POLYMORPHIC HYDROXYLATION OF DEBRISOQUINE IN MAN

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By

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To My Husband

and

In Memory of My Father

Abstract

1. The metabolism of debrisoquine in man has been investigated. A g.l.c. method was established to measure the unchanged drug and its hydroxylated metabolites namely 4-, 5-, 6-, 7- and 8-hydroxy-debrisoquine in urine. 4-Hydroxy-debrisoquine was the major metabolite, its urinary excretion showed a large inter-individual variation (2.5 - 39%). The phenolic metabolites were quantitated for the first time and were also subject to variation (2.7 - 14%).

2. The cause of large individual variation in the alicyclic 4-hydroxylation of debrisoquine was investigated. A population and familial study was carried out on 194 Caucasians. From these it could be concluded that the alicyclic 4-hydroxylation of debrisoquine exhibited a genetic polymorphism, bimodally distributed. Two phenotypes were characterized, the extensive metabolizer (EM, 93% of population) and the poor metabolizer (PM, 7% of population) phenotypes. The mode of inheritance was established through family studies. The defect was shown to be governed by a pair of autosomal mutant genes.

3. Variation in the gene frequency of the defective trait among various ethnic groups were tested. The ethnic groups studied included Nigerians, Gambians, Ghanaians, English Caucasians, Malaysians and Egyptians. The frequency of the defective allele appeared to be high among the first two ethnic groups and lowest within the last two countries.

4. The relation between debrisoquine phenotypes and the hypotensive response to the drug was investigated. Both PM and EM subjects were tested with varying doses (10-60 mg) and the hypotensive response measured. Subjects who were phenotypically PM responded at lower dose level than EM phenotypic subjects.

5. The defective alicyclic 4-hydroxylation of debrisoquine was investigated in relation to other drug oxidations namely the aromatic hydroxylation of debrisoquine and guanoxan. The defect was found to be carried over to aromatic ring hydroxylation of both these drugs.

6. It is concluded that genetic polymorphisms in carbon oxidation have various implications upon new drug development and drug usage.

CONTENTS

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CHAPTER ONE

Metabolism of Debrisoquine in Man

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Introduction

Historical Review

Debrisoquine was originally synthesized by Roche in 1961. Chemically it is described as 3,4-dihydro-2(1H)-isoquinoline carboxamidine sulphate. Pharmacologically, it is an adrenergic neurone blocker similar to other guanidine-based antihypertensive agents such as bethanidine and guanethidine.

Debrisoquine has been shown to possess a potent antihypertensive property both in man (Kakaviatos et al., 1964; Abrams et al., 1964; Bryant et al., 1965) and in experimental animals (Moe et al., 1964).

Debrisoquine was first introduced in the U.K. as an orally active antihypertensive agent in 1966 under the name of Declinax® (see Athanassiadis et al., 1966; Kitchen & Turner 1966; Editorial, 1966). Reports of the first clinical trials have indicated that debrisoquine is an effective antihypertensive agent with a remarkable freedom from side effects (Athanassiadis et al., 1966; Kitchen & Turner, 1966). Marked inter-individual variation in the optimal therapeutic dose requirement was observed. In early studies, daily dose was found to vary from 20-400 mg (Athanassiadis et al., 1966; Kitchen & Turner, 1966), and in a more recent study it was found to vary from 10 to 360 mg.

Tolerance was found to occur among hypertensive patients who received debrisoquine for long periods (more than six months) (Kitchen & Turner, 1966; Rosendorff et

al., 1968; Jackson, 1972).

Absorption and pattern of elimination

The metabolism of debrisoquine was studied by the Roche Clinical Research Department (see Kitchen & Turner, 1966). The drug appeared to be well absorbed in man, 73% of 50 mg oral dose of [^{14}C] debrisoquine was eliminated in 24 h urine of two human subjects. The total excretion of radio-activity in 8 days was 80% in urine and 12% in the faeces. No radio-activity was detected in the expired air.

In rat, the elimination of the drug was dependent on the route of administration. When the drug was injected intraperitoneally, the excretion was mainly via the urine (52%) and 42% in the faeces. After oral administration 37% of the dose was excreted in urine and 50% in the faeces.

In the dog, the pattern of elimination was similar to man as 90% of the dose was excreted in urine and 10% in the faeces.

Distribution

Distribution studies in the rat showed that the drug is rapidly taken up (30 min) by several organs such as liver, heart, kidney, and lungs, after intraperitoneal injection of the drug. Drug levels then decline rapidly in most tissues for about 4 h followed by a period of much slower decline as low concentrations could be detected in several organs 16 h after injection. Metabolic

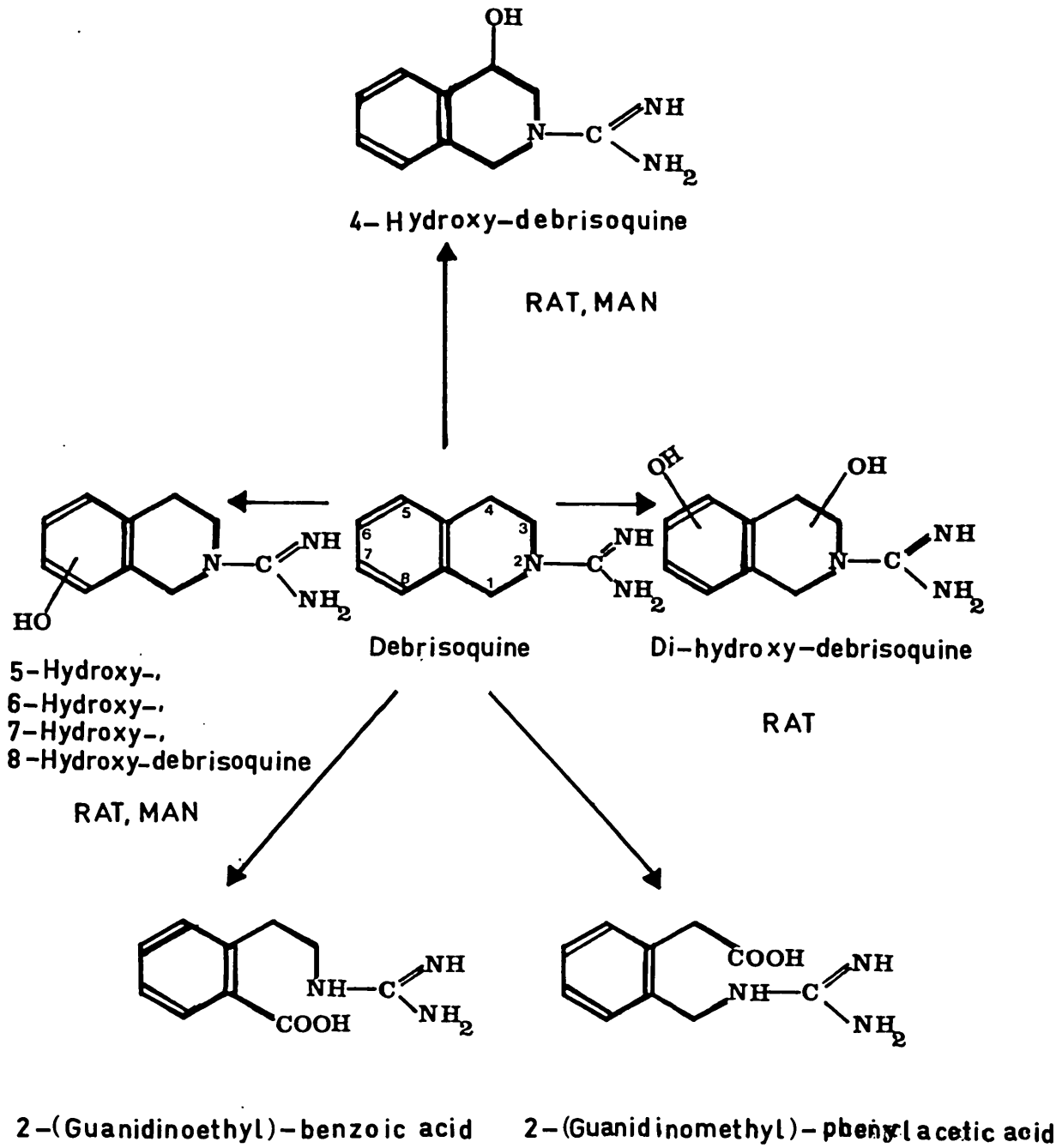
products of the drug could be found in the heart, liver and intestine 6 h after injection, but at 16 h metabolic products were found only in the intestine, urine and faeces. The drug could not be detected in brain at any time and is bound to plasma protein only to a small extent (15-30%) (Medina et al., 1969).

Metabolism

Debrisoquine was shown to undergo metabolism along two pathways, namely oxidation of the alicyclic and aromatic ring structures and by ring cleavage giving rise to two acidic metabolites, namely 2-(guanidino-ethyl)-benzoic acid and 2-(guanidino-methyl)phenyl acetic acid (Allen et al., 1975; 1976) (See Fig. 1.1).

The pattern of metabolism was found to be similar in both man and rat, the major metabolite being formed by 4-hydroxylation. Both species excrete small amounts of phenolic metabolites, namely, 5-, 6-, 7- and 8-hydroxy-debrisoquine. 6-Hydroxy-debrisoquine is the predominating phenol in rat and 7-hydroxy-debrisoquine predominates in man. In the rat small amounts of the hydroxylated metabolites are excreted as glucuronide and/or sulphate conjugates whereas there is no evidence that this occurs in man (Allen et al., 1975). A dihydroxylated metabolite was found in rat but not in human urine. Both species excrete about 10-15% of the dose as acidic metabolites arising from the cleavage of the tetrahydroisoquinoline ring at the 1- and 3- positions, and were identified by g.l.c.m.s. and n.m.r. techniques (Allen et al., 1976) (See Fig. 1.1 for metabolic fate of debrisoquine in rat

Fig. 1.1 Metabolic Fate of Debrisoquine in Rat and Man



and man)

Angelo (1976) found that there were major quantitative differences in the amounts of the hydroxylated metabolites found in human and rat urine; furthermore there also occurred a marked intersubject difference among volunteers in the amounts of hydroxylated metabolites formed. Using paper chromatography and radiochromatogram scanning they found that the amount of unchanged drug varied 19-54% and of 4-hydroxy-debrisoquine varied 5-24% of the dose. Compared to man, rats extensively metabolize the drug; 30-40% of the dose was excreted in urine as 4-hydroxy-debrisoquine and 7-12% as unchanged drug. The phenolic metabolites were identified as 5-, 6-, 7- and 8-hydroxy-debrisoquine, but the amounts not quantified.

Aim of the study

1. To confirm the metabolic fate of debrisoquine in man.
2. To separate the phenolic metabolites and quantitate them by gas-chromatographic methods.

Materials and Methods

Materials

3,4-Dihydro-2(1H)-isoquinoline [^{14}C] carboxamidine hydrochloride ([^{14}C] Debrisoquine)

The labelled compound was previously synthesized in the department and had a specific activity 0.5 µci/mg, m.p. 176-179°C. The radiochemical yield was 28.8% calculated on the potassium [^{14}C] cyanide. The radiochemical purity was greater than 97% as determined by reverse isotope dilution, paper and thin layer chromatography.

Reference compounds

3,4-Dihydro-2(1H)-isoquinoline carboxamidine (Debrisoquine sulphate, Ro. 5-3307/1, Declinax®) pKa approximately 12.5, m.p. 274-276°C.

4-Hydroxy-3,4-dihydro-2(1H)-isoquinoline carboxamidine sulphate. (Ro. 03-7594/001) m.p. 254-255°C.

5-Hydroxy-3,4-dihydro-2(1H)-isoquinoline carboxamidine sulphate (Ro. 7-6377/1) m.p. 298-300°C.

6-Hydroxy-3,4-dihydro-2(1H)-isoquinoline carboxamidine sulphate (Ro. 7-5063/1) m.p. not determined.

7-Hydroxy-3,4-dihydro-2(1H)-isoquinoline carboxamidine sulphate (Ro. 7-4800/1) m.p. 303°C.

8-Hydroxy-3,4-dihydro-2(1H)-isoquinoline carboxamidine sulphate 302-304°C.

All the above listed compounds were gifts from Roche Products Ltd., (Welwyn Garden City, Herts. U.K.).

7-Methoxy-2-guanidinomethyl-1,4-benzodioxan (Pfizer Ltd., Sandwich, U.K.)

Methods

Human investigations

Healthy normotensive adult male volunteers were given orally a mixture of debrisoquine sulphate (30 mg) and [^{14}C] debrisoquine hydrochloride (10 mg; 5 μCi) (total drug equivalent to 31.7 mg free base) as a solution in water. Urine was collected as pooled 24 h samples in plastic bottles and stored at -15°C until analysed.

Estimation of debrisoquine and 4-hydroxy-debrisoquine in urine by derivatization with hexafluoroacetylacetone and electron capture detection.

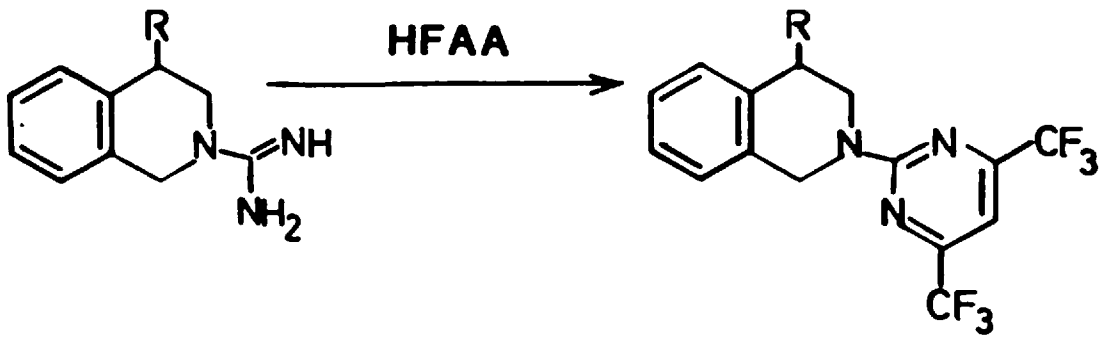
Five methods have been reported for the determination of debrisoquine and 4-hydroxy-debrisoquine in plasma and urine. These are summarised as follows:-

Method	Reference
1. Fluorimetry	Medina et al. (1969)
2. Gas chromatography mass-spectrometry (g.c.m.s.)	Hengstmann et al. (1974) Malcolm & Marten (1976)
3. Gas chromatography flame ionization detection and nitrogen sensitive detection	Lennard et al. (1977)
4. Radio-immuno-assay	Dixon et al. (1977)

In order to achieve a rapid and simple estimation of debrisoquine and 4-hydroxy-debrisoquine, the method of Erdtmansky & Goehl (1975) for the measurement of guanidine drugs was adapted for debrisoquine. To urine (0.1 ml) in a screw-capped septum vial was added aqueous 7-methoxy-guanoxan (50 µl) as internal standard (5 µg). The mixture was buffered with 1M $NaHCO_3$ (100 µl) and benzene (1 ml) and hexafluoroacetylacetone (H.F.A.A.; Koch Light Laboratories Ltd., Colnbrook, Bucks., England; 50 µl) added. The reaction vial was then heated in an aluminium block at 100°C for 1 h, the vial removed and left to cool whereupon 3M NaOH (5 ml) was added to hydrolyse the excess hexafluoroacetylacetone. The sample was vortexed, centrifuged and a portion of the benzene layer (1 µl) was injected onto the g.l.c. column. The derivatization scheme is shown in Fig. 1.2.

