85	21.2	10.4	2.0
30	31.6	10.2	3.1	86	35.4	7.2	4.9
31	38.6	3.6	10.7	87	30.2	6.8	4.4
32	43.8	1.0	43.8	88	22.2	2.4	9.3
33	19.6	20.6	1.0	89	36.6	0.8	45.8
34	45.2	14.0	3.2	90	29.8	9.2	3.2
35	24.0	26.8	0.9	91	36.8	9.8	3.7
36	36.0	17.0	2.1	92	56.0	0.4	140
37	24.8	12.4	2.0	93	35.2	5.8	6.1
38	24.8	2.4	10.3	94	38.2	2.6	14.7
39	39.8	16.8	2.4	95	28.2	14.8	1.9
40	16.2	0.6	27.0	96	39.6	9.2	4.3
41	61.0	5.0	12.2	97	30.8	8.8	3.5
42	32.2	7.6	4.2	98	40.2	2.8	14.4
43	62.6	7.0	8.9	99	26.6	9.6	2.8
44	24.8	5.8	4.3	100	22.8	7.6	3.0
45	16.8	4.4	3.8	101	23.4	10.0	2.3
46	31.8	7.0	4.5	102	42.2	10.2	4.1
47	19.0	3.0	6.3	103	36.6	9.8	3.7
48	28.8	11.2	2.6	104	32.6	8.6	4.0
49	12.8	7.2	1.8	105	30.2	7.4	4.1
50	33.4	12.2	2.7	106	25.2	11.8	2.1
51	21.4	12.2	1.8	107	29.0	14.4	2.0
52	25.6	11.2	2.3	108	30.4	31.6	1.0
53	43.2	0.8	54.0	109	42.2	1.0	42.2
54	15.4	4.6	3.3	110	41.0	7.0	5.9
55	55.2	8.4	6.6	111	51.8	1.6	32.4
56	8.6	1.2	7.2	112	36.2	12.2	3.0

Table II—*cont'd*

Subject No.	*Phenformin (%/8 hr)*	*4-OH-Phen (%/8 hr)*	*PR*	*Subject No.*	*Phenformin (%/8 hr)*	*4-OH-Phen (%/8 hr)*	*PR*
113	28.2	5.8	4.9	155	46.8	16.8	2.8
114	29.2	2.8	10.4	156	40.6	11.4	3.6
115	20.6	11.0	1.9	157	41.6	6.8	6.1
116	30.8	8.4	3.7	158	38.4	9.8	3.9
117	11.6	5.4	2.1	159	36.2	12.4	2.9
118	28.2	12.0	2.4	160	37.6	13.0	2.9
119	43.6	3.0	14.5	161	32.2	10.0	3.2
120	44.8	4.2	10.7	162	30.2	10.8	2.8
121	26.4	5.4	4.9	163	37.8	11.2	3.4
122	20.4	11.8	1.7	164	39.0	7.4	5.3
123	24.6	12.4	2.0	165	12.8	2.0	6.4
124	44.8	8.2	5.5	166	36.8	0.2	184
125	33.8	5.8	5.8	167	26.4	7.4	3.6
126	25.2	4.0	6.3	168	39.4	12.4	3.2
127	18.8	9.8	1.9	169	52.2	1.4	37.3
128	48.6	1.8	27.0	170	42.6	15.6	2.7
129	35.6	1.2	29.7	171	27.4	18.6	1.5
130	29.2	7.0	4.2	172	46.2	8.2	5.6
131	24.2	13.0	1.9	173	52.4	8.0	6.6
132	29.2	0.8	36.5	174	60.6	29.0	2.1
133	24.2	5.8	4.2	175	46.8	2.6	18.0
134	36.0	10.2	3.5	176	55.2	9.8	5.6
135	68.6	8.2	8.4	177	35.2	18.6	1.9
136	45.4	9.6	4.7	178	30.6	11.2	2.7
137	51.8	15.2	3.4	179	42.0	28.6	1.5
138	44.2	2.6	17.0	180	27.2	16.4	1.7
139	28.0	4.6	6.1	181	73.4	15.2	4.8
140	26.4	3.2	8.3	182	50.8	6.4	7.9
141	35.2	13.8	2.6	183	70.8	3.6	19.7
142	47.2	5.6	8.4	184	25.6	15.4	1.7
143	44.0	6.2	7.1	185	40.2	10.2	3.9
144	28.0	5.6	5.0	186	28.8	12.6	2.3
145	27.0	18.6	1.5	187	34.0	17.4	2.0
146	42.0	1.0	42.0	188	48.4	7.8	6.2
147	76.0	11.8	6.4	189	36.0	6.4	5.6
148	33.2	9.2	3.6	190	43.2	14.2	3.0
149	23.4	11.8	2.0	191	38.2	14.6	2.6
150	55.0	5.2	10.6	192	39.6	12.0	3.3
151	27.4	3.8	7.2	193	51.4	10.2	5.0
152	46.2	9.4	4.9	194	38.0	7.2	5.3
153	43.0	8.6	5.0	195	38.8	11.6	3.3
154	46.8	11.8	4.0				

of St. Mary's Hospital and Medical School (Table I). Their ages ranged from 18 to 52 yr (mean 22.3) and their body weights from 41.4 to 99 kg (mean 63.5), with the exception of one individual who weighed 146 kg. None consumed more than moderate quantities of alcohol, but 23 regularly smoked more than 15 cigarettes each day. Close relatives of 27 of these subjects also participated in the study; these subjects were also in good health.

Experimental procedure. Each subject was given a single oral dose of 50 mg phenformin (Dibotin) after an overnight fast and was asked to take no other medication either before or during the study. Urine was collected for 8 hr after dosing; after recording the total volume, a small sample was stored at −20° for analysis. On a separate occasion 101 of the same subjects were given 10 mg debrisoquine (Declinax) and urine was collected in an identical manner.

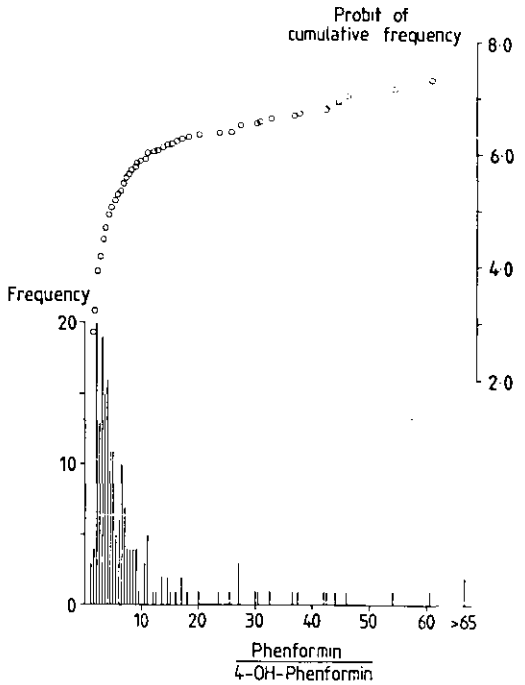

Fig. 1. The distribution of phenformin ratios in 195 subjects.

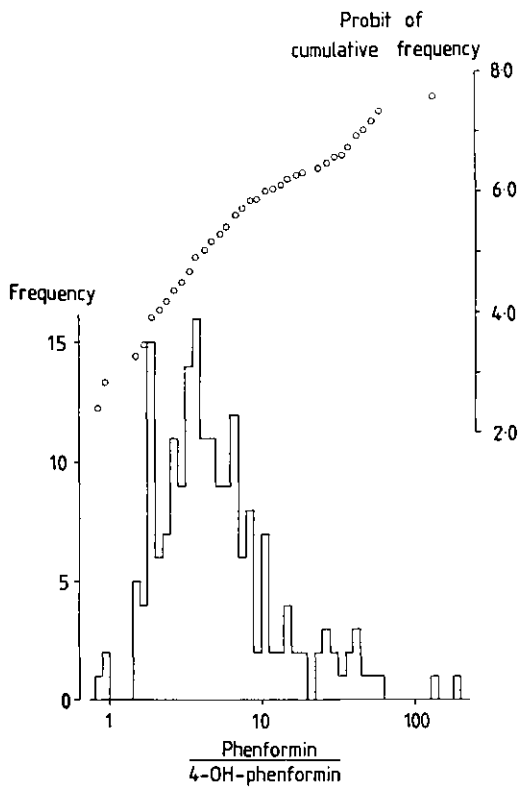

Fig. 2. The distribution of phenformin ratios in 195 subjects, plotted on a logarithmic scale.