Debrisoquine and 4-hydroxy-debrisoquine in urine were quantitated by the construction of a calibration curve in which the ratio of debrisoquine/7-methoxy-guanoxan

Fig. 1.2 Derivatization Scheme for Debrisoquine and Metabolites with Hexafluoroacetylacetone (HFAA)



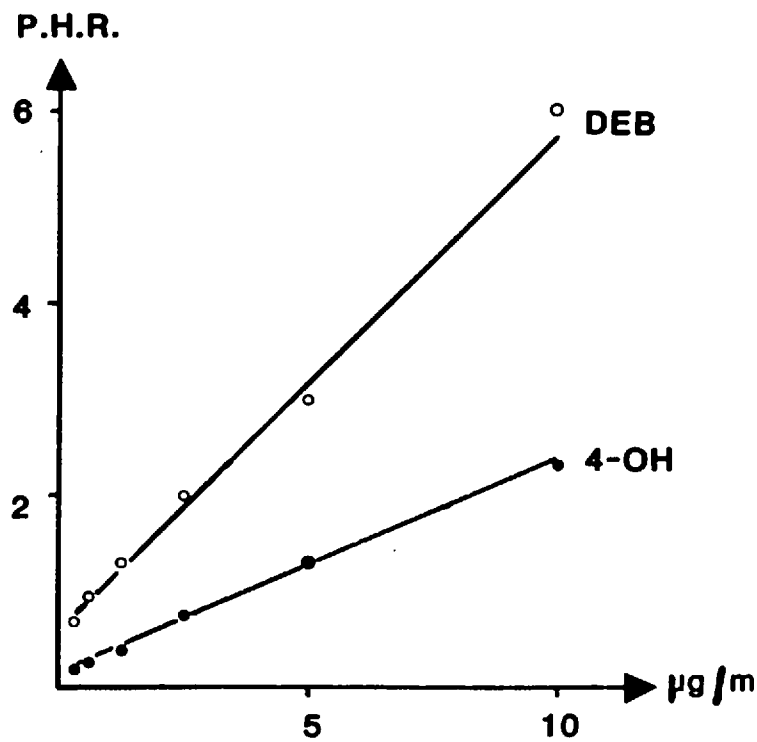


Fig. 1.3 Calibration Curves for Debrisoquine and 4-Hydroxy-debrisoquine

P.H.R. = peak height ratio

peak height ratio against different concentrations of debrisoquine (0-10 μg/ml) and the ratio of 4-hydroxy-debrisoquine/7-methoxy-guanoxan against concentrations of 4-hydroxy-debrisoquine (0-10 μg/ml) was plotted. (Fig. 1.3).

All samples were done in duplicate and a standard curve was run on every occasion samples were analysed.

G.L.C. Conditions

The chromatograph used was Packard Model 417 equipped with a tritium scandium electron capture detector (75 mCi).

Glass column: 3% OV-1 on Chromosorb W.H.P., 80-100 mesh (Phase Separation Ltd., Queensferry,Flintshire, U.K.).

Length of column: 1.83 cm (6 ft) 3 mm internal diameter.

Carrier gas: 5% argon in methane.

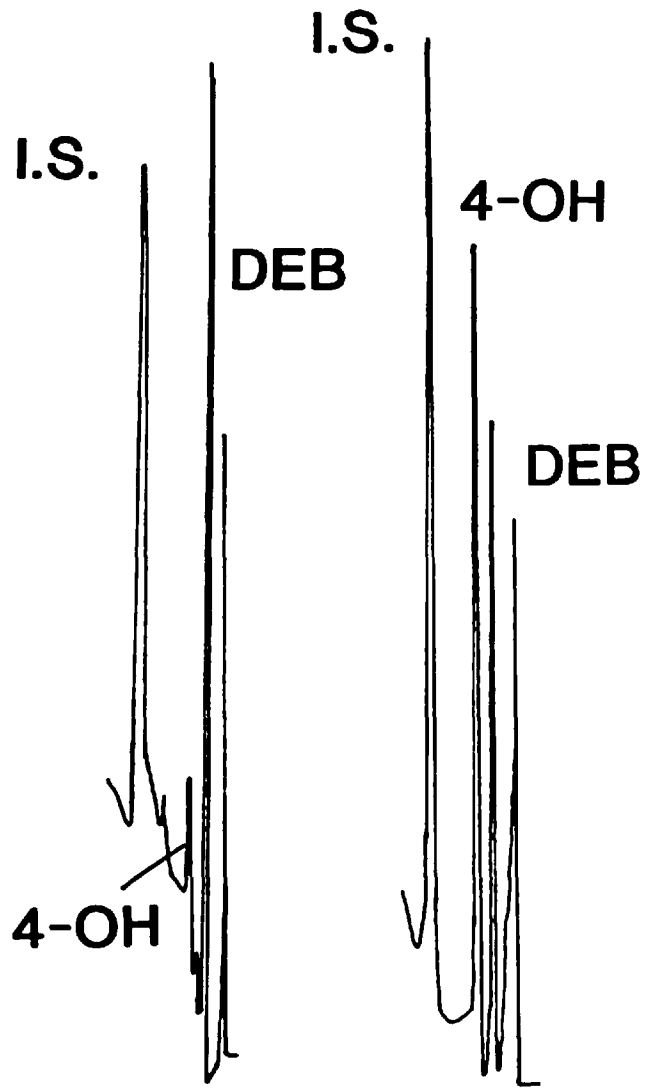
Flow rate: 60 ml/min. Pressure 30 psi.

Column temperature: 195°C.

Detector temperature: 235°C.

Manipulation of samples was relatively easy, the internal standard used gave a very stable peak. All peaks were very sharp, well separated and no interfering peaks were observed. The assay was simple and short as the retention times for debrisoquine and 4-hydroxy-debrisoquine are 1 min. and 2 min. respectively. The calibration curves were linear and the lowest amount that could be detected was 5 ng/ml urine for debrisoquine and 20 ng/ml for 4-hydroxy-debrisoquine. A typical gas chromatograph trace for debrisoquine and 4-hydroxy-debrisoquine is given in Fig. 1.4.

Fig. 1.4 Typical chromatogram trace for debrisoquine and 4-hydroxy-debrisoquine from urine of two different subjects.

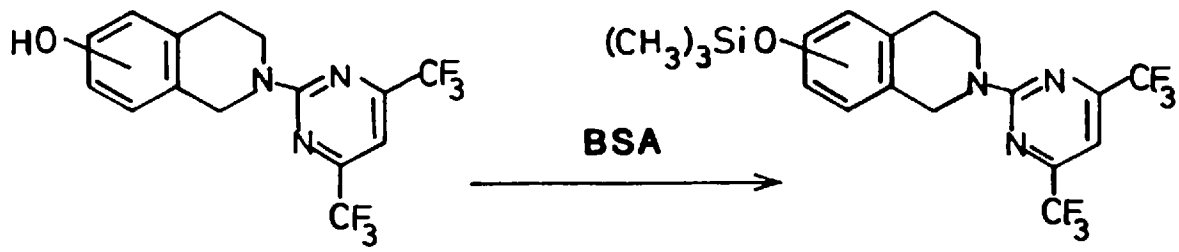


I.S. Internal Standard

Estimation of phenolic metabolites 5-, 6-, 7- and 8-hydroxy-debrisoquine by derivatization with hexafluoroacetylacetone and electron capture detection.

To the urine sample (0.5 ml) in a screw-capped vial was added aqueous 7-methoxy-guanoxan (50 µl containing 5 µg); the mixture was buffered with 1M $NaHCO_3$ (100 µl) and benzene (2 ml) and hexafluoroacetylacetone (100 µl) added. The sample was treated as previously described (vide supra). After centrifugation, 1 ml of the benzene layer was transferred to a 2 ml screw capped vial and the benzene then blown to dryness under a stream of nitrogen. The phenolic debrisoquine metabolites were then silylated (see Fig. 1.5): with 20% N, O, bis-(trimethylsilyl) acetamide (100 µl BSA; Phase Separation Ltd., Queensferry, Flintshire, U.K.) in benzene just prior to the injection of 1 µl onto the g.l.c. column. The amount of each phenol was quantitated by the construction of calibration curves in which the ratio of the peak height of each phenol (multiplied by its Retention time)/peak height of 7-methoxy-guanoxan (multiplied by its Retention time) was plotted against concentrations (50-1000 ng/ml urine; see Fig. 1.6) of each phenol. Relative retention times for the phenols (5-, 6-, 7- and 8-hydroxy-debrisoquine) are 0.58, 0.75, 0.67 and 0.54 respectively. Fig. 1.7 shows a chromatogram of the standard phenols added to urine. G.L.C. conditions: the same as described before for debrisoquine except column temperature was 165°C which allowed better separation of the four phenolic peaks. Also the flow rate was 40 ml/min. The calibration curves were curvilinear up to 500 ng/ml

Fig. 1.5 Secondary derivatization scheme for phenolic metabolites of debrisoquine



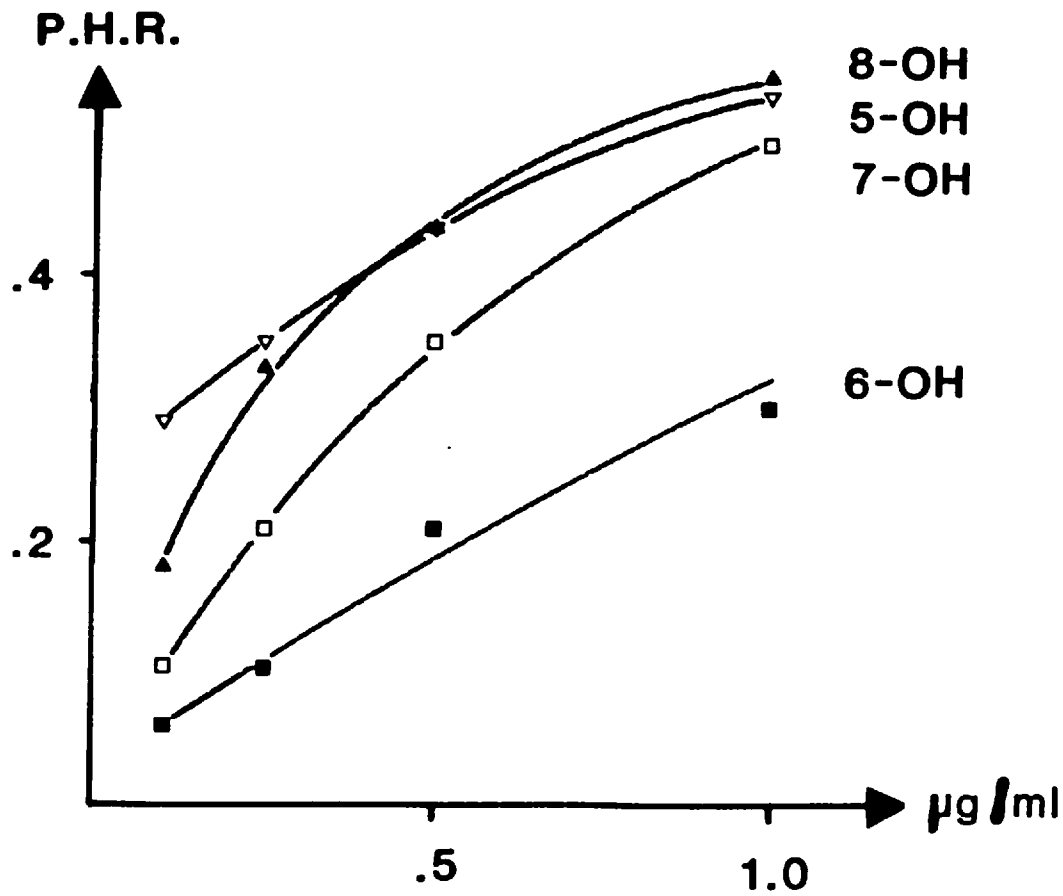
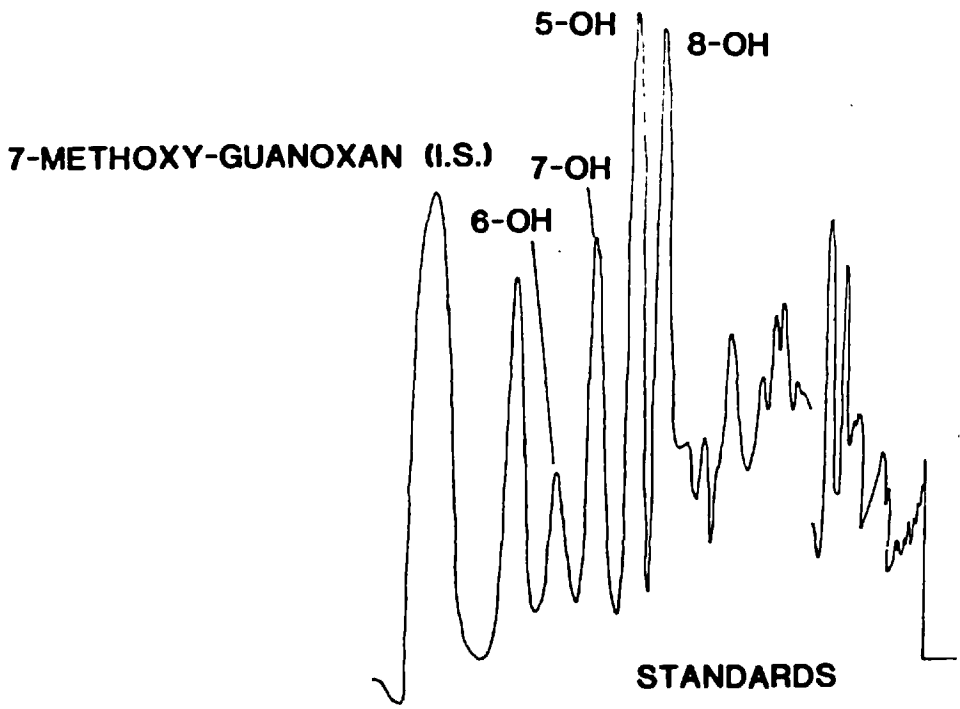


Fig. 1.6 Calibration curves for 5-, 6-, 7- and 8-hydroxy-debrisoquine.

P.H.R. = peak height ratio

Fig. 1.7 Gas-chromatographic separation of the phenolic metabolites of debrisoquine in urine.



and the lowest amount that could be detected was 50 ng/ml urine.

Radiochemical techniques

^{14}C was determined with a scintillation spectrometer (Packard Model 3385). Urine samples (0.1-1.0 ml) was counted for radioactivity in duplicates in vials containing a dioxan-based scintillation fluid. Quench correction was performed by the method of "channels ratio".

Reverse isotope dilution technique for debrisoquine

Debrisoquine sulphate (approx. 1 g accurately weighed) was added to the urine samples to be analysed (containing approx. 150,000 dpm). The compound was thoroughly dissolved by the addition of water and the solution made strongly basic (pH13) with 10M NaOH. The solution was then saturated with NaCl, and extracted with dichloromethane (3x2 vol.). The pooled extracts were evaporated to dryness in vacuo and the residue redissolved in the minimum volume of ethanol. To this was added a slight excess of HCl in ethanol. A few drops of anhydrous ether were generally required to promote the precipitation of debrisoquine hydrochloride which was then filtered and re-crystallized from ethanol to constant specific radioactivity (m.p. 178-179^{o}C).

Concentration of metabolites

Urine samples (0-24 h) were initially concentrated

by freeze drying and leaching of the solid residue with methanol. The resulting extracts were reduced to a small volume by evaporation under reduced pressure and stored overnight at -15°C to precipitate urea. After filtering under suction the solution was evaporated to dryness *in vacuo* and the residue re-dissolved in water. This was used for chromatography for identification of the phenols.

Enzyme treatment of urine samples

To the urine to be analysed for debrisoquine and its metabolites adjusted to pH5 with 2M acetic acid, was added an equal volume of either β-glucuronidase solution (Ketodase, Warner-Chilcott, Eastleigh, Hants, U.K.) or 0.2 vol. arylsulphatase preparation (type H-2; Sigma Chemical Co., St. Louis, Missouri, U.S.A.) which contained β-glucuronidase activity and 0.5 vol. 0.2M sodium acetate/acetic acid buffer (pH5). Tubes were incubated for 24 h at 37°C. Saccharic acid-1,4-lactone (2 mg, Sigma Chemical Co., Kingston upon Thames, U.K.) was added to the sulphatase incubations to inhibit the β-glucuronidase activity. A control which contained buffer added to the urine to be tested containing no enzymes was run with the test proper. After the end of incubation, both urine samples and control were derivatized with hexafluoroacetylacetone as described before and the amount of debrisoquine and 4-, 5-, 6-, 7- and 8-hydroxy-debrisoquine determined by g.l.c.