Analytical methods. The urinary excretion of phenformin and 4-hydroxyphenformin was measured as reported.[18] Briefly, this entailed the extraction of phenformin and its metabolite from urine onto Amberlite XAD-2 resin (BDH), eluting with methanol followed by high-performance liquid chromatography (HPLC) on a reversed-phase radial compression column (Waters Associates). Debrisoquine and 4-hydroxydebrisoquine were measured by gas-liquid chromatography after derivatization with hexafluoroacetylacetone.[11]

The results from the analysis of both drugs were expressed in terms of the total excretion of the parent compounds and their metabolites in the 8-hr collection period. The following ratio was calculated from these results:

$$\frac{\text{Excretion of unchanged drug in 8 hr}}{\text{Excretion of hydroxylated metabolite in 8 hr}}$$

In the case of debrisoquine this expression has been termed the "metabolic ratio."[15]

Results and discussion

The urinary excretion of phenformin and 4-hydroxyphenformin, expressed as the percentage of the 50-mg dose recovered in the 8-hr collection period, is given in Table II for each of the subjects. Total recovery of drug and metabolite varied widely between individuals and ranged from 9.8% to 89.6%. To avoid complications arising from these differences in recovery, the metabolism of phenformin was expressed as the ratio of the excretion of unchanged drug to that of the 4-hydroxy metabolite. This ratio for recovery of phenformin : recovery of 4-hydroxyphenformin (PR) (Table II) is entirely analagous to that used in studies in the oxidation of the drug debrisoquine.[15]

There were marked interindividual differences in PR, with values ranging from 0.9 to 184, the median being 4.3. The distribution of these ratios within the population studied (Fig. 1) was skewed to the left with the result that the probit transformation of the data deviated from linearity ($\chi^2 \approx 862$, $P < 0.0001$).[9] On plotting the same results on a semilogarithmic scale (Fig. 2) the distribution obtained more closely resembled a normal distribution. Once again, however,

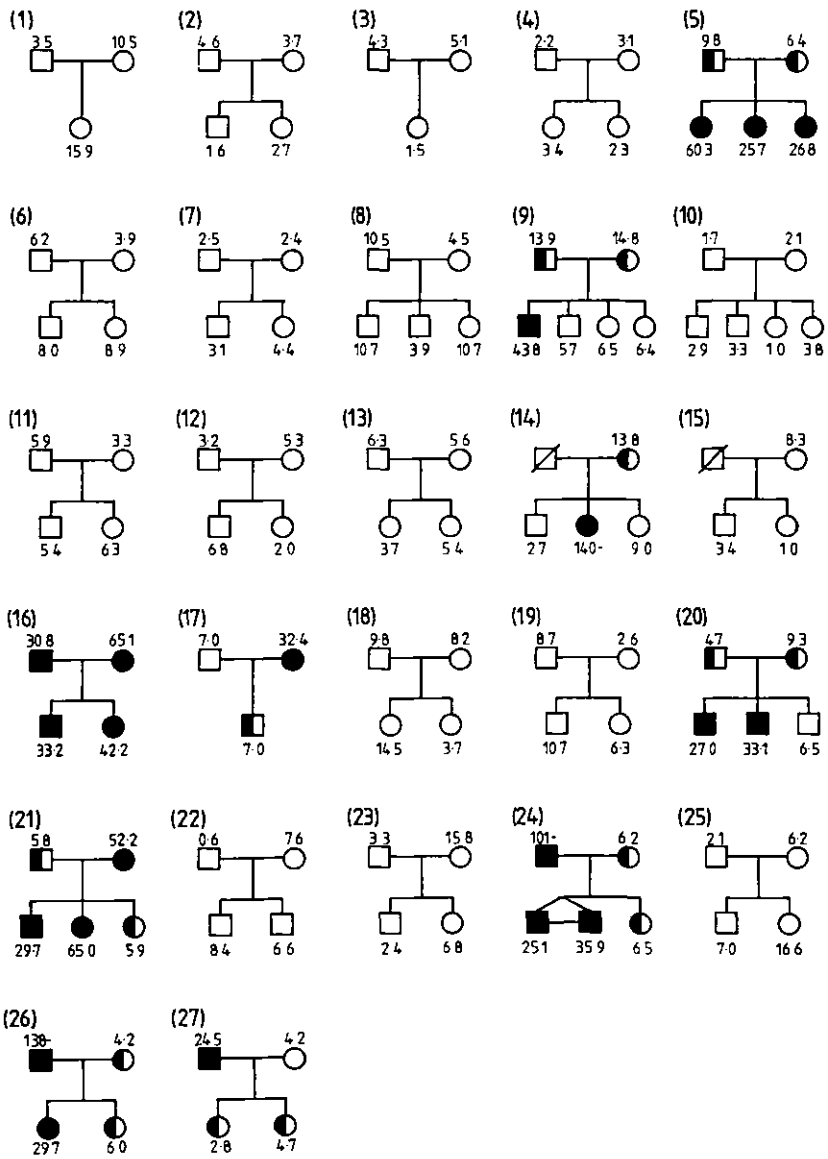

Fig. 3. The pedigrees of 27 families in whom phenformin ratios have been determined as indicated by the values shown. □ = male; ○ = female; ⁄□ = deceased; ■ = homozygous impaired metabolizer; ◧ = individuals who can be identified as heterozygotes with respect to impaired 4-hydroxylation of phenformin.

probit analysis did not display a straight line relationship with PR ($\chi^2 = 279$, $P < 0.0001$), but on this occasion there was an inflection in the line between ratios of approximately 10 and 35. This may be taken as evidence of bimodality of this distribution, with an antimode close to 20.

Such bimodal distribution provides evidence that phenformin metabolism might be under genetic control by a single pair of alleles providing two phenotypically distinct groups of individuals: those capable of a high degree of phenformin metabolism, and therefore, having low values for PR, and those displaying an impairment in phenformin oxidation having high PR. To investigate this possibility further, studies were carried out on the families of a number of the original subjects.

Twenty-seven families were studied and their

pedigrees are shown, together with PR values, in Fig. 3. Those with a PR greater than 20 were designated as impaired oxidizers of phenformin. The relationships of 21 such individuals within these families were consistent with the view that this property was inherited as an autosomal recessive trait. If this were true, the Hardy-Weinberg equation[5] would enable the calculation of genotype frequencies from the observed occurrence of impaired phenformin metabolism. Thus, the 18 subjects of the original population who had PR values above 20 were all homozygous for the recessive allele and this genotype was, therefore, observed at a frequency of 0.092. The remainder of the population comprised both heterozygotes and individuals homozygous for the dominant phenotype of high phenformin metabolism, the frequencies of these genotypes being calculated as 0.422 and 0.486. Furthermore, the allele responsible for impaired phenformin metabolism was calculated to occur at a frequency of 0.303 ± 0.034 (SEM).[5]

These calculated genotype frequencies bore a close resemblance to those reported[7] for the inheritance of debrisoquine 4-hydroxylation in the British white population, which provided values as follows: homozygous poor metabolizers 0.089, heterozygotes 0.419, and homozygous extensive metabolizers 0.492. The frequency of the allele controlling poor debrisoquine hydroxylation[7] did not differ from that calculated for phenformin ($P > 0.1$).

In view of the apparent close relationship between the genetic control of debrisoquine oxidation and that of phenformin, the ability to hydroxylate debrisoquine was also studied in 101 of the subjects. The oxidation of these drugs displayed a high degree of correlation ($r_s = 0.785$, $P < 0.0001$) indicating that, at the least, the genes controlling the metabolism of debrisoquine and phenformin are positioned closely together on the genome. Evans et al.[7] have estimated that the phenotype for extensive debrisoquine hydroxylation shows an approximately 30% dominance over the recessive; the same calculations applied to the data obtained for phenformin provided a value of 35% dominance, demonstrating again a high degree of agreement.

Further evidence on the relationship between the genetic mechanisms controlling the metabolism of the two drugs may be obtained by studies on distinct ethnic groups. The frequency at which debrisoquine poor metabolizers occur varies between races; those described, other than British Caucasians, include, Ghanaians 6%,[22] Nigerians 8.6%,[16] and Swedes 1.5%.[2] If the same genetic control is exerted over these two metabolic pathways, then similar frequencies of poor metabolizers should be found for both drugs. One example has already been described in which this appears to be true. In Saudi Arabs the frequency of poor metabolizers of debrisoquine was found to be 1%[14] and in recent preliminary findings a low incidence of individuals with impaired phenformin metabolism was observed in the same population.[10] Additional studies of this type will be necessary to confirm this relationship.

Interindividual differences in phenformin metabolism have already been described in small panels of subjects.[13] Our results confirm those findings in a large scale population study and, together with family studies, have provided evidence of single gene inheritance of this property closely related to that for debrisoquine 4-hydroxylation. The responses achieved by standard doses of phenformin exhibit wide variation between patients[17]; one of the major factors in this variation may well be differences in the metabolic drug clearance. In support of this, blood lactate levels after a single dose of phenformin have been found to be greater in subjects with impairment of phenformin metabolism than in others.[13] Those individuals who are less able to oxidize phenformin may also be more susceptible to toxic effects and impaired oxidation may be one of the predisposing factors to phenformin-induced lactic acidosis.[19]

In more general terms, the possibility of such variability should, perhaps, be recognized at an early stage in the development of new drugs before the associated kinetic, therapeutic, and toxicologic problems become apparent. In persuance of this we have advocated the use of debrisoquine to determine oxidation phenotype in volunteers and patients undergoing drug therapy or taking part in investigations.[20] The use of

phenformin, together with a simple and rapid HPLC assay, provide the basis of an alternative determination of a drug oxidation phenotype, thus identifying subjects with various degrees of related drug oxidation impairment that may have a bearing upon the kinetics of, and response to, a variety of drugs.

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Prediction of subclinical perhexiline neuropathy in a patient with inborn error of debrisoquine hydroxylation

Rashmi R. Shah, B.Sc., M.R.C.P., Nicholas S. Oates, Ph.D., Jeffrey R. Idle, Ph.D., Robert L. Smith, D.Sc., and J. Dennis F. Lockhart, M.B.B.Ch., D.P.H. *Paddington and Middlesex, England*

Perhexiline is an effective antianginal agent.[1] Its use however, has been associated with occasional serious side effects, notably peripheral neuropathy,[2] severe weight loss,[1] and effects on hepatic function, including cirrhosis.[3] Perhexiline is metabolized by oxidation to more polar hydroxylated metabolites prior to its elimination.[4] Recently, human studies of debrisoquine metabolism have shown the occurrence of a genetic polymorphism in drug oxidation, approximately 9% of British white persons being defective in this respect.[5] Furthermore, individuals with impaired debrisoquine oxidizing ability cannot metabolize normally a number of other drugs.[5] As a consequence, they are susceptible to various adverse drug reactions such as debrisoquine-induced postural hypotension, phenformin-induced lactic acidosis, phenacetin-induced methemoglobinemia, and nortriptyline-induced vertigo, dizziness, and confusional state.[6] We describe a patient for whom the determination of debrisoquine hydroxylation status was of value in alerting us to investigating and demonstrating the existence of subclinical peripheral neuropathy, which might otherwise have escaped recognition until it had developed to the point of becoming clinically overt.

G.A., a 60-year-old man with a past medical history of duodenal ulcer and treatment with cimetidine, was admitted in 1978 with extensive anterior myocardial ischemia. His ECG showed severe ST-T changes without any accompanying changes in serum enzyme levels. Following recovery, he continued to experience angina at rest, during

From the Department of Biochemical and Experimental Pharmacology, St. Mary's Hospital Medical School, and Merrell Pharmaceuticals Limited.

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Reprint requests: Rashmi R. Shah, B.Sc., M.R.C.P., Department of Biochemical/Experimental Pharmacology, St. Mary's Hospital Medical School, Paddington, London W2 1PG, England.

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Table I. Electromyographic data after treatment with perhexiline, 300 mg/day

	*Patient G.A.: age 60 yr, male, ratio 18.1**		*Patient K.B.: age 62 yr, male, ratio 0.6**		*Patient M.C.: age 50 yr, male, ratio 0.6**	
	33 wk†	*16 wk‡*	*29 wk†*	*48 wk†*	*22 wk†*	*41 wk†*
Sensory conduction						
Right median nerve						
Latency (msec)	4.8	3.5	3.6	3.0	2.5	2.4
Amplitude (μV)	3.0	5.0	16.0	10.0	22.0	10.0
Right ulnar nerve						
Latency (msec)	α	3.4	3.4	2.8	2.5	2.3
Amplitude (μV)	0	2.5	13.0	8.0	15.0	10.0
Motor conduction						
Velocity (m/sec)	50.0	51.0	n.d.	55.0	n.d.	n.d.
EMG conclusion	Demyelinating, predominantly sensory, neuropathy	Neuropathic changes, particularly sensory, resolving	No evidence of neuropathy	No evidence of neuropathy	No evidence of neuropathy	No evidence of neuropathy

n.d. = not determined; α = action potentials undetectable.
*Debrisoquine metabolic ratio.
†Duration of perhexiline treatment.
‡After stopping treatment with perhexiline.

emotional stress, and at night, with a total of about 12 to 15 episodes daily. Beta blockers and nifedipine did not prove to be of substantial benefit. There were no abnormal cardiovascular, respiratory, or neurologic physical signs, and his routine hepatic and renal biochemical tests were normal. Coronary angiography revealed normal left ventricular function without major coronary arterial obstruction.

The patient began receiving perhexiline, 100 mg twice daily, and in view of encouraging therapeutic response and normal liver function tests 4 weeks later, the dose was increased to 100 mg three times a day. At regular follow-up visits, he reported considerable reduction in anginal episodes and had no neurologic complaints. During this follow-up, he maintained normal liver function tests on five occasions apart from one minor elevation of serum aspartate aminotransferase concentration to 75 U/L (normal range 7 to 40 U/L), which dropped to 46 U/L during continued perhexiline therapy. Following a total of 33 weeks of perhexiline therapy, the patient's phenotype was determined with 10 mg oral debrisoquine for his hydroxylation status.[5] Treatment continued with atenolol, cimetidine, perhexiline, and isosorbide dinitrate. He was found to have a metabolic ratio of 18.1, confirming that he was of poor metabolizer phenotype. In view of this finding, an electromyogram (EMG) was obtained 3 days later, and it revealed definite evidence of subclinical demyelinating, predominantly sensory, neuropathy. Perhexiline was therefore discontinued and replaced by prenylamine lactate, 60 mg three times a day, although this agent has not proved equally beneficial. The plasma elimination half-life of perhexiline in this patient was estimated to be 9.5 days. A repeat EMG 16 weeks later confirmed considerable resolution of neuropathy, the improvement being particularly marked in the sensory component.

The patient's EMG data are summarized in Table I, which, for comparison, also contains the results of EMGs of two other patients (K.B., M.C.) of extensive metabolizer phenotype, both of whom have debrisoquine metabolic ratios of 0.6 each. These two patients have been receiving perhexiline, 100 mg three times a day, without any evidence of side effects for more than 40 weeks. A previous study reported high plasma perhexiline levels as well as high ratios of plasma perhexiline to 4-hydroxy-perhexiline in neuropathic patients compared to non-neuropathic controls.[7] Wide interindividual variations in the rate of its oxidative metabolism in healthy volunteers have been noted,[4] and the plasma half-life of perhexiline has been shown to be longer in neuropathic patients than in non-neuropathic patients.[7] It has been suggested that the neuropathic reaction associated with perhexiline usage represents an individual susceptibility secondary to a latent inborn metabolic disorder.[2] The finding of impaired debrisoquine oxidation status in our patient is of great interest when viewed with all other available evidence and compared to the status of the two patients with normal oxidative ability. The phenotype of our first-mentioned patient was again determined on two separate occasions, and his ratios were 27.4 (5 weeks after stopping perhexiline) and 24.2 (after discontinuing cimetidine for 5 days 12 weeks later). It is also worth noting that liver function tests in our patient remained essentially normal, thereby excluding initial hepatic damage to account for the impaired debrisoquine, and by inference perhexiline, hydroxylation. The ability to oxidize many drugs has been shown to be controlled by the same pair of gene alleles as

defined by debrisoquine,[5] and the high metabolic ratio in the patient described suggests that impaired drug oxidation may be the inborn metabolic disorder predisposing an individual to perhexiline accumulation and the associated neurotoxic sequelae.

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response from the ulnar or median nerves. Lumbar puncture not done. The patient improved after withdrawal of perhexiline and administration of diuretics for his ascites. Liver function tests returned to normal within 4 weeks. He was discharged from hospital after 6 weeks when he was able to walk unaided, and at follow-up 4 months later he still showed some weakness of the legs and the tendon reflexes remained absent.

These cases illustrate the severe toxicity of perhexiline, a drug which, in our opinion, should no longer be marketed. Both patients had severe proximal motor and distal sensory neuropathy with slowing of nerve conduction, indicating segmental demyelination.[1] In patient 1 the CSF protein was considerably raised, suggesting Guillain-Barré syndrome. A raised CSF protein in perhexiline toxicity has been previously described.[2] The neuropathy improved slowly when the drug was withdrawn. The older patient also had micronodular cirrhosis of the liver with impaired liver function which reverted to normal a month after drug withdrawal. He was a confirmed teetotaller. Liver damage due to perhexiline has been described by Kopelman and Morgan[3] and others, but a combination of liver damage with severe sensorimotor neuropathy in a patient taking this drug has not, to our knowledge been described.

Whipps Cross Hospital, London E11
K. W. G. HEATHFIELD
F. CARABOTT

ARGON LASER PHOTOCOAGULATION IN BLEEDING PEPTIC ULCERS

SIR,—Professor Piper (Feb. 13, p. 401) takes exception to our statement that the mortality rate in our controls (16%) was not unduly high. We point out that this should be compared with 15% in a similar group of patients with signs of recent haemorrhage (SRH) described by Foster et al.[4] Our trial was designed to concentrate on patients at highest risk of rebleeding and dying (those with SRH) and was limited to these patients. If all the patients in our series with bleeding peptic ulcers are considered, 11 out of 155 (7%) died, while the total mortality rate for all patients with acute gastrointestinal bleeding was 18 out of 330 (5·5%). 11 of the deaths were in the 108 patients with ulcers plus SRH: 7 were in the control group (40) and 4 in the group excluded because of inaccessibility (32). There were no deaths in the treated group (36) or in those with ulcers without SRH (47). The other 7 deaths were in the no ulcer group (175)—2 with oesophageal varices, 2 with mesenteric infarction (1 postoperative), 2 with gastric erosions (both postoperative), and 1 with gastric carcinoma (postoperative).