Paper chromatography for debrisoquine and its metabolites

Standards were dissolved in 2M HCl. in methanol at pH2.

Solvent systems

Solvent A: Butan-2-ol: Formic acid: Water (100:12:10 v/v)

Solvent B: Propan-2-ol: Ammonia (sp.gr.0.88): Water (20:1:2 v/v).

Whatman No.3 paper was used.

Spray reagents

Sodium nitroprusside-hydrogen peroxide: (Hoffman & Wunsch, 1958). This reagent has the following composition:
5% aqueous solution of sodium nitroprusside (20 ml)
+ 10% NaOH (10 ml) + 3% hydrogen peroxide (50 ml)
+ water (150 ml).
Guanidines appear as coloured spots (see Table 1.1).

Diazotized 4-nitroaniline:

4-Nitroaniline (0.25 g) was dissolved in 1M HCl (25 ml) and the solution diluted with ethanol (25 ml). Sodium nitrite (0.1 g) was dissolved in this solution (10 ml) and the chromatograms sprayed. After drying, the chromatograms were resprayed with 0.5M NaOH in ethanol (Wickström & Salversen, 1952).

The phenols appeared as red spots.

Table 1.1 RF values and colour reactions of debrisoquine and its metabolites

Compound	RF values in solvent		Colour reaction with	
	A	B	Diazotised 4-nitroaniline	Sodium nitroprusside
Debrisoquine	0.73	0.47	none	pink
4-hydroxy-debrisoquine	0.28	0.33	none	pink
5-hydroxy-debrisoquine	0.58	0.25	reddish-brown	purple
6-hydroxy-debrisoquine	0.57	0.22	reddish-brown	purple
7-hydroxy-debrisoquine	0.57	0.22	reddish-brown	purple
8-hydroxy-debrisoquine	0.58	0.22	reddish-brown	purple

Radiochromatogram scanning:

Paper chromatograms were scanned on a Packard Model 7201 radiochromatogram scanner.

Quantitation of metabolite peaks:

Each metabolite peak was quantified by cutting up paper chromatograms of the urine samples into bands (1 cm wide) and counting in the scintillation spectrometer. (Paper chromatogram in system A was used).

Results

The excretion pattern after a single oral dose of [^{14}C] debrisoquine (40 mg; 5 μCi) was studied in 4 normotensive subjects. The drug is well absorbed in man, 69 ± 1.8 (± S.D.)% of the dose being excreted in the urine in 24 h. (See Table 1.2).

Table 1.2 Elimination of ^{14}C in four normotensive subjects after an oral dose (40 mg) of [^{14}C] debrisoquine

Subject	Recovery of ^{14}C expressed as % of the dose
1. JI	66.8
2. RL	70.6
3. AL	70.4
4. RS	69.0
Mean	69 ± 1.8 (± S.D.)

Gas chromatography of urine samples after derivatization with HFAA revealed two peaks corresponding to debrisoquine and 4-hydroxy-debrisoquine (retention times 1 and 2 mins. respectively).

Quantitation of these as described before gave the following results; in three subjects the amount of debrisoquine excreted in 24 h urine varied from 27 to 45% with a mean of 33 ± 10.3 and for 4-hydroxy-debrisoquine 30 to 39% with a mean of 35.0 ± 4.8% . The results are shown in Table 1.3.

Table 1.3 Metabolites of Debrisoquine in 3 Normotensive Subjects expressed as % of dose in 0-24 h urine (determined by g.l.c. method)

	Human subjects			
Compound	JI	AL	RL	Mean ± S.D.
Debrisoquine	27.7	26.5	45.0	33.1 ± 10.4
4-Hydroxy-debrisoquine	37.0	38.8	29.8	35.2 ± 4.8
5-Hydroxy-debrisoquine	1.3	2.3	0.5	1.4 ± 0.9
6-Hydroxy-debrisoquine	1.0	3.6	0.7	1.8 ± 1.6
7-Hydroxy-debrisoquine	1.9	6.3	1.2	3.1 ± 2.8
8-Hydroxy-debrisoquine	0.6	1.5	0.3	0.8 ± 0.6
Total phenols	4.8	13.7	2.7	7.1 ± 5.8
Urinary recovery	69.5	79.7	77.5	75.6 ± 5.4

Gas chromatography after secondary derivatization with BSA showed four drug related peaks with relative retention times of 0.58, 0.75, 0.67 and 0.54, corresponding exactly to the standards 5-, 6-, 7- and 8-hydroxy-debrisoquine. Total amount of phenolic metabolites excreted in three subjects varied from 2.7 to 13.7% with a mean of (7.1 ± 5.8). (See table 1.3).

One subject, however, was found to be different from the other three subjects studied, as radiochromatogram scanning of his 24 h urine showed that he excreted the drug mainly as unchanged debrisoquine (54.8%) and only a small proportion as 4-hydroxy-debrisoquine (5.4%). This 'aberrant' subject was reinvestigated; 10 mg debrisoquine sulphate was given orally and the 8 h urine collected, and analysed for debrisoquine, 4-hydroxy and the four phenolic metabolites using the g.l.c. method described previously. The results are shown in Table 1.4.

Table 1.4 Metabolism and excretion of a single oral dose of 10 mg debrisoquine sulphate in an 'Aberrant' Metaboliser (RS)

Compound	% of dose excreted in 8 h urine
Debrisoquine	45.9
4-hydroxy-debrisoquine	2.5
5-hydroxy-debrisoquine	4.1
6-hydroxy-debrisoquine	0.9
7-hydroxy-debrisoquine	1.0

cont./

Table 1.4 continued

Compound	% of dose excreted in 8 h urine
8-hydroxy-debrisoquine	0.2
Total phenols	6.2
Total recovery	60.8

Incubation of urine with β-glucuronidase or sulphatase did not increase the amounts of debrisoquine, 4-hydroxy-debrisoquine or the phenolic metabolites indicating that these are excreted unconjugated in man.

A good correlation was found between the different methods used for the estimation of debrisoquine and 4-hydroxy-debrisoquine. (See Table 1.5).

Table 1.5 Estimation of debrisoquine and 4-hydroxy-debrisoquine by different techniques

	Gas-chromatography		Radiochromatogram scanning		Reverse isotope dilution technique
	debrisoquine	4-hydroxy-debrisoquine	debrisoquine	4-hydroxy-debrisoquine	debrisoquine
1. JI	27.8	37.0	25.8	29.1	-
2. RL	45.0	29.8	48.5	24.3	-
3. AL	26.5	38.8	23.0	33.4	-
4. RS	-	-	54.8	5.4	54.0
	33.1 ± 10 S.D.	35.2 ± 4.8 S.D.	32.4 ± 14 S.D.	28.9 ± 4.6 S.D.	

- Means not performed

Discussion

A single oral dose of debrisoquine is well absorbed in man as judged by the high urinary mean recoveries for the four normotensive subjects studied (70% in the 24 h urine).

Debrisoquine is metabolized in man mainly by hydroxylation. (See Fig. 1.1). 4-Hydroxy-debrisoquine is the main metabolite which agrees with the finding of Allen et al. (1975) and Angelo et al. (1975). However a large inter-individual variation in the amount of 4-hydroxy-debrisoquine excreted in the 24 h urine (5.5-39%) was observed. Three subjects had a good capacity to hydroxylate the drug hence excreting in their urine mainly 4-hydroxy-debrisoquine. On the other hand, one apparently aberrant subject had a limited capacity to effect 4-hydroxylation of the drug, excreting the drug mainly as unchanged debrisoquine (45.9%) with only a small amount as 4-hydroxy-debrisoquine (2.5%).

A significant amount of the four phenolic metabolites was excreted which also showed individual variation (2.7-14%). 7-Hydroxy-debrisoquine is the main phenol excreted in man.

No evidence was found for conjugation of any of the metabolites in man. Because all the ^{14}C excretion could be accounted for by gas chromatography as the metabolites given in Table 1.3, then metabolism by ring-fission must be a minor pathway in man.

To explain the wide inter-individual variation in the

extent of debrisoquine 4-hydroxylation it was decided to investigate the possibility that a genetic component determines the extent of this reaction. Accordingly, the study was extended to a larger population. Chapter 2 elucidates the pharmacogenetics of debrisoquine, which includes population and familial studies.

CHAPTER TWO

Pharmacogenetics of Debrisoquine

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Introduction

Pharmacogenetics

Pharmacogenetics is a science which deals with those variations in response to drugs that are under hereditary control. The term pharmacogenetics was first introduced by Vogel in 1959 for the study of genetically determined variations that are revealed solely by the effects of drugs.

Variation in drug response has been recognized for many years, Motulsky in 1957 called attention to those hereditary disorders in which symptoms may occur spontaneously but are often precipitated or aggravated by drugs. These pharmacogenetic studies have supplied evidence that there exist wide individual differences in the role of drug metabolism, the pattern of drug metabolites and the response to drugs in man. From these studies it seems that each individual has a particular pharmacological individuality, like his biochemical individuality.

Genetically transmitted variations in drug response arise mainly from mutations of DNA, through which structural alterations occur in a protein that functions directly on drug absorption, distribution, biotransformation, interaction with receptor sites, excretion or a combination of these. In a given individual, more than one pharmacogenetic mutation can exist. In such a person these pharmacogenetic mutations can exert their effects simultaneously on several processes, since each of the five processes may be controlled by different proteins, each under separate genetic control.

Two terminologies are usually used in describing any genetic disorder; first genotype, which refers to the genetic constitution of the individual, the second is the phenotype, which means the appearance or expression of an individual, and is the outcome of the interaction between the environment and the genotype over many generations.

Genetic polymorphism

Genetic polymorphism is the condition of having two or more inherited variants of the same trait or locus co-existing in the same population. Ford in 1940 pointed out that a polymorphism exists whenever a second genetic variant exists with a frequency of 1% or more, with some ambiguity as to whether the 1% refers to gene or phenotype. If no second variant or combination of variants exceeds 1% the population is said to be monomorphic. A typical example of genetic polymorphism is the ABO blood group system. There is an increasing list of drugs through which new examples of genetic polymorphisms were uncovered some of which will be discussed here.

A genetic problem is usually dealt with by various methods such as population, family, and twin studies. After establishing the mode of inheritance of the trait concerned, the need arises for estimating how frequently the trait occurs in a certain population, that is the mathematical approach or quantitative genetics.

Population studies

To study the inter-individual variation in response to a particular drug, the procedure usually adopted is to give a standard dose of the drug and to examine the drug's pharmacokinetics or, after a suitable interval, determine the response to the drug. Different methods are used, such as determination of plasma half-life (Vesell, 1973), steady state plasma concentration (Evans et al., 1960; Alexanderson et al., 1969) rates of urinary metabolite excretion (Evans & White, 1964) and direct measure of the response, as in case of induction and maintenance of anaesthesia (Keeri-Szanto & Pomeroy, 1971). Also clinical observation could be a fair estimate of drug metabolism as by measuring pulse or blood pressure.

When the response of a large number of individuals is plotted in the form of a histogram, either a continuous (a unimodal distribution) or a discontinuous variation (either a bimodal or trimodal), may be found.

Continuous variation

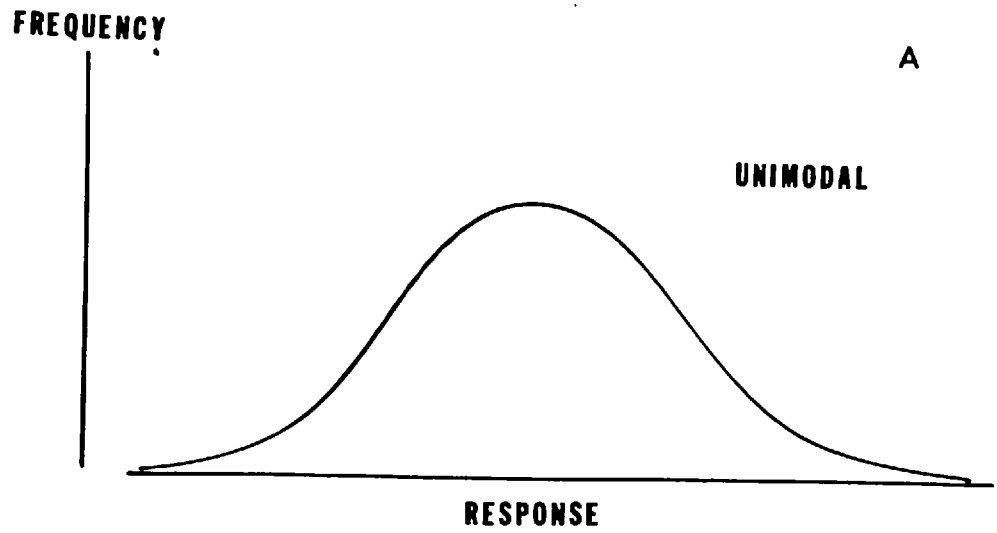
This may be described as a Gaussian distribution where the variation is represented by a single humped-curve (see Fig.2.1a). This type of variation has three possible interpretations, firstly the population under study is genetically similar in respect to the genes controlling the response being measured. Secondly, the trait being measured is controlled by genes at multiple loci (polygenic) such as genes controlling the height of individuals, stature, intelligence quotient, blood pressure, and the elimination rates of various drugs such

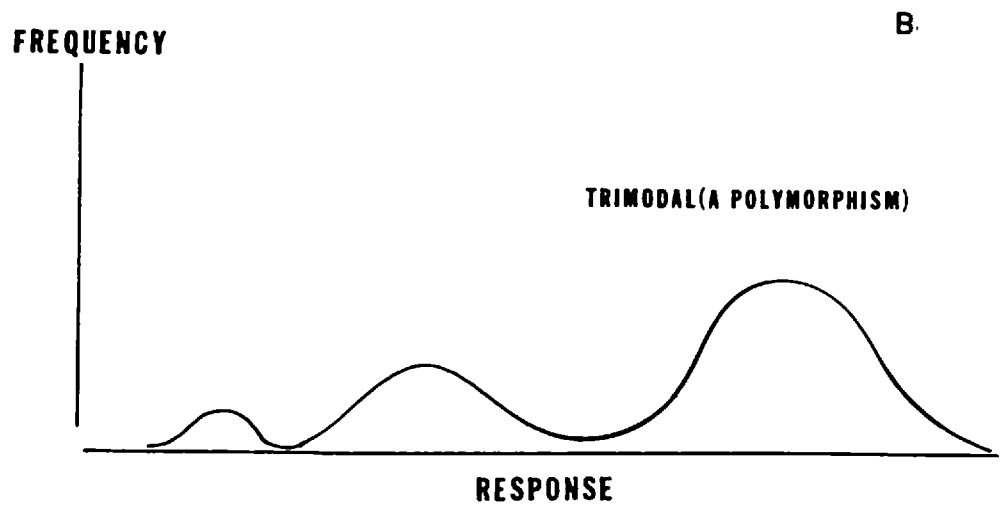
as antipyrine, phenylbutazone, bishydroxycoumarin, ethanol and halothane. The third possibility is that the observed variation is a result of insensitivity of the method used to separate inherently different populations from one another. This situation has occurred several times in pharmacogenetics. For example, when plasma pseudocholinesterase was measured in a large population, using benzoylcholine as a substrate and fluoride as the inhibitor, a unimodal distribution resulted; while when the same population was studied using a methocaine derivative (Dibucaine) as the inhibitor, a trimodal distribution occurred.