Piper cites series where the mortality rate was 2% or less when acute stress ulcer was excluded. By this qualification he excludes patients defined as having "bleeding which began in hospital" and which "is known to have a worse prognosis compared with the remainder of the patients". We did not exclude these high-risk patients. If these are included, then the total mortality in his series of 184 patients[5] in 1976–77 was 9·8% (for chronic duodenal ulcer it was 6·1% and in chronic gastric ulcer it was 7·0%). A review[6] of substantial European reports on gastrointestinal haemorrhage emphasised that total mortality rate generally remains between 7% and 10%, and that in pooled data from four recent studies it is 8·7% for gastric ulcer and 5·1% for duodenal ulcer.

Norman Tanner Gastroenterology Unit, St James' Hospital, London SW12 and Department of Gastroenterology, University College Hospital, London WC1
T. C. NORTHFIELD
C. P. SWAIN
J. S. KIRKHAM
D. W. STOREY
S. G. BOWN
P. R. SALMON

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BETA-BLOCKERS AND DRUG OXIDATION STATUS

SIR,—The unusually high plasma concentrations of three β-blockers (metoprolol, alprenolol, and timolol) in individuals of deficient debrisoquine hydroxylation status reported by Dr Alvan and colleagues (Feb. 6, p. 333) are clinically very important. As these workers suggest, some patients may be at risk of concentration-dependent side-effects, such as unacceptable bradycardia, on conventional dosage regimens of these β-blockers. We have studied the effect on pulse rate of even lower doses of metoprolol in individuals of the two oxidation phenotypes, and would add propranolol to the β-blockers listed by Alvan et al.

Two healthy volunteers, one an extensive and one a poor metaboliser of debrisoquine, took a single 25 mg oral dose of metoprolol after a light breakfast. Standard maximal effort tests on a bicycle ergometer were done 0, 2, 4, 6, and 8 h after dosing. Pulse rates were electrocardiographically recorded every minute (six×10 s period) for 8 min. β-blockade at time t was calculated by subtracting the peak pulse rate at time t from that at time zero and expressing the difference as a percentage of peak pulse rate at time zero. The individual of poor debrisoquine oxidation status was subject to more intense and prolonged β-blockade due to metoprolol (see figure).

A 70-year-old woman with ischaemic heart disease was prescribed propranolol 20 mg three times daily, nifedipine 10 mg three times daily, and cyclopenthiazide (as 'Navidrex-K') two tablets daily. After 3 weeks she started to feel tired and experienced dizziness and syncope after even the mildest exertion. Her radial pulse was regular 44/min. Full blood count, liver function tests, and serum urea, electrolytes, and creatinine were all normal. Her electrocardiogram confirmed sinus bradycardia with normal PR and QT_C intervals. An electroencephalogram showed no abnormality. In view of the low doses of propranolol, excessive β-blockade was overlooked. Insertion of a permanent pacing system was considered but deferred while propranolol withdrawal was tried. Stepwise reduction in propranolol dose was associated with the following pulse-rates: 3×20 mg daily, 44/min; 3×10 mg daily, 54/min; 10 mg once daily, 60/min; no propranolol, 72/min.

After discontinuation of propranolol, the tiredness, dizziness, and syncope progressively disappeared. On phenotyping for debrisoquine oxidation status[1] she eliminated 20·1% of a single oral dose of 10 mg debrisoquine as unchanged debrisoquine and 0·6% as 4-hydroxydebrisoquine. Her metabolic ratio was thus 33·5, confirming her as a poor metaboliser.[2]

In all trials of β-blockade (with metoprolol, timolol, alprenolol, oxprenolol, and propranolol) in the post-myocardial infarction period there have been drop-outs because of excessive β-blockade. The findings of Alvan et al. and ours emphasise the inadequacies of

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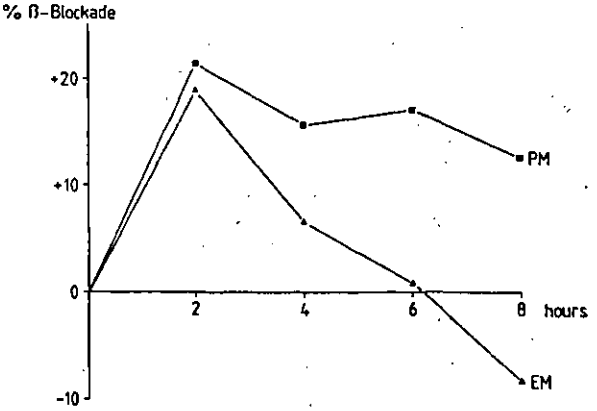

β-blockade due to 25 mg oral metoprolol in an extensive (EM) and a poor (PM) metaboliser of debrisoquine.

fixed-dose multicentre trials. Poor metabolisers may be at risk either of excessive β-blockade or of being deprived of a drug that could help them. The only way round this is for the dose to take into account the patient's drug oxidation status. Ischaemic heart disease shows no predilection for any particular oxidation phenotype;[3] and, although the frequency of poor metaboliser phenotypes may be only about 3·2% in Sweden, it is about 9% in Whites in Britain.[2]

Department of Biochemical and Experimental Pharmacology, St Mary's Hospital Medical School, University of London, London W2 1PG

R. R. SHAH
N. S. OATES
J. R. IDLE
R. L. SMITH

SIR,—Genetically determined variability in drug oxidation was first described for hydroxylation of the antihypertensive drug debrisoquine. Dr Alvan and colleagues (Feb. 6, p. 333) suggest that oxidation of some β-adrenoceptor blocking drugs may be dependent on the same genetic control. We came to an identical conclusion when studying the pharmacokinetics of the β-blocker bufuralol (Ro-3-4787).[4-7] In poor metabolisers of debrisoquine the changes in blood behavour of bufuralol were so striking[5] (very high concentrations of the parent drug and very low concentrations of the main metabolite) that we tried to phenotype individuals using bufuralol plasma data instead of the debrisoquine urinary metabolic ratio.[6,7] The figure shows the frequency distribution of the metabolic ratios (plasma bufuralol divided by plasma 1'-hydroxybufuralol concentrations 3 h after absorption of a 30 mg bufuralol tablet) in 154 healthy Swiss volunteers.[7] 12 (8%) were poor metabolisers for bufuralol and debrisoquine.

Poor metaboliser status was associated with an increased risk of side-effects of bufuralol, even after a single dose in healthy volunteers.[5] In poor metabolisers plasma bufuralol concentrations 3 h after a 30 mg tablet are 165±80 SD ng/ml (n = 12) compared with 69±36 ng/ml (n = 142) in extensive metabolisers.[7] In the same poor metabolisers the mean 1'-hydroxybufuralol concentration is 27±9 ng/ml versus 60±21 ng/ml in extensive metabolisers.[7] The low concentration of metabolite in poor metabolisers may be relevant when considering drugs with active derivatives such as β-adrenoceptor blocking drugs or pro-drugs. The antiarrhythmic drug encainide, for instance, may be ineffective, due to the absence of active metabolite, in poor metabolisers of debrisoquine.[8]

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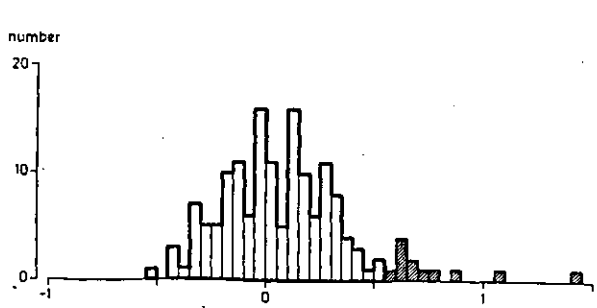

Plasma metabolic ratios of bufuralol in 154 healthy Swiss volunteers.

Hatched = poor metaboliser for bufuralol and debrisoquine.

Our findings accord with those of Alvan et al., except on one point. In our experience the side-effects encountered in poor metabolisers are not solely of the β-adrenoceptor blocking type, but may also be less specific, being related to other pharmacological effects of the parent drug or metabolite.

Research Laboratory, Medical Polyclinic, Hôpital Cantonal Universitaire, 1211 Geneva 4, Switzerland

PIERRE DAYER
FRANÇOIS COURVOISIER
LUC BALANT
JEAN FABRE

PLASMA BOMBESIN CONCENTRATIONS IN PATIENTS WITH EXTENSIVE SMALL CELL CARCINOMA OF THE LUNG

SIR,—We have shown[1] that a high content of the neuropeptide bombesin characterises small cell carcinoma of the lung (SCCL); we found bombesin in all of 17 SCCL cell lines examined and in none of 8 non-small cell lines. Like other neuropeptides, bombesin is secreted by selective depolarising stimuli,[2] an observation recently confirmed by T. W. Moody, A. F. Gazdar, and J. D. Minna (unpublished) in cultured small cell lines. In the light of this and the high bombesin content of tumour metastases obtained at necropsy,[1] it seemed likely that bombesin is secreted in vivo in large quantities in SCCL patients.