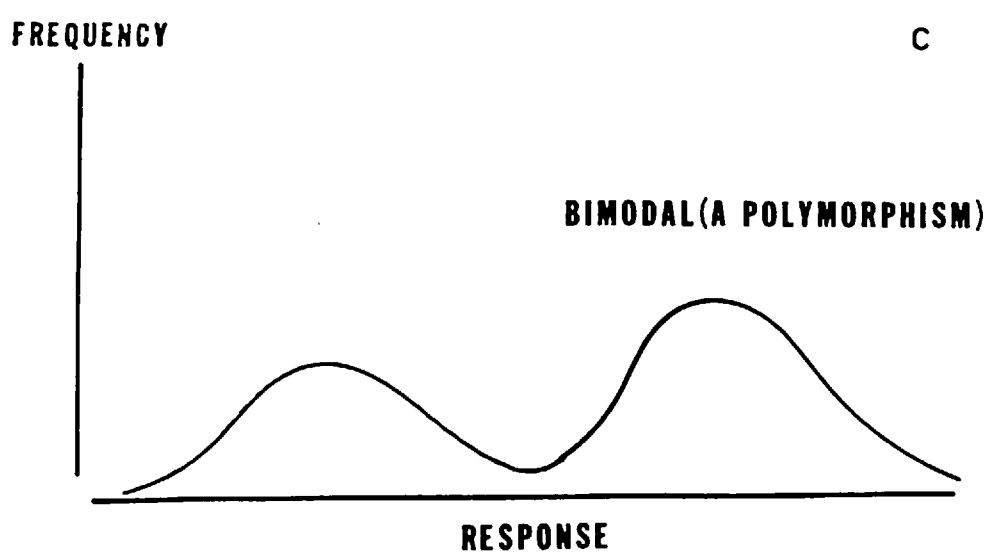
Discontinuous variation

If the discontinuous variation is under the control of a single mutant gene (monogenic), either a bimodal or a trimodal distribution can occur. Each mode represents a different genotype. In a typical condition transmitted as an autosomal recessive in which a double dose of the mutant gene is required for full expression, one hump of the distribution curve corresponds to affected individuals who have two mutant genes (homozygous defective), the second intermediate hump represents heterozygotes who are clinically or phenotypically normal and are carriers that can transmit the mutant gene to their progeny; the carriers possess one normal and one mutant gene. The third hump of the distribution curve corresponds to entirely normal individuals who have two normal genes (homozygous normal). (See Fig.2.1b).

Fig. 2.1 Various types of response to different drugs. A. Continuous variation; B. and C. Discontinuous variation.







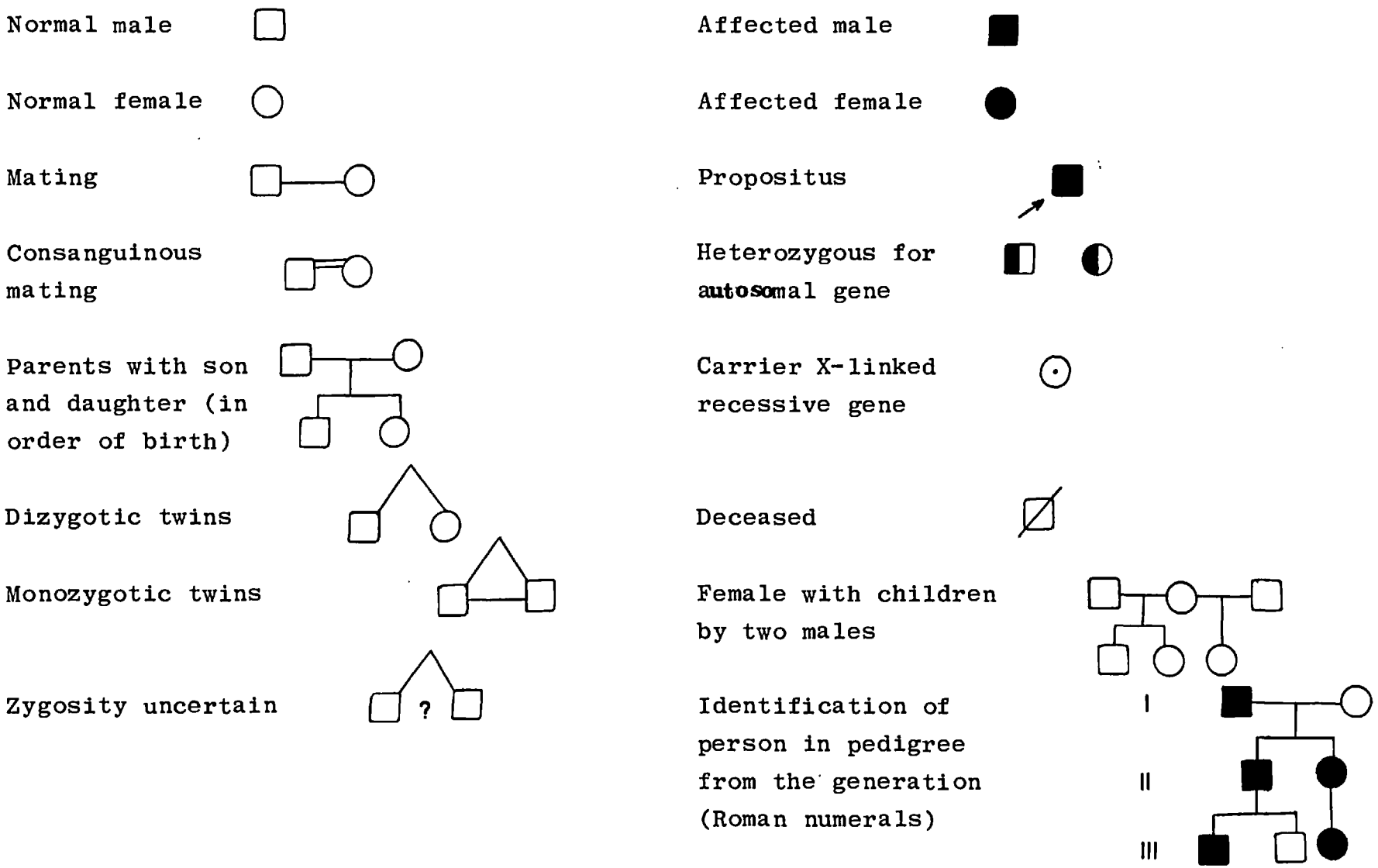
Sometimes a two hump distribution curve occurs, one hump corresponds to homozygous affected and the second includes both homozygous and heterozygous normal individuals which are unresolved (see Fig. 2.1c). Examples of drugs under monogenic control will be mentioned, and the possible mode of inheritance discussed.

Family studies

This is a useful method through which a proper understanding may be obtained of how a particular trait or disease is inherited. The drawing up of a family tree or pedigree chart begins with the affected person first found to have the trait, and through whom the family came to the attention of the investigator. This person is referred to as the propositus, if a male, and proposita if a female, and his or her position is indicated by an arrow in the pedigree chart. The next step is to investigate all brothers and sisters (siblings) and if possible, all paternal and maternal relatives. Relative information is put on the pedigree chart, the symbols which are used are shown in Fig. 2.2.

As the number of chromosomes in man is 23 pairs, 22 pairs are known as autosomes, the other pair as the sex chromosomes, hence a trait which is determined by a gene on an autosome is inherited as an autosomal trait, and it may be either dominant or recessive. A trait which is determined by a gene on one of sex chromosomes is said to be sex-linked, and may also be either dominant or recessive. There are two rare types of inheritance; in some heterozygous persons where a gene is partially expressed

Figure 2.2 Symbols used in pedigree charts (Emery, 1975)



and is said to be partially dominant or autonomous; for example the heterozygosity in sickle cell anaemia is called sickle cell trait. The second form of phenotype expression is codominance, in which two traits are both expressed in the heterozygous state, for example the ABO blood groups.

Twin studies in pharmacogenetics

A twin study is a useful method through which the environmental and heritable components of individual variations in rates of drug metabolism can be determined. It was first introduced by Galton in 1875. Recently many drugs have been studied using a twin method, such as phenylbutazone (Vesell & Page, 1968 a), antipyrine (Vesell & Page, 1968 b), bishydroxycoumarin (Vesell & Page, 1968 c), nortryptyline (Alexanderson et al., 1969), ethanol (Vesell et al., 1971) and halothane (Cascorbi et al., 1971)

In these studies both identical and fraternal twins were used, the twin zygosity was determined by typing for 30 blood groups. All twins were adults, receiving no medicine during, or at least one month before, the study. The two members of a twin pair lived apart in different houses, eating different food and having different jobs. No hospitalization was used for the subjects so that proper assessment of the environmental components of the variation could be seen. In a study of the three drugs, bishydroxycoumarin, antipyrine and phenylbutazone Vesell & Page(1968 a,b,c) used both fraternal and identical twins. Plasma half-life measurements for fraternal and identical

Table 2.1 Plasma half-lives of Bishydroxycoumarin, antipyrine and phenylbutazone in Fraternal and Identical twins (Vesell & Page, 1968c).

Twin	Age	Sex	Bishydroxy-coumarin (h)	Anti-pyrine (h)	Phenyl-butazone (days)
Fraternal twins					
AM	21	F	45.0	15.1	7.3
SM	21	F	22.0	6.3	3.6
DL	36	F	46.5	7.2	2.3
DS	36	F	51.0	15.0	3.3
SA	33	F	34.5	5.1	2.1
FM	33	F	27.5	12.5	1.2
Ja H	24	F	7.0	12.0	2.6
Je H	24	F	19.0	6.0	2.3
FD	48	M	24.5	14.7	2.8
PH	48	M	38.0	9.3	3.5
LD	21	F	67.0	8.2	2.9
LW	21	F	72.0	6.9	3.0
EK	31	F	40.5	7.7	1.9
RK	31	M	35.0	7.3	2.1
Mean differences			10.1	5.3	1.0
Identical twins					
HOM	48	M	25.0	11.3	1.9
HOM	48	M	25.0	11.3	2.1
DT	43	F	55.5	10.3	2.8
VW	43	F	55.5	9.6	2.9
JG	22	M	36.0	11.5	2.8
PG	22	M	34.0	11.5	2.8
JaT	44	M	74.0	14.9	4.0
JaT	44	M	72.0	14.9	4.0
CJ	55	F	41.0	6.9	3.2
FJ	55	F	42.5	7.1	2.9

cont.,/

Table 2.1 continued

Twin	Age	Sex	Bishydroxy-coumarin (h)	Anti-pyrine (h)	Phenyl-butazone (days)
Identical twins					
GeL	45	M	72.0	12.3	3.9
GuL	45	M	69.0	12.8	4.1
DH	26	F	46.0	11.0	2.6
DW	26	F	44.0	11.0	2.6
Mean differences			1.5	0.2	0.1

The difference between identical and fraternal twins in intrapair variance is significant $P < 0.005$

twins were 10.1 and 1.5 h respectively for bishydroxycoumarin, 5.3 and 0.2 h for antipyrine, 1.0 and 0.1 days for phenylbutazone. Obviously a hereditary factor predominates (see Table 2.1).

A mathematical expression of the relative control of a trait by hereditary factors (heritability) is given from the formula:

$$\frac{\text{(Variance within pairs of fraternal twins)} - \text{(Variance within pairs of identical twins)}}{\text{Variance within pairs of fraternal twins}}$$

(Neel & Schull, 1954; Osborne & De George, 1959).

Values ranging from zero indicating negligible hereditary and complete environmental contribution, to unity indicating complete hereditary influence. For the above three drugs studied and also ethanol, the values exceeded 0.97; halothane had a value of 0.88, nortryptyline had a value of 0.98 (Alexanderson et al., 1969).

Hardy-Weinberg Law

Having identified the trait concerned, and its mode of inheritance whether recessive or dominant, the necessity of quantitation of the observed effects arises. Hardy and Weinberg independently in 1908 put forward their law, in which they assumed that in a large randomly-mated population, the gene frequencies and genotype frequencies are constant from generation to generation. Such a population is said to be in a Hardy-Weinberg equilibrium. It follows that if the parent generation has gene A_1, A_2 and genotype frequency p and q, in random mating the various gametic contributions can be presented as:

		male gametes	
		A_1	A_2
		(p)	(q)
	A_1 (p)	A_1A_1	A_1A_2
female gametes		$(p)^2$	(pq)
	A_2 (q)	A_2A_1	A_2A_2
		(qp)	$(q)^2$

The frequencies of the various offspring from such matings are therefore $p^2 + 2pq + q^2 = 1$. Because p + q must equal unity by definition, then knowing one single gene frequency will give information on the relative proportions of the three genotypes.

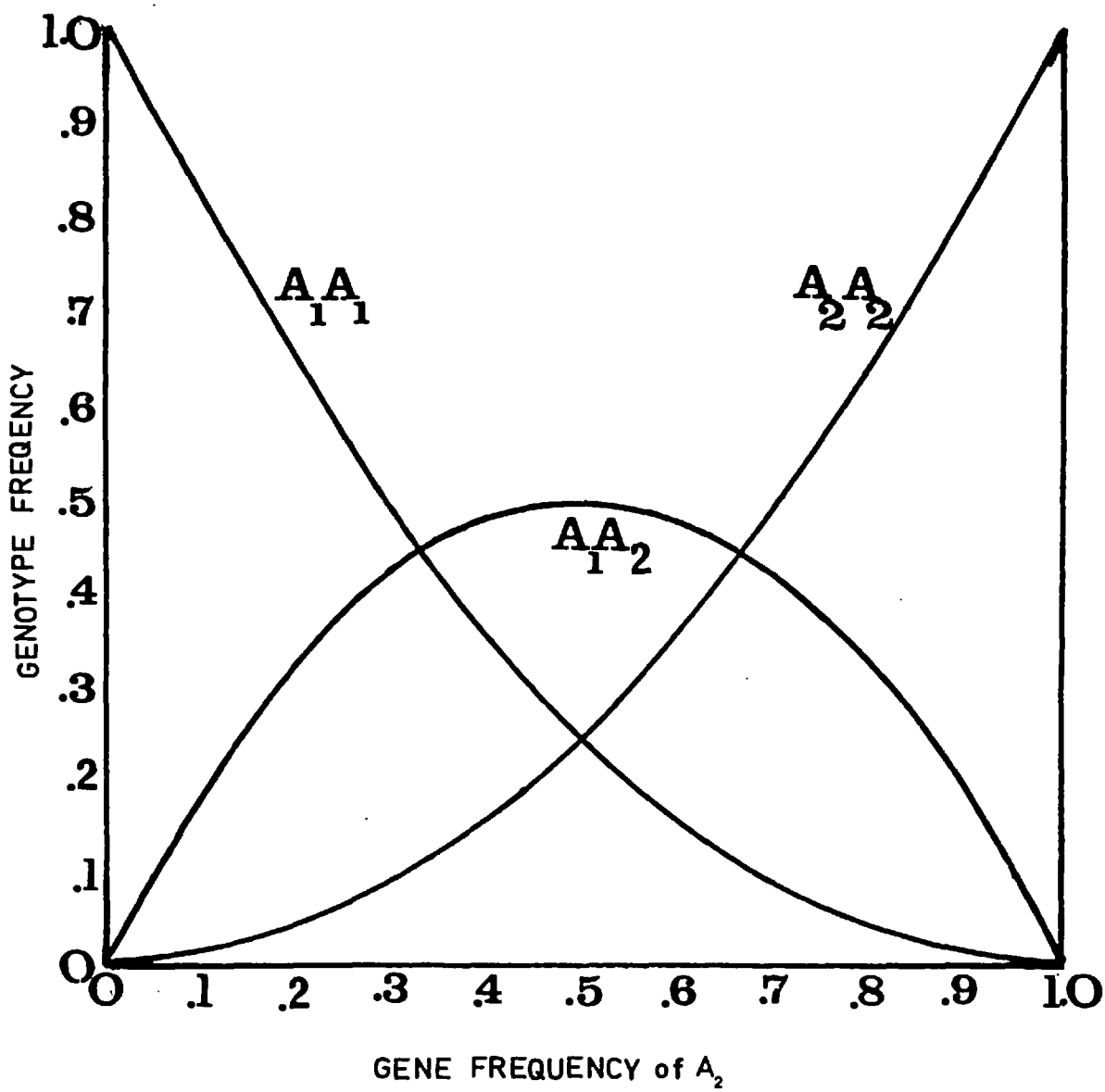
The relationship between gene frequency and genotype frequencies expressed in the above equation is illustrated graphically in Fig. 2.3, which shows how the frequencies of the three genotypes for a locus with two alleles depend on the gene frequency.

Classification of pharmacogenetic disorders known in man

Pharmacogenetic disorders have been reviewed by different authors (Kalow, 1962, 1971; Evans, 1963; Motulsky, 1964, 1971; Vesell, 1969, 1972, 1973; La Du, 1972; Goldstein et al., 1974) all of whose classifications for these disorders were quite arbitrary.

The classification which will be represented here is also arbitrary and is given in the form of tables where the name of the condition, the abnormal enzyme

Fig. 2.3 Relationship between genotype frequencies and gene frequency for two alleles in a population in Hardy-Weinberg equilibrium. (From Falconer, 1960)



responsible and its location, the mode of inheritance of the defect, its frequency among the population concerned (if known) and the drug which produces that abnormal response is given.
Accordingly, pharmacogenetic disorders could be classified into:

I - Disorders due to decreased drug detoxication.
II - Disorders characterized by increased resistance to drugs.
III - Disorders induced by administration of drugs.
IV - Miscellaneous disorders.

I Disorders due to decreased drug detoxication

The underlying defect here is caused by decreased or total absence of enzyme activity. Clinical symptoms usually result from high plasma levels or tissue concentrations of the parent drug which is not cleared or detoxicated as rapidly as in normal individuals (see Table 2.2)

II Disorders characterized by increased resistance to drugs

Disorders classified in Table 2.3 are those where the genetic defect may be due to a defective receptor, an absorption defect, an atypical enzyme, or a partial or complete absence of the enzyme.

III Disorders induced by the administration of drugs

Drug-induced disorders may arise due to enzyme deficiency, abnormal structure of the enzyme, or when an

Table 2.2 Disorders due to a genetically controlled deficiency in drug detoxication

Condition	Abnormal enzyme and location	Mode of inheritance	Frequency	Drugs that produce the abnormal response	Reference
Slow acetylation	N-acetyl-transferase (Liver)	Autosomal recessive	50% USA population	Isoniazid	Knight et al., 1959; Evans et al. (1960); Sunahara et al. (1961)
				Hydralazine	Evans & White (1964); Reidenberg et al. (1973)
				Procainamide	Reidenberg et al. (1975); Karlsson & Molin (1975); Frislid et al. (1976)
				Sulphapyridine	Das & Eastwood (1975); Schroder & Evans (1972); Schroder & Campbell (1972).
				Sulphazalazine	Schroder & Campbell (1972)
				Sulphadimidine	Evans (1962); Evans & White (1964)
				Dapsone	Gelber et al. (1971); Peter & Levy (1971).
				Phenelzine	Evans et al. (1965); Johnstone & Marsh (1973).
				Nitrazepam	Karim & Evans (1976).
Succinylcholine apnea of long duration	Pseudocholine-estrase (Plasma)	Autosomal recessive	Several aberrant alleles most common disorder occurs 1 in 2500	Succinylcholine (Suxamethonium)	Lehmann & Ryan (1956); Kalow & Genest (1957); Kalow & Staron (1957); Lehmann & Liddell (1964); Harris & Whittaker (1961); Lubin et al. (1973); Scott (1973)
Diphenylhydantoin toxicity due to deficient para-hydroxylation	?Microsomal mixed function oxidase (Liver)	Autosomal or X-linked dominant	Only one small pedigree	Diphenyl-hydantoin	Kutt et al. (1964; 1970)
Bishydroxycoumarin sensitivity	?Microsomal mixed function oxidase (Liver)	Unknown	Only one small pedigree	Bishydroxy-coumarin	Vesell (1975)

Table 2.3 Genetically determined disorders characterised by an increased resistance to drugs

Condition	Abnormal enzyme and location	Mode of inheritance	Frequency	Drugs that produce the abnormal response	Reference
Warfarin resistance	?altered receptor or enzyme in liver with increased affinity for vit.K	Autosomal dominant	Two large pedigrees	Warfarin	O'Reilly et al., 1964 O'Reilly (1970,1971)
Inability to taste phenyl-thiourea or phenyl-thiocarbamate	Unknown	Autosomal recessive	Approximately 30% of Caucasians	Drugs containing NCS group e.g. phenyl-thiouromethyl and propyl-thiouracil	Snyder (1932); Blakeslee (1932)
Increased capacity to metabolize ethanol	A typical alcohol dehydrogenase in the liver	Unknown	20% in liver specimens from Switzerland, 4% from England	Ethanol	Edward & Evans (1967) Von Wartburg & Schürch (1968), Stamatoyannopoulos et al. (1975)
Resistance to antihypertensive agent α-methyl DOPA	Dopamine β-hydroxylase	Autosomal recessive	-	α-methyl DOPA	Weinshilboum et al. (1975, 1973); Ross et al. (1973); Weinshilboum & Axelrod (1971); Kopin (1968).
Acatalasia	Catalase (erthrocytes)	Autosomal recessive	Mainly in Japan and Switzerland reaching 1% in certain small areas of Japan	Hydrogen Peroxide	Takahara, 1952; Nishimura et al., 1959; Takahara, 1961; Aebi et al., 1962.
Phenacetin induced methaemoglobinemia	?Microsomal mixed function oxidase (Liver)	Autosomal recessive	Only one small pedigree	Phenacetin	Shahidi, 1968
Crigler-Najjar syndrome	Microsomal glucuronyl transferase (Liver)	Autosomal recessive	-	Bilirubin	Crigler & Najjar, 1952; Childs et al., 1959; Axelrod et al., 1957; Arias et al., 1969.
				Paracetamol	Axelrod et al., 1957
				Tetrahydrocortisol	Peterson & Schmid, 1957
				Chloral hydrate, trichloroethanol and ethanol	Childs et al., 1959
Plasma Paraoxonase low activity	Arylesterase (plasma)	Autosomal recessive	65% of white English population	Paraoxon (O,O-diethyl-O-P-nitrophenyl phosphate	Geldmacher et al., 1973; Playfer et al., 1976.

enzyme is normal in structure but is induced by certain drugs. Here subjects are clinically normal, but the condition reveals itself on the administration of the drug concerned. Among this group is glucose 6-phosphate dehydrogenase deficiency, also known as favism or drug induced haemolytic anaemia. The condition is very common, approximately 100 million subjects are affected in the world with a high frequency in areas where malaria is endemic. The condition is induced by a wide variety of drugs which include, analgesics such as acetanilide acetylsalicylic acid and phenacetin (Hockwald et al., 1952; Beutler et al., 1955), antimalarials such as primaquine (Beutler, 1969) and sulphonamides (Motulsky et al., 1971; Vesell, 1972).

IV Disorders characterised by abnormal distribution of drugs

Certain conditions are known to be due to defective binding of a substance to plasma proteins, such as copper, iron and thyroxine. In the case of Wilson's disease the enzyme responsible for plasma protein binding of copper is deficient or completely absent, so copper is deposited in tissues (Scheinberg & Sternlieb, 1965; Bearn, 1972). In the case of haemochromatosis also known as Bronz diabetes, the plasma iron binding protein transferrin is normal or even high (up to twice normal) but plasma iron concentration is very high resulting in saturation of transferrin, and hence iron will be deposited in the tissues leading to the pathologic condition mentioned (Dreyfus & Shapira, 1964; Pollycove, 1972).

In the case of thyroxine which is normally bound to three types of protein namely, albumin, prealbumin and thyroid bound globulin (TBG), two types of defects have been described. The first type in which there is an increase in TBG and in a second form TBG is deficient leading to increase in the amount of circulating unbound thyroxine and increased tissue levels, particularly in the liver (Ingbar, 1961; Editorial, 1968).

V Miscellaneous Disorders

This includes a group of conditions in which an abnormal response occurs as a result of the administration of a drug. The underlying mechanism or enzyme defect is not yet known or characterised. Examples of these include, dexamethasone induced glaucoma (Armaly, 1968) and malignant hyperthermia which follows the administration of anaesthetic agents such as halothane (Denborough et al., 1962; Britt & Kalow, 1970); succinycholine, ether and cyclopropane (Kalow, 1972).

From the previous classification of pharmacogenetic disorders in man it is shown that metabolism of few drugs exhibit the phenomenon of genetic polymorphism. Among these is acetylation of isoniazid and sulphonamides, the hydrolysis of esters such as succinylcholine and paroxon. Others were shown to be under a genetic control. For example the conjugation of bilirubin and oxidation reactions such as C-parahydroxylation of diphenylhydantoin, N-hydroxylation of amorbarbital and O-de-ethylation of phenacetin. The consequences of

certain of the better understood examples will be discussed in more detail.

Defective acetylation of isoniazid

Isoniazid is a potent antituberculer drug, exhibiting a wide inter-individual variation in its metabolism, the hereditary nature of which was reported by Bönicke & Reif (1953) and Hughes et al.,(1954). Two phenotypes were recognised, namely, rapid and slow acetylators. In rapid acetylators about 94% of isoniazid is converted to acetylisoniazid which is further metabolized to isonicotinic acid and free acetylhydrazine (Michell et al., 1976). In slowinactivators, only 63% was converted to acetylisoniazid and subsequently to the two other metabolites. Slow acetylators were found to be more liable to develop peripheral neuropathy. In the study of Devadatta et al., (1960) he reported an incidence of 20% amongst the slow phenotype and 3% among the rapid phenotype. On the other hand, slow acetylators showed a higher cure rate than rapid acetylators when a once or twice a week standard dosage regimen was employed. Alarcon-Segovia et al. (1971) reported the higher occurrence of systemic lupus erythematosus and demonstrated the antinuclear antibodies in the serum of the slow acetylator phenotype.

Phenytoin toxicity among slow acetylators was also shown by Kutt et al. (1970) due to the inhibitory effect of isoniazid on phenytoin metabolism. By contrast, rapid acetylators were found to be more prone to liver injury

after a prolonged term treatment with isoniazid (Michell et al., 1975), where it is claimed that acetylhydrazine metabolites are responsible for the hepatic injury by binding covalently to macromolecules in the liver cells.

In a population study of isoniazid where 484 subjects were given a dose of the drug (9.8-10 mg/kg body weight) and the plasma concentration of the drug was measured 6 h after dosing, a bimodal distribution was found to occur. A plasma level of 2.5 μg/ml was taken to differentiate the two modes. Those above that level were described as slow inactivators, and those below as rapid inactivators.

Using twin methods, Bönicke & Lisboa (1957) found that the inter-individual variation in isoniazid metabolism is 97% governed by genetic factors.

By using the family study method, Knight et al. (1959) in a large family pedigree reached the conclusion that the slow inactivators were homozygous for a recessive allele, while rapid inactivators were either homozygous or heterozygous for a dominant allele, in other words the slow inactivator allele is recessive. Evans et al. (1960) in his study of 53 families arrived at the same conclusion and he reported further that the mode of inheritance is not sex linked nor age dependent.

Racial differences in the incidence of isoniazid slow phenotype has been reported by many authors and this will be discussed in Chapter 3.

Deficient C-para-hydroxylation of diphenylhydantoin

Diphenylhydantoin (phenytoin) is widely used as

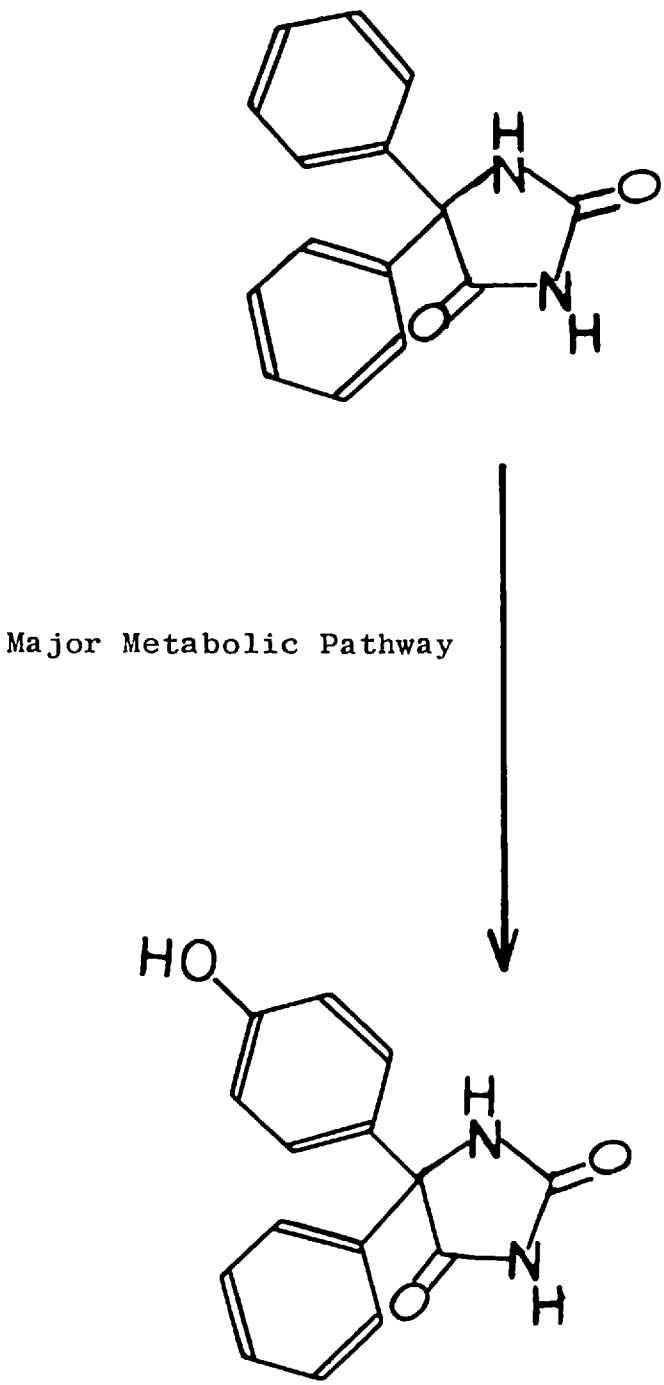
an antiepileptic drug. It has a structure which is common to all anticonvulsants, similar to phenobarbitone, the oldest anticonvulsant. (See Fig. 2.4).

The absorption of the drug from the intestine is slow and variable, peak plasma concentrations after a single oral dose could be as early as 3 h or as late as 12 h after dosing. Phenytoin is 70% to 95% bound to plasma protein, mainly albumin; the drug is widely distributed in all tissues; fractional binding in tissues including brain, is about the same as in plasma. Its concentration in CSF is equal to the unbound fraction in plasma.

The drug is extensively metabolized in man, less than 5% is excreted unchanged in urine, the remainder is metabolized primarily by the hepatic microsomal enzymes. The para-hydroxy-phenyl derivative which is inactive represents the major metabolite of phenytoin accounting for 60 to 70% of a single dose and is excreted in urine partly unchanged and as its glucuronic acid conjugate. Other minor and apparently inactive metabolites are, the dihydroxy (catechol), its 3-methoxy derivative and the dihydrodiol derivatives of phenytoin.

A genetically determined limitation in ability to metabolize phenytoin has been detected (Kutt et al., 1964). In his family study of a patient who developed overdose toxicity symptoms when given the usual dose of the drug, he found a reduced ability to hydroxylate diphenylhydantoin in several members of the family and he assumed that the defect might be due to defective microsomal hydroxylase activity. As diphenylhydantoin could accelerate, by induction, its own rate of metabolism

Fig. 2.4 Metabolic pathway of diphenylhydantoin.



Para-hydroxy-phenyl derivative

upon repeated administration (Conney, 1967), so these individuals may have a hereditary deficiency which prevents the usual increase in microsomal enzyme activity. Conversely, the same authors found a few patients who metabolize the drug much more rapidly than usual, but it is uncertain whether they inherited a more efficient microsomal hydroxylase or have a greater capacity for enzyme induction on chronic treatment.

As previously discussed, slow acetylators of isoniazid are more prone to develop diphenylhydantoin toxicity when they receive both drugs concomitantly. Thus this type of drug-drug interaction is genetically determined.

Deficient O-de-ethylation of phenacetin

The usual pathway of phenacetin metabolism in man involves predominantly a P-450 mediated O-de-ethylation and the excretion of large amounts of the phenolic metabolite 4-acetamidophenol (paracetamol) excreted mainly conjugated with glucuronic acid and sulphate together with small amounts of the mercapturic acid derivative. Ring epoxidation by a P-450 mediated mono-oxygenase also presumably occurs and usually results in the excretion of small amounts of 2-hydroxyphenacetin and 2-hydroxyphenetidine in the form of conjugates.