In a blind experiment, we examined the plasma concentration of bombesin-like immunoreactivity of fifteen patients on the oncology ward of the Washington Veterans Administration Hospital. 5 ml of blood was rapidly mixed with bacitracin (final concentration 0·5 mg/ml), centrifuged, and frozen. The extraction procedure was modified from Böhlen et al.[3] and a bombesin radioimmunoassay kit (Immunonuclear, Stillwater, Minnesota) was used. Recovery of bombesin standards was 95% efficient, plasma levels of 100 pmol/l could be reliably detected and the intra-assay variation coefficient was less than 5%.

Bombesin-like immunoreativity was detected in all fifteen samples, but there was no significant difference between the plasma concentrations of six SCCL patients with limited stage disease[4] and six patients with assorted non-SCCL carcinomas, including squamous cell, adenocarcinoma, prostate, and breast carcinoma. Values were, respectively, 33±17 and 38±12 pg in 200 μl plasma, mean ±SD. Three of the patients were at an extensive, metastasised stage of the disease[4] and all three had very high plasma bombesin levels of 640±72 pg in 200 μl, about 200 times higher than those of patients with limited stage disease or other forms of cancer.

Thus very high bombesin levels may be characteristic of SCCL patients with extensive tumour burden, and it is possible that some clinical manifestations of extensive disease, such as anorexia, hyperglycaemia, and hypothermia, are mediated by hypersecretion of bombesin.[1] Unfortunately, bombesin levels in early stage SCCL and non-SCCL patients are indistinguishable, at least with the peptide extraction and assay we used. If subtle chemical differences between tumour and non-tumour secreted bombesin were to be found it might be possible to exploit them to produce a clinically valuable early detection assay for SCCL. In collaboration with our National Cancer Institute colleagues, we plan continued efforts in this direction.

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Biological Psychiatry Branch, National Institute of Mental Health, Bethesda, Maryland 20205, U.S.A.

CANDACE B. PERT
UWE K. SCHUMACHER

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Impaired oxidation of debrisoquine in patients with perhexiline neuropathy

R R SHAH, N S OATES, J R IDLE, R L SMITH, J D F LOCKHART

Abstract

The use of perhexiline maleate as an antianginal agent is occasionally associated with side effects, particularly neuropathy and liver damage. The reason why some individuals develop these toxic reactions is not clear, though some evidence suggests that they may result from impaired oxidative metabolism, due to genetic or hepatic factors, and consequential accumulation of the drug in toxic concentrations. Drug oxidation was measured with an oxidation phenotyping procedure in 34 patients treated with perhexiline, 20 of whom had developed neuropathy and 14 of whom had not. Most of the 20 patients with neuropathy, but not the unaffected patients, showed an impaired ability to effect metabolic drug oxidation. This impairment was independent of hepatic function, concurrent drug therapy, or tobacco or alcohol consumption.

The fact that the ability to oxidise several drugs is genetically controlled points to a genetic susceptibility to developing neuropathy in response to perhexiline. Routine determination of the drug oxidation phenotype might lead to safer use of perhexiline by predicting patients who may be more at risk of developing a neuropathic reaction associated with its long-term use.

Introduction

Perhexiline maleate (Pexid), a synthetic antianginal agent, was first marketed in the UK in 1975. Clinical trials have shown its efficacy in reducing both anginal attacks and the consumption of glyceryl trinitrate in patients with myocardial ischaemia.[1-3] Its mode of action is not fully understood, but it reduces exercise-induced tachycardia and improves exercise tolerance without effect on blood pressure or resting heart rate.[4 5] It has calcium antagonistic properties[6] and may exert some intracellular biochemical effect to reduce high-peak demand for oxygen during exercise or stress.[7]

The use of perhexiline has, however, become increasingly associated with adverse effects, both minor and major. The minor side effects, which include nausea, vomiting, dizziness, lethargy, insomnia, tremor, and loss of libido,[8-11] have not been too troublesome, and if they do not disappear spontaneously,[9 11] they can easily be controlled by reducing the dose or stopping the drug.[8 9] The major side effects comprise peripheral neuropathy[12 13]; hepatic damage,[14 15] including cirrhosis[16]; hypoglycaemia[17]; and weight loss.[8 9] Proximal myopathy[18] and papilloedema[19 20] have also been reported. Most of these effects are reversed when the drug is withdrawn, but the return of neurological and hepatic functions to normal may be slow or incomplete.

The reason why some people develop serious toxic reactions to perhexiline is not clear, though some evidence suggests that the reactions may result from impaired metabolism of the drug. Perhexiline is a lipophilic drug and its elimination depends on metabolic oxidation to the more polar monohydroxylated and dihydroxylated metabolites. The large interindividual variations in plasma half lives of perhexiline have been interpreted as being due to interindividual differences in the rate of metabolism of the drug.[21] L'Hermitte *et al* suggested that perhexiline-induced neuropathy might represent individual susceptibility, perhaps secondary to some latent inborn metabolic disorder,[22] and Singlas *et al* later showed that patients with peripheral neuropathy had higher perhexiline plasma concentrations and longer plasma half lives than those without peripheral neuropathy.[23] Furthermore, the mean ratio of plasma concentrations of the parent drug to those of its major metabolite, monohydroxy-perhexiline, was some nine times higher in the patients with neuropathy than in the controls, and they concluded that this toxic effect of perhexiline was associated with accumulation of the parent drug due to different patterns of distribution or metabolism in affected individuals.[23] The metabolic differences, in turn, were attributed

Department of Biochemical and Experimental Pharmacology, St Mary's Hospital Medical School, London W2 1PG
R R SHAH, BSC, MRCP, Wellcome clinical research fellow
N S OATES, PHD, Wellcome research fellow
J R IDLE, PHD, lecturer in biochemical pharmacology
R L SMITH, DSC, PHD, professor of biochemical pharmacology

Merrell Pharmaceuticals Limited, Hounslow, Middlesex TW5 9QY
J D F LOCKHART, MB, DPH, director of clinical studies

to either genetic factors or the hepatic damage that may be associated with perhexiline therapy.[23]

Recently it has been shown that several drug oxidations in man are under single gene control and exhibit genetic polymorphism.[24 25] The genetic structure of a population with respect to oxidation is readily ascertained by the use of the drug debrisoquine, which is extensively oxidised by most individuals (extensive metaboliser phenotype) to its major metabolite, 4-hydroxy-debrisoquine. About 9% of white British subjects, however, show impaired oxidation of debrisoquine (poor metaboliser phenotype).[24 26] The oxidation is regulated by two alleles which have been referred to as the D^{H} (rapid and extensive oxidation) and the D^{L} alleles (slow impaired oxidation).[25] Individuals homozygous for the D^{L} allele have an impaired

TABLE I—*Details and results of the two groups of patients who received long-term perhexiline and those who never received perhexiline*