Shahidi (1968) found that in a 17 year old Swiss female taking daily 2-5 gm (40-100 mg/kg) as phenacetin suppositories for the treatment of dysmenorrhea, episodes of cyanosis developed with a methaemoglobin level ranging from 9% to 50%. When her urine was incubated at 37°C for 3 h with normal washed red cells there was a

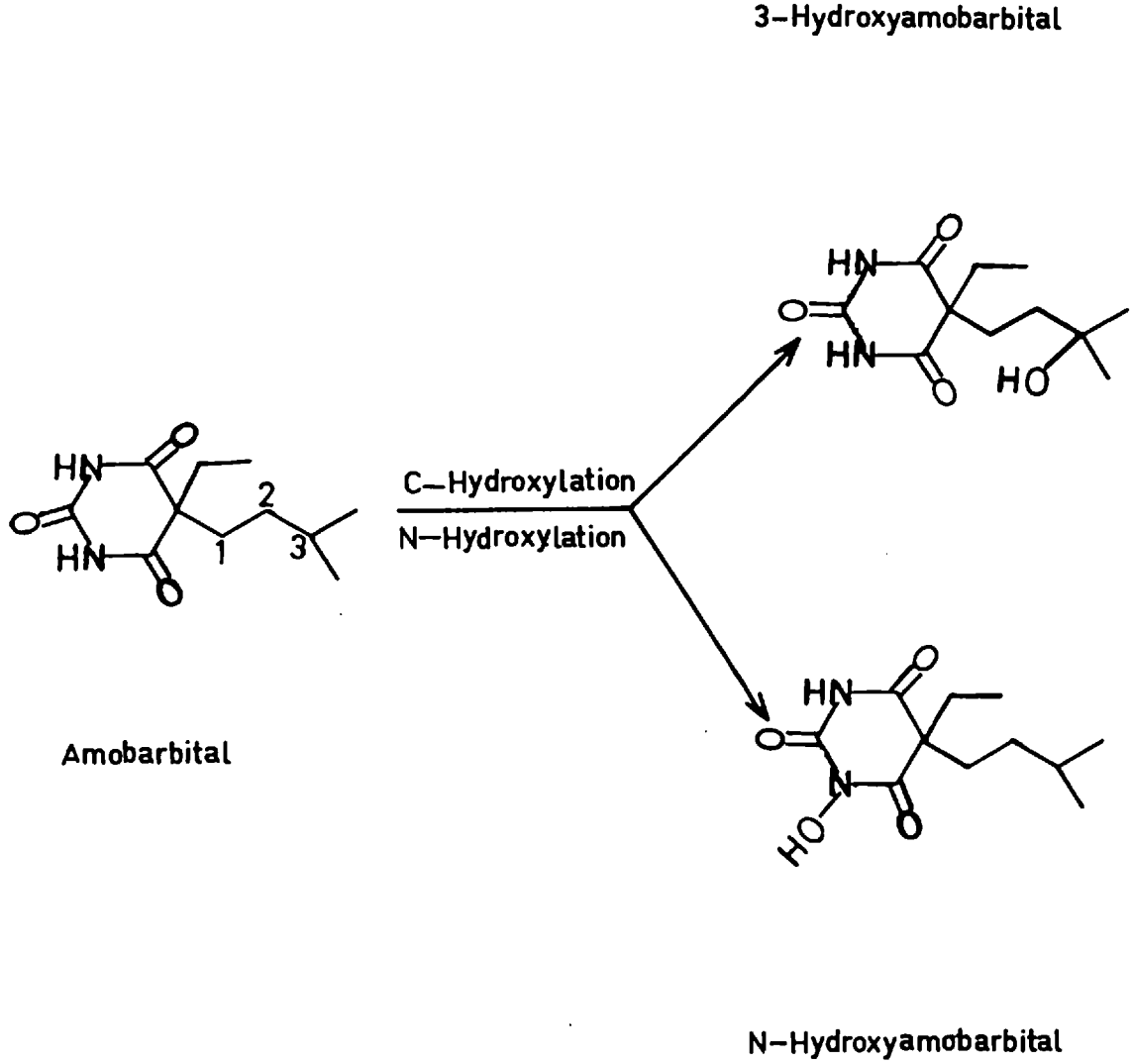
25% methaemoglobin formation; pre-treatment of her urine with β-glucuronidase increased this to 65%. A family study revealed that one sister was similarly affected, but the other sister, brother, father and mother behaved as normal volunteers. The sister excreted only 30% of the total dose as paracetamol (normal about 60%) and excreted large amounts of 2-hydroxyphenacetin glucuronide, 2-hydroxyphenetidine sulphuric acid ester, and 2-hydroxyphenetidine glucuronide. 2-Hydroxyphenetidine and its glucuronide in the presence of β-glucuronidase were shown to cause marked methaemoglobinemia, with red cells *in vitro*, (Shahidi, 1968).

The mechanism of methaemoglobinemia is presumably due to direct chemical oxidation of oxyferrohaemoglobin by the *o*-aminophenol. So this disorder appears to be the result of decreased P-450 mediated *O*-de-ethylase activity leading to the formation of haemotoxic metabolites. No population study was done so the frequency of the disorder is unknown.

Defective *N*-hydroxylation of amobarbital

Amobarbital is a short acting barbiturate with a half life varying from 14-42 h. The drug is completely absorbed when given orally with no first pass metabolism and no major biliary excretion (Inaba *et al.*, 1976). The drug is moderately bound to plasma protein (55 to 60%) (Inaba & Kalow, 1975), and it is extensively metabolized, almost no unchanged amobarbital is excreted (Maynert, 1965; Tang *et al.*, 1975). All of the drug can be accounted for as metabolites, the main metabolite being 3-hydroxy-

Fig. 2.5 Metabolic pathway of amobarbital.



amobarbital, a product of side chain hydroxylation and the second major metabolite is N-hydroxy-amobarbital. These two metabolites account for about 92% of the drug in urine and the hydroxylated amobarbitals are not conjugated (see Fig. 2.5).

Kalow et al., (1977) in their early studies, found that N-hydroxylation of amobarbital is under genetic control. In a family study he showed that the amount of N-hydroxy-amobarbital excreted in urine was very low in a pair of twin mothers, while their husbands excreted the highest amounts, and all offsprings in both families as well as the grandmother ranged between (Table 2.4). He concluded that N-hydroxylation capacity is determined by an autosomal recessive allele. Therefore the grandmother and all offsprings were heterozygous (see Fig. 2.6). Also there was no evidence of compensatory or linked activities between the two types of hydroxylation, namely N and C-hydroxylation; each appearing to be independent and catalysed by a cytochrome P-450 containing mono-oxygenase system in human liver.

Since publication, the exact nature of the "N-hydroxy" metabolite has been the subject of debate. There is now some evidence that this metabolite may be an N-glucoside (Kalow, 1978).

Because debrisoquine is extensively metabolized by oxidation (see Chapter 1) it is important to discuss here the mechanism of oxidation.

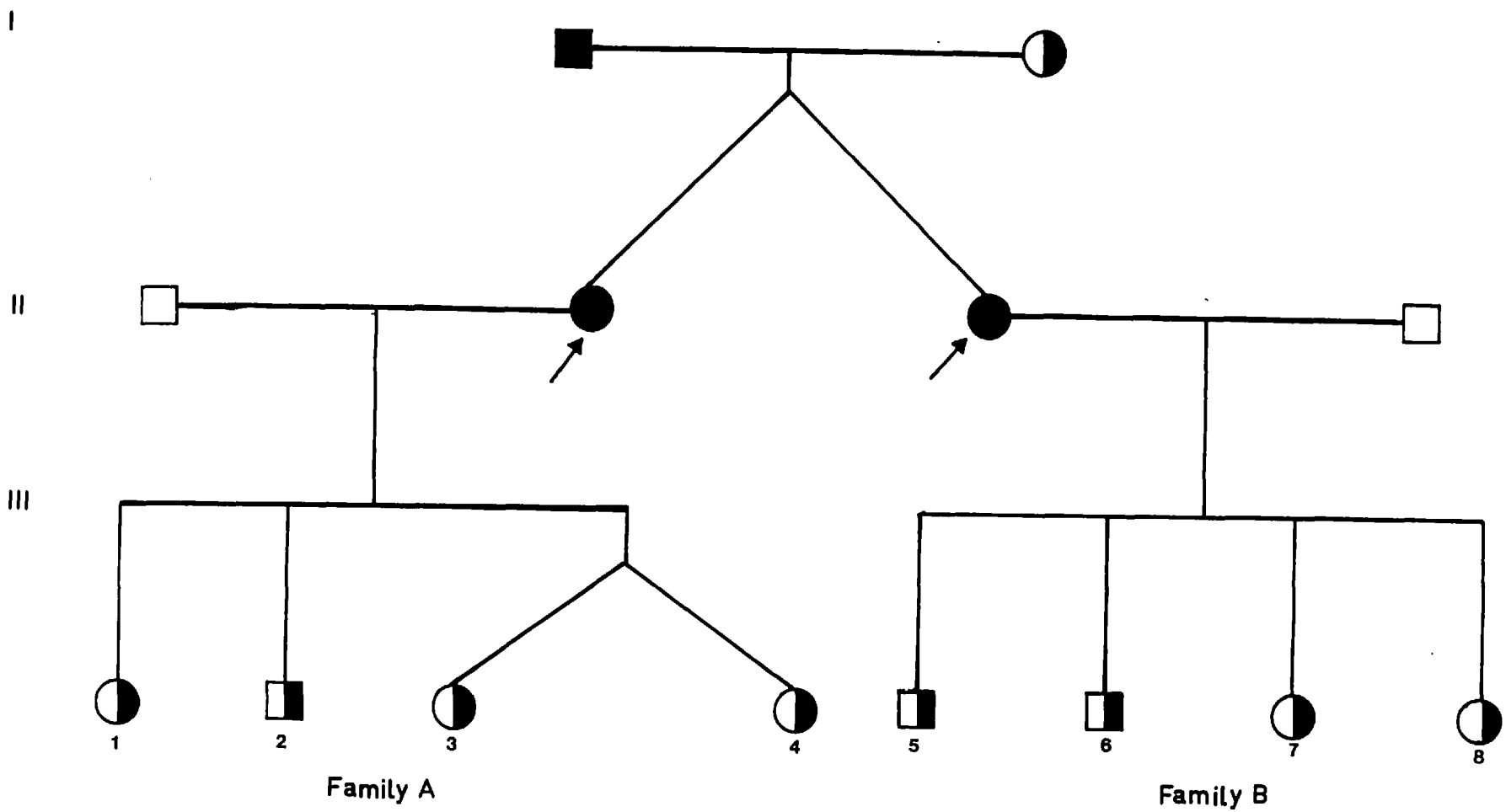
Mechanisms of drug oxidation

Oxidation appears to be the most common metabolic

Table 2.4 Amobarbital metabolism in a family (Kalow et al., 1977)

	Oral dose (mg)	Age (yr)	% dose excreted in urine (0-48 h) as N-OH	C-OH	Total
Grandmother	120	80	8.6	19.1	27.7
Mother A (twin) ←	120	52	0.2	30.2	30.4
Mother B (twin) ←	120	52	0.7	38.0	38.7
Husband A	120	45	21.7	24.7	46.4
Husband B	120	56	18.9	30.7	49.6
1 Daughter A	120	18	12.7	17.2	29.9
2 Son A	120	16	10.3	24.4	34.7
3 First twin A	60	13	10.7	33.7	44.7
4 Second twin A	60	13	12.3	34.8	47.1
5 First son B	120	28	6.4	42.8	49.2
6 Second son B	120	25	11.4	41.1	52.5
7 First daughter B	120	23	9.4	39.6	49.0
8 Second daughter B	120	20	4.6	40.5	45.1

Fig. 2.6 Family pedigree of N-hydroxylation of amobarbital. (Kalow et al., 1977)



event for drugs and has been extensively studied *in vitro*. These reactions include, carbon oxidation (both aromatic and aliphatic hydroxylation), *N*-oxidation, *N*-hydroxylation, sulfoxidation, *N*- and *O*-dealkylation, *S*-dealkylation, deamination and desulfuration (Williams, 1959).

Drug oxidation reactions could be classified into two main groups. The first group, where drugs are catalyzed by nonmicrosomal enzyme systems, such as benzyl alcohol oxidation and the aromatization of quinic acid (Adamson *et al.*, 1970) to benzoic acid. The second and major group includes drugs that are catalyzed by a group of oxidative enzymes called mixed function oxidases (Mason, 1957), or mono-oxygenases (Hayaishi, 1969).

Mixed function oxidases or mono-oxygenases

This enzyme system is mainly found in the endoplasmic reticulum of the hepatic parenchymal cells. When this system is isolated by ultra-centrifugation from a liver homogenate, the endoplasmic reticulum forms small lipoprotein vesicles known as microsomes. Other tissues with notable mono-oxygenase activity are, kidney, adrenal, lung and intestinal mucosa.

Lu *et al.*, (1970) showed that mono-oxygenase systems of the rat liver is composed of 3 components, a heat stable phosphatidyl choline lipid factor which is essential for the proper transfer of electrons from NADPH to cytochrome P-450. The second factor is known as NADPH-cytochrome P-450 reductase. The third component is called

cytochrome P-450 which is a carbon monoxide binding haemoprotein, first reported by Klingenberg (1958) and by Garfinkel (1958). The carbon monoxide pigment complex has an intense absorption band at 450 nm. The interaction of substrate with oxidised cytochrome P-450 has shown that there are two distinct types of spectral changes, namely type I and type II spectra. This indicates that there exists either two different binding sites or there are two types of cytochrome P-450 (Remmer et al., 1966; Imai & Sato, 1966).

The interaction of a drug and mono-oxygenase has been rationalised by Hildebrandt & Estabrook (1971) and is shown in Fig. 2.7. The reaction could be represented as follows:-

$$\text{drug} + \text{NADPH} + H^+ + O_2 \longrightarrow \text{drug-O} + \text{NADP}^+ + H_2O$$

$\frac{1}{2}$ mol of molecular oxygen is incorporated into the substrate (1 mol) the other $\frac{1}{2}$ mol being reduced to H_2O. As molecular oxygen per se does not interact with reduced P-450 drug complex, it has been suggested (Bray, 1970) that the form of activated oxygen is the superoxide anion O_2^- which has been detected in biological systems by e.s.r. spectroscopy. This oxygen species is unstable in aqueous media as it gives rise to hydrogen peroxide but it is believed (Ullrich et al., 1971) that the lipid (hydrophobic) environment of the endoplasmic reticulum membrane stabilizes this superoxide.