	Details				Liver function tests†							
Case No	Age and sex	Weight (kg)	Daily smoking*	Alcohol consumption	Bilirubin (μmol/l) (5-17)	AST (U/l) (7-40)	Alkaline phosphatase (U/l) (30-115)	Albumin (g/l) (35-51)	Globulin (g/l) (25-45)	ALT (U/l) (7-40)	Debrisoquine metabolic ratio	Approximate weekly consumption of perhexiline (g/week)
Patients treated with perhexiline												
Neuropathic patients												
1	57 F	70	0	0	5	—	78	35	31	43	0·5	3·0
2	52 M	75	10 c	+	7	—	86	40	32	18	0·8	2·8
3	62 M	54	1 cg	0	6	—	72	39	33	12	0·9	1·3
4	58 M	57	1 cg	±	8	—	113	41	26	18	1·6	2·2
5	68 M	80	8 c	+	11	31	69	43	31	—	2·6	2·2
6	54 M	65	0	±	10	67	234	24	43	—	2·6	2·1
7	62 M	72	0	±	<17	55	25	40	25	—	4·5	2·1
8	55 M	76	40 c	0	<17	<40	<115	>35	<45	—	5·7	2·1
9	68 M	73	Pipe	±	12	23	54	40	32	—	8·5	2·1
10	69 M	89	Pipe	0	8	45	9	38	31	62	10	2·4
11	60 M	76	0	0	8	46	112	45	27	—	19	2·0
12	73 F	45	0	0	4	106	67	32	28	—	25	1·4
13	60 M	79	0	±	8	123	10	40	32	176	27	2·3
14	56 M	86	0	±	14	18	160	51	20	—	28	2·0
15	66 M	77	Pipe	±	<17	34	<115	>35	<45	61	30	1·4
16	63 M	68	0	±	6	—	85	29	31	16	32	1·4
17	68 M	62	0	±	11	152	110	36	34	—	35	1·8
18	68 M	65	6 c	±	8	15	50	>35	<45	—	69	1·4
19	60 M	76	0	±	<17	<40	<115	>35	<45	—	77	1·4
20	67 M	76	5 c	±	9	25	94	47	25	—	>100	2·3
Mean	62·3	71·1			10·2	54·7	88·8	38	33·1			1·99
±SD	±5·8	±10·7			±4·2	±40·8	±51·5	±6·1	±7·6			±0·48
Non-neuropathic patients												
21	66 M	70	0	+	4	33	110	43	29	—	0·4	3·1
22	57 M	78	0	±	<17	23	23	29	34	—	0·4	3·8
23	76 F	57	0	0	12	17	92	42	29	11	0·5	1·4
24	55 M	69	Pipe	±	<17	<40	<115	>35	<45	—	0·5	1·5
25	62 M	58	0	0	9	64	87	39	29	—	0·6	2·1
26	50 M	91	0	±	6	36	57	44	28	—	0·6	2·1
27	67 M	75	6 c	±	11	23	79	40	24	22	0·6	2·1
28	58 M	65	0	±	3	15	191	44	23	—	0·7	3·7
29	71 M	74	0	±	9	23	85	43	32	—	1·0	2·1
30	71 F	53	0	±	14	28	92	41	27	—	1·1	2·1
31	74 M	71	0	0	12	21	110	42	32	—	1·1	2·8
32	71 F	57	0	0	7	—	74	38	30	21	1·6	1·4
33	60 M	52	0	0	<10	16	25	41	27	—	1·7	3·8
34	73 F	67	0	+	8	112	93	40	26	—	4·6	1·4
Mean	65·1	66·9			9·9	34·7	88·1	40·1	29·6			2·39
±SD	±8·1	±10·9			±4·3	±26·7	±41	±4·0	±5·4			±0·90
Patients who never received perhexiline												
35	68 M	67	0	±	15	105	74	45	28		0·1	
36	58 M	107	0	±	7	22	42	44	23		0·1	
37	74 M	71	0	0	9	26	140	42	27		0·1	
38	70 M	65	0	0	8	19	102	45	29		0·2	
39	59 M	89	0	+	8	77	297	41	27		0·2	
40	59 M	92	5 c	±	5	20	100	43	25		0·2	
41	53 M	76	2 cg	+	12	27	221	42	37		0·2	
42	58 M	69	0	±	7	20	58	41	23		0·2	
43	39 M	83	0	+	7	36	101	48	26		0·2	
44	54 M	61	0	±	7	26	110	42	29		0·3	
45	60 M	68	0	+	14	36	78	43	23		0·3	
46	71 M	60	7 c	±	8	29	98	41	24		0·3	
47	48 M	80	0	0	7	29	108	45	23		0·3	
48	68 F	47	0	±	7	20	112	46	30		0·4	
49	50 M	86	0	+	9	32	99	49	24		0·4	
50	51 M	54	6 c	0	4	23	98	40	20		0·5	
51	77 M	65	0	0	5	25	111	39	26		0·5	
52	38 M	76	0	±	4	26	57	44	32		0·5	
53	71 M	81	10 c	±	8	19	92	41	28		0·5	
54	32 M	69	0	±	9	25	83	48	18		0·6	
55	45 M	73	0	±	7	22	95	44	24		0·6	
56	41 M	86	15 c	0	4	23	140	45	29		0·6	
57	46 M	65	0	0	15	32	95	48	34		0·7	
58	53 M	69	1 cg	±	9	32	56	43	23		1·0	
59	79 M	52	0	±	6	22	151	46	31		1·0	
60	51 M	83	3 c	0	3	32	104	44	27		1·1	
61	66 M	67	0	+	7	21	82	43	18		1·4	
62	55 M	82	0	±	11	45	101	45	27		1·7	
63	69 M	87	14 c	±	5	28	139	44	29		1·9	
64	70 M	69	0	0	7	33	114	43	25		2·3	
65	67 M	57	15 c	±	5	21	126	41	33		2·6	
66	47 M	83	0	±	3	15	76	46	26		2·8	
67	62 M	73	15 c	+	6	19	89	45	29		4·0	
68	52 F	71	10 c	±	5	22	78	44	26		4·5	
69	70 F	55	0	±	3	21	130	44	26		5·6	
70	62 M	74	5 c	±	7	38	65	44	28		15	
71	59 M	67	0	±	13	27	97	41	24		26	
72	59 M	80	0	±	5	19	74	40	23		39	
Mean	58·2	72·6			7·4	29·3	102	43·7	26·4			
±SD	±11·5	±12·3			±3·2	±16·4	±47·7	±2·4	±4·0			

*c = cigarette; cg = cigar; 0 = none; ± = only occasionally; + = mild to moderate; — = not determined. †Normal reference range in parentheses.
AST = Aspartate aminotransferase. ALT = Alanine aminotransferase. *Conversion: SI to traditional units*—Bilirubin: 1 μmol/l ≈ 0·06 mg/100 ml.

ability to oxidise several other drugs besides debrisoquine.[25 27 28] This genetic polymorphism in drug oxidation reactions has several important implications. One of the more significant is that in subjects with impairment drugs that are normally eliminated by oxidation are more likely to accumulate, possibly to toxic concentrations.[29]

We therefore investigated patients who had developed perhexiline-induced neuropathy for their debrisoquine oxidation status on the supposition that the toxicity could be the result of an impaired ability to eliminate drugs through oxidation.

Patients and methods

Twenty patients with past or present perhexiline-induced neuropathy confirmed by nerve conduction studies (18) or definite clinical evidence (2), were identified from various cardiac outpatient departments. Another 14 patients who received perhexiline for angina and who had had no serious side effects on long term treatment served as controls. A further 38 patients with ischaemic heart disease who had never had perhexiline were also included in the study to assess the influence of the disease on oxidation pattern and also to determine whether the disease had predilection for any particular oxidation status. Approval was obtained from the St Mary's Hospital ethical committee, and each patient gave his or her informed consent before participating in the study. The details of these patients and, where appropriate, the approximate weekly intake of perhexiline are given in table I.

All the patients were phenotyped for oxidation status,[24] and each took a single 10-mg oral dose of debrisoquine (Declinax 10, Roche). The urine passed in the subsequent eight hours was collected in bulk, the volume measured, and a 20-ml sample stored at −20°C before analysis. Each sample was analysed for its content of parent drug and its major metabolite, 4-hydroxy-debrisoquine using electron-capture gas chromatography. From the results, the metabolic ratio was calculated. This ratio defines an individual's ability to metabolise debrisoquine (and by inference several other drugs) and is derived as follows:

$$\frac{\%\text{ dose excreted as unchanged debrisoquine}}{\%\text{ dose excreted as 4-hydroxy-debrisoquine}} \text{ in the 0-8 h urine}$$

after a single 10-mg oral dose of the drug. Low values (<1) represent extensive oxidative ability, while values greater than 12·6 indicate the grossly impaired ability found in the individuals homozygous for the D^L allele.[28] Intermediate ratios indicate varying degrees of impairment.

To assess their hepatic function, routine biochemical liver function tests were carried out on all the patients included in this study. Most of these tests were performed within one week of the debrisoquine phenotyping test.

Results

Table I shows the details of the 34 patients who received long-term perhexiline treatment for angina, together with the results of their liver function tests and their debrisoquine metabolic ratios. The 20 patients who developed neuropathy consisted of 18 men and 2 women while the 14 patients who did not develop this complication included 10 men and 4 women. There were no significant differences between the two groups in terms of their age, weight, or alcohol consumption. Ten of the 20 neuropathic patients and two of the 14 non-neuropathic individuals consumed tobacco in some form. Both groups were exposed to comparable dosages of perhexiline. In the eight hours the mean (±SD) urinary recoveries of debrisoquine plus 4-hydroxy-debrisoquine were 29·8±13·1% (range 14-67%) in the neuropathic group and 26·1±10·7% (range 12-53%) in the non-neuropathic group. The patients fell into two fairly well defined groups with respect to their oxidation status and the development of neuropathy.

The patients who developed neuropathy showed metabolic ratios in the range 0·5- > 100 with a median value of 14·4 whereas the range for the non-neuropathic group was 0·4-4·6 with a median value of 0·65. The median metabolic ratio of the neuropathic group was significantly greater than that of the non-neuropathic group as determined by the Wilcoxon rank test. There were many more patients with ratios of more than 12·6 (50%) and fewer with ratios of less than 1·0 (15%) among those who developed neuropathy. This preponderance of individuals with high metabolic ratios was significantly greater (χ^2= 37·9; $p<0·0001$) in the neuropathy group than would have been expected in normal healthy population[24 26] or in a random sample of patients with ischaemic heart disease (see below). Correspondingly, the neuropathy group contained significantly fewer ($\chi^2=7·6$, $p<0·01$) individuals with metabolic ratios of less than 1·0.

Table I also shows details of the 38 patients (35 men and 3 women) with ischaemic heart disease who were not prescribed perhexiline and the results of their liver function tests and debrisoquine metabolic ratios. This group of patients did not differ in age, weight, alcohol or tobacco consumption, or biochemical liver function values from the two perhexiline-treated groups. Their mean eight-hour urinary recovery of debrisoquine plus 4-hydroxy-debrisoquine (33·9±9·3%, range 11-62%) was also comparable. These patients had metabolic ratios of 0·1-39 with a median value of 0·55. Only three patients had a ratio greater than 12·6 (8%), whereas 25 had a ratio of less than 1·0 (66%). The remaining 10 patients (26%) had intermediate ratios.