Factors which affect the activity of the mono-oxygenases include species, sex, and the presence or absence of certain inducers and inhibitors. Species differences occur mainly in phase II reactions. For example

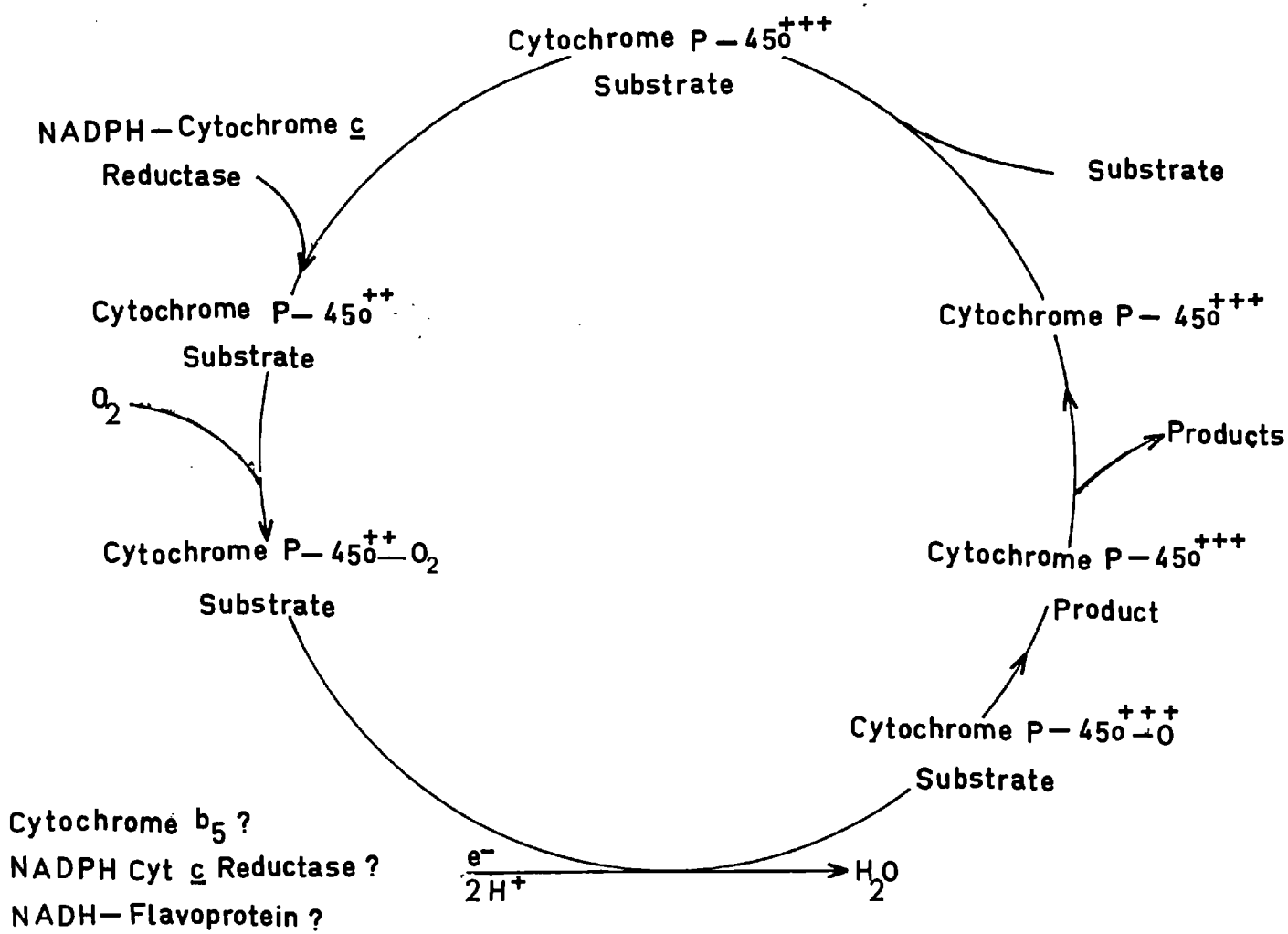


Fig. 2.7 Mechanism of cytochrome P-450 enzyme systems. (by Hildebrandt & Eastabrook, 1971)

the conjugation of phenylacetic acid (James et al., 1972) and benzoic acid (Bridges et al., 1970) with glucuronic acid and certain amino acids shows wide and varied species differences. In contrast, there are few documented examples of large species differences e.g. defects in oxidative pathways (see Table 2.5)

Table 2.5 Common animal species defective in certain conjugations (after Williams, 1967)

Conjugation	At low level or absent in
Glucuronide synthesis	Cat
Acetylation of aromatic amines	Dog
Mercapturic acid synthesis	Guinea pig, man, hen
Hippuric acid synthesis	Hen
Sulphate conjugation	Pig

One example of an oxidative difference is amphetamine. It is metabolized mainly by oxidative deamination in many species. In man, rhesus monkey, dog, guinea pig and mouse the end product of this process is benzoic acid and its conjugates hippuric acid and benzoyl glucuronide. In rabbit, beside these metabolites two major products are found, one being 1-phenyl-2-propanol and a product yielding phenylacetone on hydrolysis. However in the rat, aromatic hydroxylation is the major metabolic pathway resulting in the excretion of 4-hydroxyamphetamine and its conjugates. Very little benzoic acid or its conjugates are formed. 4-Hydroxyamphetamine and its conjugates are excreted in small amounts in urine of other

species as also was unchanged amphetamine (Dring et al., 1970).

Liver microsomes from male rats metabolize a wide variety of drugs more rapidly than do those from female rats. Quinn et al. (1958) showed that the biological half life of hexobarbital was significantly longer in female than in male rats, and that the activities of the hexobarbital, aminopyrine and antipyrine metabolizing enzymes of the liver were lower in female than in male rats. In man sex differences in foreign compounds metabolism is quite limited. For example, the metabolism of antipyrine in women has been reported to be slightly faster than in men (plasma half-lives of 10.3 and 13.4 h respectively) (O'Malley et al., 1971).

One of the characteristics of the microsomal enzyme system is that its activity is liable to be increased by the administration of certain drugs or by chemicals in the environment. The administered drug may not only increase its own metabolism but also increase that of other drugs and endogenous substrates. The first observed stimulants of enzyme activity were the polycyclic hydrocarbons (Brown et al., 1954). Conney & Burns (1959) and Remmer (1959) found that phenobarbital and a number of other common drugs also acted as inducers. Most classes of drugs were implicated, e.g. hypnotics, tranquilizers, sedatives, CNS stimulants, anticonvulsants, hypoglycemic agents, anti-inflammatory agents, muscle relaxants, analgesics, antihistamines, anaesthetic agents, steroid hormones, certain food additives and insecticides.

In man susceptibility to induction is believed to

be genetically determined and is greatest in individuals with slowest rates of drug metabolism prior to induction (Fingel & Dixon, 1975). As drug metabolizing activity may increase as much as four-fold phenobarbital and other microsomal inducers have been employed in the treatment of hyperbilirubinemia in infants.

The hepatic mono-oxygenase system was found to be inhibited either competitively or non competitively the best known compound in this respect is SKF 525-A (Anders, 1971).

It is concluded that any observed inter-individual variation in drug oxidation is due to differences in enzymic activity which is the outcome of the interaction of many factors, such as physiological (e.g. liver blood flow), pathological (e.g. liver disease), environmental (e.g. diet and smoking) and genetic factors. The most dramatic should be the genetic component, since the presence or absence of enzymic activity, as genetically determined, will play a larger role than perturbation of enzyme activity by physiological, pathological or environmental determinants. This is exemplified by the observation that inter-species variations in drug metabolism are generally more profound than intra-species differences.

Investigative Procedures

Population studies

(i) London study:

97 Healthy volunteers all of Caucasian origin, among staff and students at St. Mary's Hospital Medical School were included in this study, after having the approval of the Ethical Committee of the Medical School.

Subjects were male and female of the age group 19-50, none of the subjects was taking any medicine during, or for at least one week before the experiment. Volunteers were informed that they were taking a drug which is used to lower the blood pressure of patients suffering from hypertension, that the dose of 10 mg used in the investigation was below the therapeutic dose, and that the only side effect of this drug is postural hypotension, an exaggeration of the clinical response.

All experiments started at 9 a.m. After emptying their bladder, each subject was given a 10 mg Declinax® tablet orally, on top of a light breakfast. Urine was collected in plastic bottles for a period of 0-8 h, during which subjects went about their normal activities, having unlimited amounts of food and drink with the exception of alcohol which was not allowed during the investigation. Records kept for each subject included name, age, sex, smoking habits and any family history of hypertension. Urine volume was measured and a sample of 20 ml was kept in plastic vials at $-20^{o}C$ until analysed for

debrisoquine and 4-hydroxy-debrisoquine by gas chromatography (vide infra).

(ii) Liverpool study:

97 Normal healthy volunteers were among Caucasians living in Liverpool, including members of staff and students at the Medical School, University of Liverpool. Subjects were both male and female of the age group 18-60 years.

The protocol was the same as in the London study. After giving a 10 mg Declinax tablet, urine was collected for 8 h and the volume measured. A 20 ml sample of each urine was kept at -20°C until transported to London in dry ice to be analysed.

Family studies

(i) London study:

Available members of families of subjects defective in their ability to 4-hydroxylate debrisoquine, (the propositi) after being repeated to demonstrate consistency, were given a 10 mg Declinax tablet. The two subjects in family A aged 9 and 12 were given 5 and 7.5 mg respectively. Urine collection and urinary analysis was as for the population study. Four families were included denoted families A, B, C and D.

(ii) Liverpool study:

The four families of the propositi found to be defective in 4-hydroxylation of debrisoquine during the Liverpool population study were denoted families E,F,G and H.

Analytical Methods

All urine samples were analysed by e.c.g.c. after derivatization with hexafluoroacetylacetone as described in Chapter 1. The urinary excretion of debrisoquine and 4-hydroxy-debrisoquine was estimated and a metabolic ratio was then calculated as defined by the formula:

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

and the results were plotted in the form of a histogram, which relates the number of individuals against the metabolic ratio plotted on a semilog plot basis.

Results

Population Study

London study:

From the study of 97 Caucasian subjects living in the London area, the mean population recovery encountered as debrisoquine and 4-hydroxy-debrisoquine in the 8 h urine was 58.0% $\pm$ 19.2. The mean recovery for 68 males included in the study was 58.0% $\pm$ 19.2 while that of 29 females was 58.4% $\pm$ 19.6.

From the histogram illustrated in Fig. 2.8 it is apparent that the distribution of the metabolic ratio in the population exhibits a discontinuous variation, with metabolic ratios ranging 0.2 to 33. A first small group with metabolic ratio from 20 to 33 consisted of six subjects, 3 males and 3 females. Subjects in this group excreted mainly debrisoquine (18.1 - 56.1%) and very little amounts of 4-hydroxy-debrisoquine (0.8 - 2.5%), hence they are designated "poor metabolizers" (PM) and comprise the PM phenotype. The mean 0-8 h urinary recovery of this group as debrisoquine and 4-hydroxy-debrisoquine was 44.6% $\pm$ 15.8.

The largest number of individuals (91) tested fell into a Gaussian-like distribution with metabolic ratios ranging from 0.2 - 8.0 and a mean of 0.5 - 0.7. The mean 8 h urinary recovery as judged by debrisoquine and 4-hydroxy-debrisoquine excretion was 59.6% $\pm$ 18.6. These subjects excreted debrisoquine (9.7 - 65%) and 4-hydroxy-debrisoquine (1.3 - 58%) both in substantial

Fig. 2.8 Frequency distribution of the metabolic ratio, urinary debrisoquine/4-hydroxy debrisoquine in 97 London Caucasian volunteers.

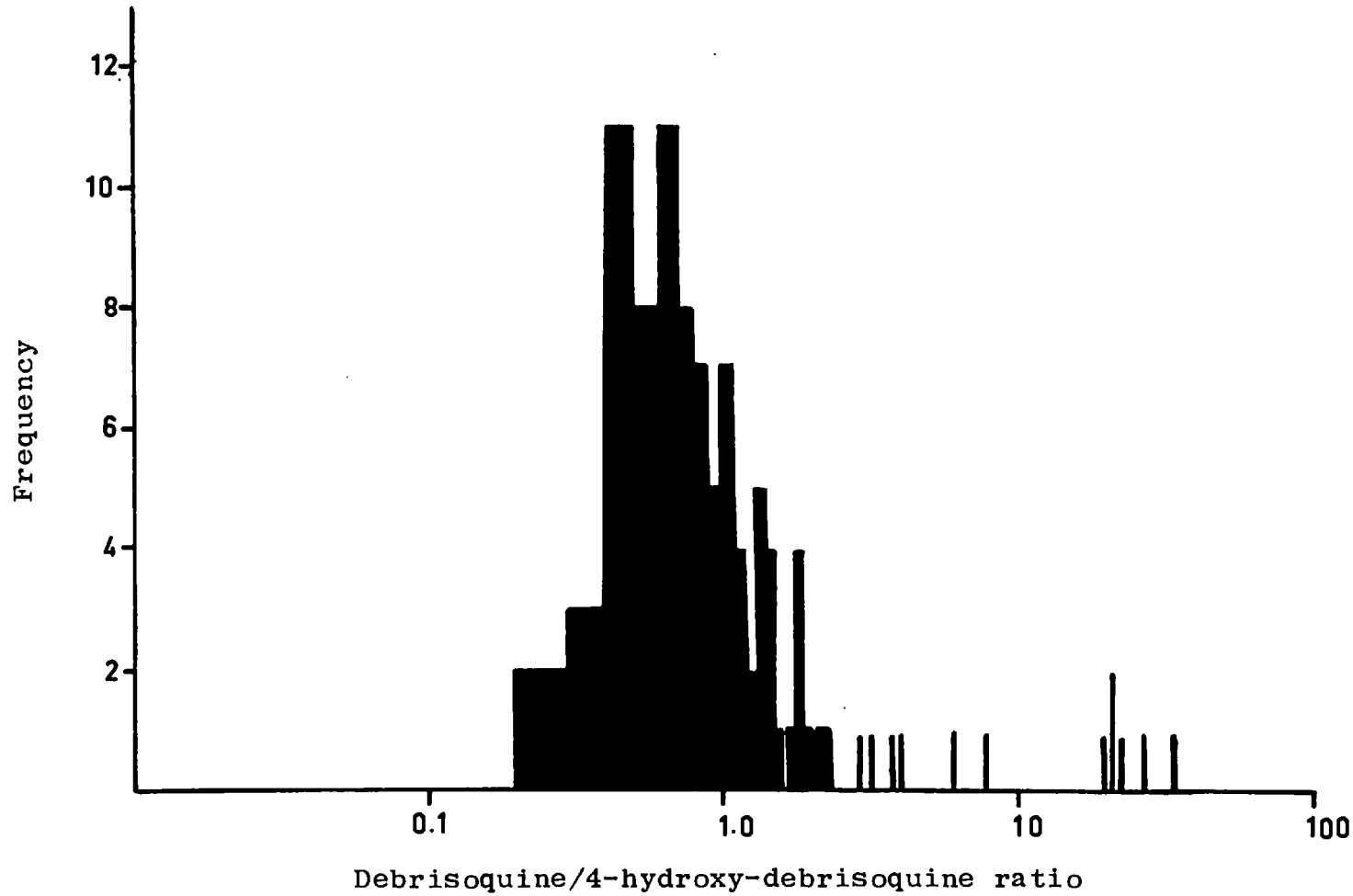


Table 2.6 Metabolic ratios of repeated subjects in the London study

	Subject		% dose excreted in 8 h as: Debrisoquine	4-hydroxy-deb.	Recovery (0-8 h)	Ratio
	Extensive metabolizers (EM)					
1.	T.M.	(i)	15.7	25.8	41.5	0.6
		(ii)	41.3	55.8	97.1	0.7
2.	M.C.	(i)	16.6	30.5	47.1	0.5
		(ii)	32.3	45.0	77.3	0.7
3.	R.D.	(i)	28.9	29.5	58.4	1.0
		(ii)	20.0	23.8	43.8	0.8
4.	P.L.	(i)	45.1	45.4	90.5	1.0
		(ii)	33.4	46.3	79.7	0.7
5.	G.K.	(i)	28.6	18.7	47.3	1.5
		(ii)	10.4	8.1	18.5	1.3
6.	L.W.	(i)	24.8	48.2	73	0.5
		(ii)	11.2	22.4	33.6	0.5
	Poor metabolizers (PM)					
7.	R.S.	(i)	42.7	2.0	44.7	21.4
		(ii)	39.6	2.0	41.6	19.8
8.	H.D	(i)	18.1	0.8	18.9	22.6
		(ii)	59.7	3.1	62.8	19.3
9.	M.E.	(i)	36.7	1.6	38.3	22.9
		(ii)	18.0	0.9	18.9	20.0
		(iii)	56.4	2.7	59.1	20.9

amounts and were thus designated "extensive metabolizers" (EM) and comprised the EM phenotype. It was noted that the urinary recoveries for the two phenotypes were not significantly different.