Discussion

The question arises whether the metabolic impairment in the neuropathic patients was due to hepatic dysfunction produced by perhexiline treatment. It is impossible to generalise about the effect of liver dysfunction on drug oxidation since the findings are inconsistent and sometimes contradictory.[30] Thus, the rate of metabolism of phenylbutazone,[31] aminopyrine,[32] bishydroxycoumarin,[32] antipyrine,[33] tolbutamide,[33] and pentobarbital[34] were not significantly changed, while other investigations have shown the half lives of phenylbutazone[35] and meprobamate[36] to be prolonged in patients with liver dysfunction. A leading article in the *Lancet*[37] concluded that even patients with advanced chronic liver disease metabolise drugs surprisingly normally. There was no significant difference between our three groups in their various liver function values. In the 72 patients investigated there was no correlation between the measured metabolic ratios and any of the liver function values. Therefore the difference in debrisoquine metabolic ratios between the two perhexiline-treated groups of patients probably did not originate in hepatic dysfunction. Furthermore, we have found in unpublished studies that the metabolic ratio for debrisoquine is unaffected by mild-to-moderate hepatic damage unless accompanied by hyperbilirubinaemia. Similarly, no difference was discernible between the two groups of patients on perhexiline in terms of their smoking habits or alcohol consumption.

All except four of the perhexiline-treated patients had discontinued perhexiline at least four weeks before being phenotyped. The concurrent drug treatment of all the patients studied at the time of the assessment of their oxidation status is shown in table II. It may be suggested that certain drugs when taken concurrently may influence each other's metabolic fate and therefore that the metabolic ratios of patients with neuropathy may be artefactually raised. Evidence against this suggestion is provided by our group of 38 patients with ischaemic heart disease. The pattern of drug treatment in this group was similar to that in the two perhexiline-treated groups (table II); they had metabolic ratios in the range of 0·1-39 and a median metabolic ratio of 0·55, which was lower than that of normal healthy population (0·8) receiving no medication. The proportion of individuals with metabolic ratios greater than 12·6 (7·9%) among these 38 patients was also close to that in healthy population.[26] Therefore the reason for the pronounced differences in the distribution of the metabolic ratios between the neuropathy group compared and the other two groups was not the result of concurrent drug treatment.

These results show a clear association between the occurrence of perhexiline-induced neuropathy and diminished drug metabolic ability as shown by debrisoquine hydroxylation. They suggest that individuals of poor metaboliser phenotype are at greater risk of developing neuropathy from long-term perhexiline therapy. This risk is strikingly reduced in extensive metabolisers who have metabolic ratios of less than 1.

The association between diminished drug oxidation status and propensity to develop a neuropathic reaction to perhexiline

makes it plausible to suggest that perhexiline would tend to accumulate in individuals with impaired drug oxidising ability because of defective metabolic elimination. The accumulation of the parent drug may then produce toxic sequelae. The mechanism of these toxic reactions is unknown, but drug-induced disturbances of lipid metabolism have been implicated.[38]

Without any obvious causes three of our 20 patients developed neuropathy despite their metabolic ratios being less than 1. It therefore seems likely that, although an individual's drug oxidation status may be a major pharmacokinetic determinant of drug response (toxic or therapeutic), other, as yet unidentified, factors may also contribute to producing toxic sequelae in a few patients. Such factors may include disorders affecting the immune system or lipid metabolism and pharmacodynamic sensitivity.

These findings suggest that routine determination of oxidation status using debrisoquine could be of predictive value in determining perhexiline dosage and controlling its neurotoxicity. Significant associations have already been shown between impaired debrisoquine oxidation and susceptibility to debrisoquine-induced postural hypotension[39]; phenacetin-induced methaemoglobinaemia[29]; phenformin-induced changes in blood lactic acid concentrations[40 41]; and nortriptyline-induced vertigo, dizziness, and confusional state.[42] Bertilsson *et al* found that nortriptyline could be reintroduced successfully at much lower doses in patients with high debrisoquine metabolic ratios.[42] Our findings suggest that, similarly, perhexiline can be used successfully in the treatment of angina pectoris without any appreciable danger of precipitating peripheral neuropathy provided the dose is tailored to the patient's oxidation status.

TABLE II—*Details of concurrent drug therapy in 34 perhexiline-treated patients at the time of phenotyping and in 38 patients who had never received perhexiline*

Case No (metabolic ratio)	Drugs
	Patients treated with perhexiline
Neuropathic patients	
1 (0·5)	Frusemide, nitrates, potassium supplements
2 (0·8)	Propranolol, nifedipine
3 (0·9)	Propranolol, nifedipine, digoxin
4 (1·6)	Metoprolol, frusemide, potassium supplements
5 (2·6)	Nitrates
6 (2·6)	Warfarin, salbutamol, thiazide, spironolactone
7 (4·5)	Nifedipine
8 (5·7)	Atenolol, nifedipine, nitrates, warfarin, dipyridamole
9 (8·5)	Propranolol
10 (10)	Oxprenolol, thiazide, potassium supplements
11 (19)	Atenolol, perhexiline, nitrates
12 (25)	Propranolol
13 (27)	Acebutolol, thiazide, potassium supplements
14 (28)	None
15 (30)	Oxprenolol, metformin, chlorpropamide
16 (32)	Metoprolol, thiazide, amiloride
17 (35)	Oxprenolol, perhexiline
18 (69)	Metoprolol
19 (77)	Propranolol, nifedipine, thiazide, amiloride
20 (> 100)	Dipyridamole, theophylline, chlordiazepoxide, cinnarizine
Non-neuropathic patients	
21 (0·4)	None
22 (0·4)	Nitrates, frusemide, spironolactone
23 (0·5)	Atenolol, nifedipine, frusemide, potassium supplements
24 (0·5)	Aspirin, allopurinol, dipyridamole
25 (0·6)	Digoxin, perhexiline, nitrates, thiazide, potassium supplements
26 (0·6)	Metoprolol, nitrates, triamterene
27 (0·6)	Nitrates
28 (0·7)	Bumetanide, potassium supplements, phenytoin, phenobarbitone, salbutamol, naproxen
29 (1·0)	Propranolol, thiazide, potassium supplements
30 (1·1)	Propranolol, nitrates, thiazide, potassium supplements
31 (1·1)	Propranolol, nifedipine, allopurinol
32 (1·6)	Triamterene, oxazepam
33 (1·7)	Perhexiline
34 (4·6)	Metoprolol, perhexiline, nitrates
	Patients who never received perhexiline
35 (0·1)	Nitrates, thiazide, frusemide, potassium supplements
36 (0·1)	Thyroxine, dipyridamole
37 (0·1)	None
38 (0·2)	Nitrates, thiazide, potassium supplements
39 (0·2)	Digoxin, metoprolol, warfarin
40 (0·2)	None
41 (0·2)	Digoxin, frusemide, spironolactone, tetracycline
42 (0·2)	None
43 (0·2)	None
44 (0·3)	Digoxin, labetalol, thiazide, amiloride
45 (0·3)	Digoxin
46 (0·3)	Propranolol, thiazide, potassium supplements
47 (0·3)	Metoprolol, nitrates, warfarin
48 (0·4)	Metoprolol
49 (0·4)	Digoxin, frusemide, spironolactone, prazosin
50 (0·5)	None
51 (0·5)	Digoxin, metoprolol, nitrates, frusemide, metformin, chlorpropamide, potassium supplements
52 (0·5)	Propranolol
53 (0·5)	Metoprolol, nitrates
54 (0·6)	Propranolol
55 (0·6)	Warfarin
56 (0·6)	Propranolol
57 (0·7)	Digoxin, nitrates, bumetanide, potassium supplement, clofibrate
58 (1·0)	Nitrates
59 (1·0)	Digoxin, metoprolol, nitrates, bumetanide, potassium supplements
60 (1·1)	Digoxin, propranolol, nitrates, thiazide, amiloride, warfarin
61 (1·4)	Metoprolol, nitrates, amiloride
62 (1·7)	None
63 (1·9)	Nifedipine, thiazide
64 (2·3)	Digoxin, nitrates, thiazide, frusemide, amiloride, potassium supplements
65 (2·6)	Digoxin, nitrates, thiazide
66 (2·8)	Propranolol, nitrates, frusemide, dipyridamole, potassium supplements
67 (4·0)	None
68 (4·5)	Labetalol
69 (5·6)	Propranolol
70 (15)	Atenolol, nitrates, chlorthalidone
71 (26)	None
72 (39)	Propranolol, prenylamine, thioridazine

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double-blind cross-over study after a single dose (3 h) and 2 weeks treatment (24 h after the last dose). Thromboxane B_2 (TXB_2), a metabolite of TXA_2, and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), a metabolite of PGI_2, were measured by RIA from venous blood. Platelet aggregation induced by ADP (5 μM) and adrenaline (10 μM) were studied by measuring optical density (OD) according to the method of Born.

The results (mean ± s.e. mean) after the smallest and highest doses of ASA are given in Table 1. All doses of ASA suppressed completely the production of TXB_2 after acute intake with the exception of the 50 mg dose which gave a 61% suppression ($P < 0.001$). After 2 weeks treatment the suppression was complete with the smallest dose also. None of the ASA doses studied had a significant effect on plasma 6-keto-$PGF_{1\alpha}$. ASA completely blocked the aggregation induced by adrenaline and the secondary phase of ADP induced aggregation.

It is concluded that 50 mg/day of ASA is sufficient to have a maximal antithromboxane and anti-aggregatory effect, and even a dose of 1000 mg/day does not influence the production of prostacyclin.

Captopril-induced agranulocytosis associated with an impairment of debrisoquine hydroxylation

N.S. OATES, R.R. SHAH, P.L. DRURY[1], J.E. IDLE & R.L. SMITH

Department of Pharmacology, St Mary's Hospital Medical School, London W2 1PG and [1]Department of Medicine, St Bartholomew's Hospital, London EC1 7BE

The importance of polymorphisms of drug oxidising enzymes in relation to adverse reactions has now been suggested on several occasions. A number of such relationships have already been described including phenformin-induced lactic acidosis (Oates *et al.*, 1981), the confusion and vertigo associated with nortryptiline (Bertilsson *et al.*, 1981) and perhexiline neuropathy (Shah *et al.*, 1982). In each of these examples patients most at risk of developing the toxic effects could have been identified prior to treatment using debrisoquine as a marker of the polymorphism.

In recent months the newly-introduced anti-hypertensive drug, captopril, has been found to be associated with a few cases of agranulocytosis (Staessens *et al.*, 1981) with occasional fatal results. Since oxidation to a dimeric form is one of the major determinants of captopril disposition (Kripalani *et al.*, 1980), the possibility was investigated that this adverse reaction was also associated with the genetic polymorphism of drug oxidation characterised using debrisoquine (Mahgoub *et al.*, 1977).

A total of fifteen patients (aged between 19 and 61 years) were studied. All were receiving or had previously been given captopril (Capoten; Squibb) for the treatment of otherwise resistant hypertension in doses in the range 25–100 mg three times a day. Of these patients, thirteen responded well to treatment with no untoward effects while, in contrast, captopril treatment had to be stopped in the other two patients (on doses of 50 and 100 mg three times a day) since both developed profound agranulocytosis. The reduction in the number of circulating white blood cells was particularly marked with regard to the neutrophils; withdrawal of the drug resulting in total recovery.

Drug oxidising ability was investigated in all the patients using a single 10 mg dose of debrisoquine followed by analysis of the subsequent 8 h urine to determine the metabolic ratio (excretion of unchanged debrisoquine/excretion of 4-hydroxy-debrisoquine) (Mahgoub *et al.*, 1977). While the thirteen patients who displayed no side-effects to captopril had metabolic ratios in the range 0.2–3.3 (median 0.6) and were, thus, extensive metabolisers, the two who suffered agranulocytosis both exhibited an impairment of debrisoquine oxidation having metabolic ratios of 49 and 63, confirming their poor drug metabolising status.

While these results can only be regarded as preliminary, there being no direct evidence of any concordance between oxidations of debrisoquine and captopril at present, the findings are highly suggestive that genetic impairment of captopril oxidation might be one of the factors involved in the development of this adverse reaction and merit further investigation.

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more specific binding: n is small and the sites are rapidly saturated. The second negative slope corresponds with the binding to the second class of sites which are of lower affinity but in greater number; these sites are not saturable. These Scatchard plots are in complete disagreement with those obtained previously by Judis whose positive Scatchard plots indicate that n and/or K_a decrease as the protein concentration increases. One could attribute (Bowmer & Lindup 1978) this phenomenon of positive slopes to the contaminants of the albumin commercial preparation, like fatty acids or tryptophan. We have used simultaneously a normal albumin and an albumin free from fatty acids with no change in the binding percentage. The phenomenon of positive slope can also be attributed to a cooperative binding.

This would be obvious when the protein concentration increases. The Scatchard plot of methadone is the same for a 0·4% albumin or 4% albumin: n is only a little smaller for the 4% albumin, this could be due to the masking of some sites by the folding of the protein molecules.

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On the urinary disposition of phenformin and 4-hydroxy-phenformin and their rapid simultaneous measurement

N. S. Oates, R. R. Shah, J. R. Idle*, R. L. Smith, *Department of Biochemical and Experimental Pharmacology, St Mary's Hospital Medical School, London, W2 1PG, U.K.*

Phenformin (β-phenethylbiguanide) is an orally active hypoglycaemic agent used in the treatment of maturity-onset diabetes. It has been found to be metabolized by oxidation to form a single hydroxylated derivative, 4-hydroxy-phenformin, which is excreted, together with unchanged parent drug, in the urine (Beckmann 1967). We have studied the urinary disposition of phenformin and its metabolite in a single subject given phenformin and we describe the method used. A normal male volunteer was given an oral dose of 50 mg phenformin (Dibotin, Winthrop Laboratories). Urine samples were then collected hourly for the first 8 h following this dose and at 10, 13, 24 and 26 h. After recording the volume of each sample an aliquot was stored at —20 °C before analysis as described below. Both parent drug and metabolite could readily be detected in all the urine samples collected and the rates of excretion for the two substances were plotted on a logarithmic scale against time (Fig. 1). Maximum rate of excretion for phenformin and 4-hydroxy-phenformin coincided in the same urine sample, that obtained from 1 to 2 h after dosing, suggesting the existence of a significant first-pass effect. Thereafter the rates of excretion declined exponentially with time enabling estimates of elimination half-life to be estimated which for phenformin was 3·7 h and 4-hydroxy-phenformin 3·8 h. The total recovery of the drug in this subject was 27·6 mg (56·2%) comprising 18·5 mg unchanged phenformin and 9·1 mg metabolite.

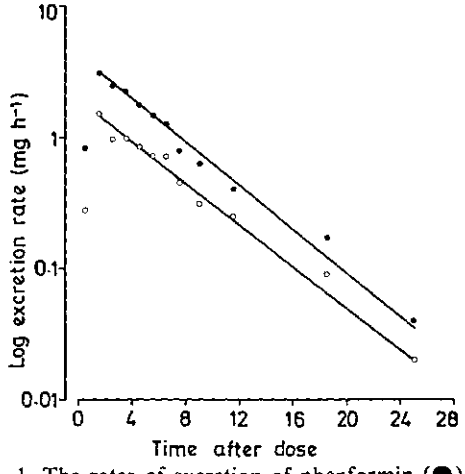

FIG. 1. The rates of excretion of phenformin (●) and 4-hydroxy-phenformin (○) by a single male subject following an oral dose of phenformin (50 mg).

Whereas a number of methods have been described for the estimation of phenformin (Matin et al 1975; Alkalay et al 1976; Hill & Chamberlain 1978), this has not been so for the metabolite. 4-Hydroxyphenformin possesses the strongly basic biguanide group and a weakly acidic phenolic hydroxyl residue, thereby

* Correspondence.

giving it an amphoteric nature which severely limits its extraction into non-polar solvents. It is not possible, therefore, to derivatize it in the same way as the parent drug (Matin et al 1975). We were unsuccessful in our attempts to derivatize the metabolite in the dried residues of urine samples as described by Mottale & Stewart (1975) so an h.p.l.c. method was devised.

In normal urine there is an unidentified substance with similar chromatographic properties to those of 4-hydroxy-phenformin and separation of drug and metabolite from this is essential. The ability of Amberlite XAD-2 resin to adsorb polar aromatic compounds such as phenformin and metabolite suggested to us that this could be a means of purification.

Small columns (5 × 0·5 cm) were packed with Amberlite XAD-2 resin (BDH) which were prepared for use by washing successively with 3 ml each of acetone, methanol and water. To each column was applied 1 ml of a solution of phenacetin (100 μg ml^{-1}) which acted as an internal standard directly followed by a urine sample (1–5 ml) or calibration standard within the range 40–200 μg phenformin and 4–20 μg 4-hydroxy-phenformin. After the columns had been washed with water (3 ml), elution was carried out with methanol (3 ml) and the eluates collected in 50 ml ground-glass stoppered tubes. These extracts were then dried by rotary evaporation under vacuum at 50 °C and chromatographed using an h.p.l.c. column packed with a bonded reversed phase material (Waters Radial Compression Column Pak A) through which a mobile phase of 30% (v/v) acetonitrile in 0·05 M KH_2PO_4 was pumped (Pye Unicam LC-XPS) at ambient temperature at 3 ml min^{-1}. Detection was achieved by u.v. absorption at 230 nm (Pye Unicam LC-UV). The dried residues obtained from the eluates from the XAD-2 columns were redissolved in the h.p.l.c. solvent (100 μl), 5 μl of which was injected on to the column. Retention volumes: 4-hydroxy-phenformin, 5·1 ml; internal standard (phenacetin), 12·6 ml; and phenformin, 18·9 ml.

From the calculated peak height ratios, calibration curves were obtained for both phenformin and 4-hydroxy-phenformin. The curves were linear over the range of concentrations used and enabled quantitation of as little as 1·0 μg ml^{-1} phenformin and 0·5 μg ml^{-1} 4-hydroxy-phenformin. Recovery from urine was assessed by analysis of six control urine samples to which phenformin and 4-hydroxy-phenformin had been added at concentrations of 200 and 20 μg ml^{-1} respectively. For both compounds the recovery was high, giving mean (with s.d.) values of 97·0 (2·5%) for phenformin and 95·5 (2·0%) for 4-hydroxy-phenformin respectively.

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