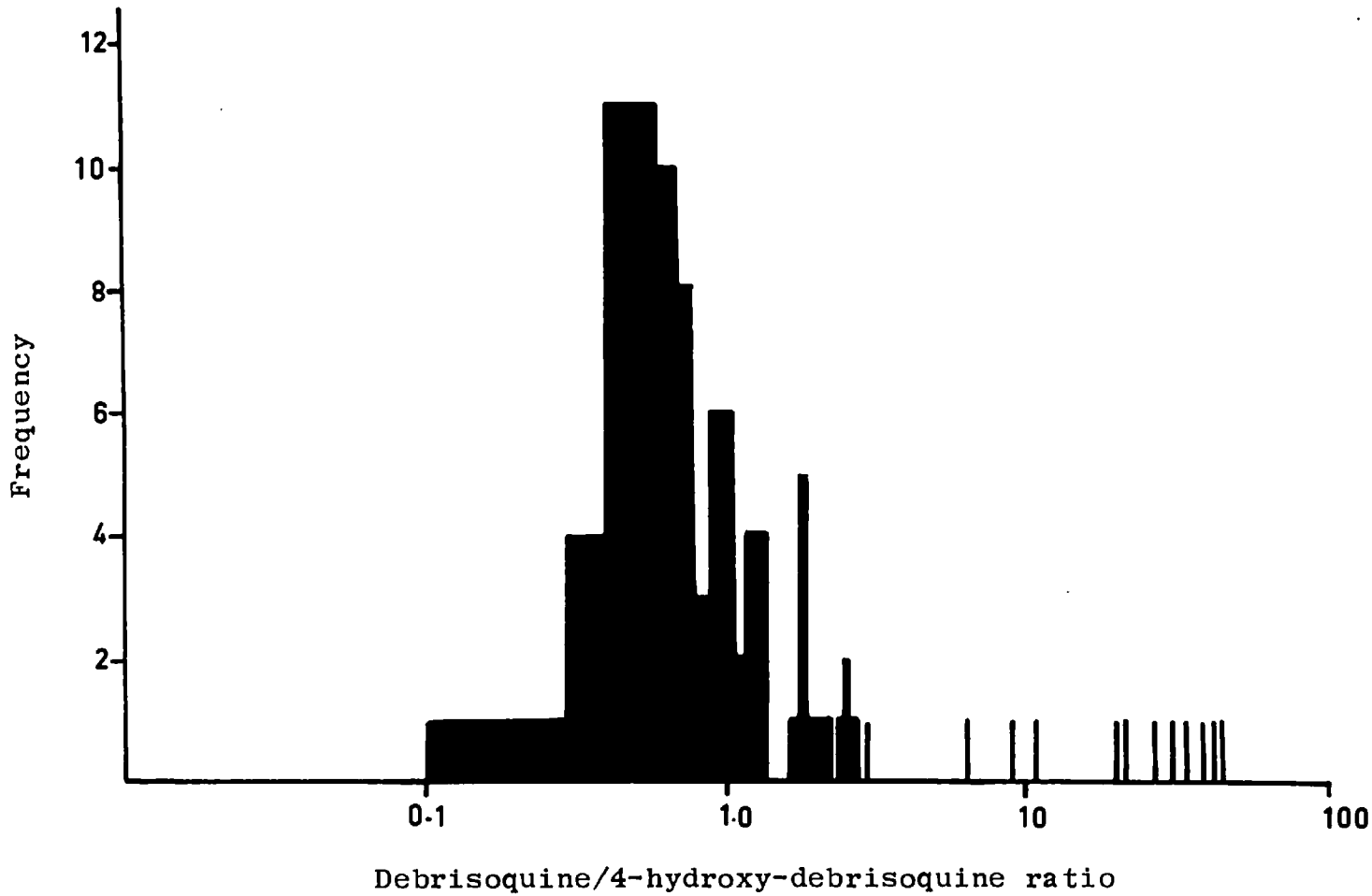
A number of subjects (6 EM and 3 PM) were repeated to test reproducibility. Table 2.6 shows that the observed metabolic ratio is largely an individual characteristic showing little or no intra-subject variation.

Liverpool study:

In order to confirm the findings of the London study, a trial was done in a second centre, viz. Liverpool. In 97 subjects investigated, it was found that the mean 0-8 h urinary recovery as debrisoquine and 4-hydroxy-debrisoquine was 36.1% $\pm$ 15.4. The mean recovery of 75 males studied was 37% $\pm$ 13.4, while that for 22 females was 33% $\pm$ 20.5.

From the histogram plotted in Fig. 2.9, it is evident that the distribution of the metabolic ratio in the population is showing a discontinuous type of variation, with a wide range of metabolic ratios from 0.1 - 42.3, similar to that of the London study. A first group which is sharply distinct and consisting of 8 subjects, 6 males and 2 females, with metabolic ratios varied from 19.6 - 42.3. Subjects in this group excreted in their urine mainly unchanged debrisoquine (18.2 - 98.8%) and very small amounts of 4-hydroxy-debrisoquine (0.7 - 2.2%). So this group was designated "poor metabolizers" (PM)and corresponded to the PM phenotype of the London study. The mean 0-8 h urinary recovery of PM phenotype was 43.4% $\pm$ 20.6.

Fig. 2.9 Frequency distribution of the metabolic ratio, urinary debrisoquine/4-hydroxy-debrisoquine in 97 Liverpool Caucasian volunteers.



The remaining 69 individuals tested showing a Gaussian-like distribution with metabolic ratios ranging from 0.1 - 10.7, with the main variation around the ratio of 0.4 - 0.6. The mean 0-8 h urinary recovery of this group was 35.0% $\pm$ 14.3. These subjects excreted a considerable amounts of debrisoquine (1.5 - 56%) and 4-hydroxy-debrisoquine (1.8 - 35.1%) so were designated "extensive metabolizers" (EM) and corresponded to the EM phenotype of the London study. As was observed in the London study there were no significant differences between the urinary recoveries for the PM and EM phenotypes.

As in the London study 6 PM and 2 EM subjects were repeated to test reproducibility and the results are shown in Table 2.7.

As for the repeated London subjects, the metabolic ratio showed little intra-subject variability, except for volunteers 2 and 8, whom on both occasions excreted, and therefore presumably absorbed, different proportions of the dose (see Chapter four). Such differences seemed to give rise to differences in excretion of unchanged drug, and thus metabolic ratio. However, phenotype assignment was unaffected (see Fig. 4.5).

Table 2.7 Metabolic ratios of repeated subjects in the Liverpool study

Subject			% dose excreted in 8 h as: Debrisoquine	4-hydroxy-deb.	Recovery (0-8 h)	Ratio
	Extensive metabolizers (EM)					
1.	L.E.	(i)	21.8	5.7	27.5	3.8
		(ii)	45.7	12.0	57.7	3.8
2.	J.H.	(i)	56.0	4.4	60.4	12.7
		(ii)	8.4	1.9	10.3	4.4
	Poor metabolizers (PM)					
3.	C.G.	(i)	20.0	0.4	20.4	48.8
		(ii)	35.7	0.9	36.6	39.7
4.	K.H.	(i)	34.9	1.5	36.4	23.0
		(ii)	41.2	2.2	43.4	18.7
5.	M.H.	(i)	35.2	1.8	37.0	19.6
		(ii)	34.2	1.7	35.9	20.1
6.	S.K.	(i)	56.3	2.2	58.5	25.6
		(ii)	18.2	0.7	18.9	26.0
7.	M.S.	(i)	40.0	1.3	41.3	30.8
		(ii)	26.3	0.9	27.2	29.2
8.	P.H.	(i)	98.8	1.7	100.5	58.0
		(ii)	36.0	1.9	37.9	18.9

Family study

(i) London study

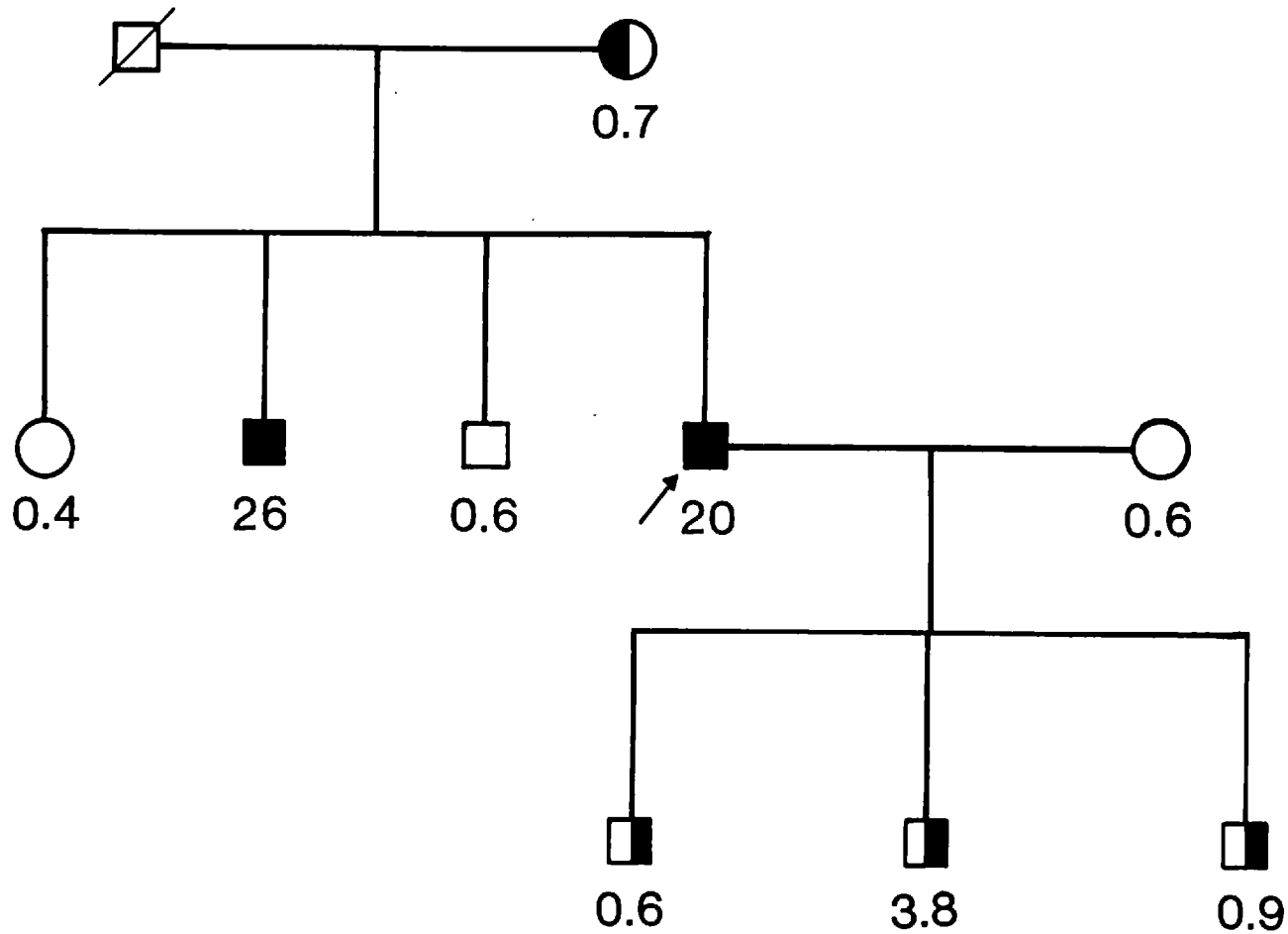
Family A

Calculated metabolic ratios and phenotype assignment for members of three generations (I, II and III) of this family are given in Table 2.8. One brother of the propositus was found to be phenotypically PM. From this data the pedigree chart shown in Fig. 2.10 was drawn. For purposes of graphical representation at this stage the affected phenotype (PM) is assumed to be homozygous for a recessive allele. This argument will be further elaborated

Table 2.8 Debrisoquine metabolism in Family A

			% dose in 0-8h urine				
		Age	Debrisoquine	4-hydroxydeb.	Recovery	Metabolic ratio	Phenotype
I	grandmother	80	12.8	17.4	30.2	0.7	EM
II	daughter	54	20.7	50.3	71.0	0.4	EM
	first son	52	47.0	1.8	48.8	26.0	PM
	second son	49	12.6	19.8	32.4	0.6	EM
	third son ←	43	42.7	2.0	44.7	21.0	PM
			45.9	2.5	48.4	18.5	
			39.6	2.0	41.6	19.5	
	wife	41	24.3	39.0	63.3	0.6	EM
III	first son	14	20.4	35.3	55.7	0.6	EM
	second son	12	33.3	8.8	42.1	3.8	EM
	third son	9	23.1	26.1	49.2	0.9	EM

Fig. 2.10 Pedigree chart of family A.



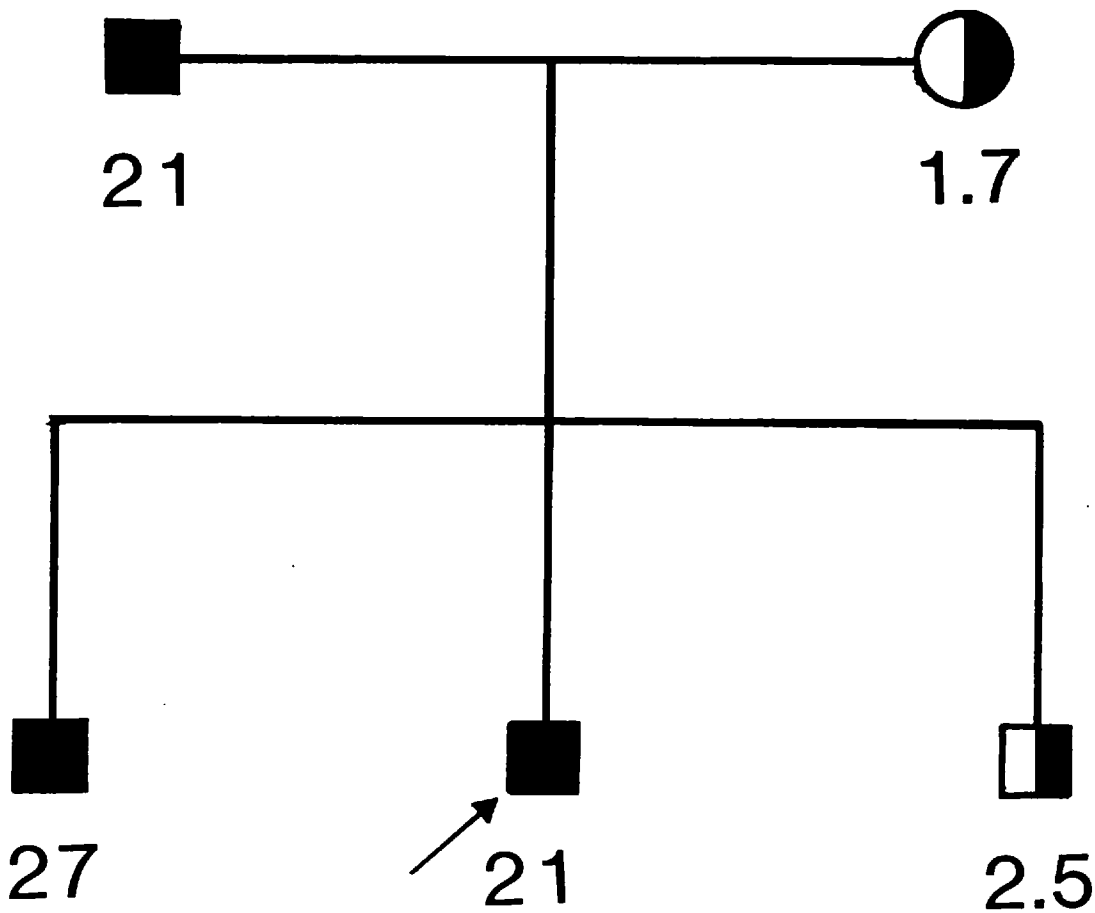
Family B

Table 2.9 represents the metabolic ratios and phenotypes of members of family B. The father of the propositus was found to be phenotypically PM (ratio 21); the mother was phenotypically EM (metabolic ratio 1.7). One brother was found to be a poor metabolizer with a metabolic ratio of 27.0; and the other phenotypically EM as his metabolic ratio was 2.5. The family pedigree chart is drawn in Fig. 2.11 from the calculated metabolic ratios presented in Table 2.9.

Table 2.9 Debrisoquine metabolism in Family B

	Age	% dose in 0-8h urine: Debrisoquine	4-hydroxy-deb.	Recovery	Metabolic ratio	Phenotype
Father	54	71.8	3.4	75.2	21.0	PM
Mother	50	47.3	27.7	75.0	1.7	EM
First son	27	43.3	1.6	44.9	27.1	PM
Second son ←	21	56.4	2.7	59.1	20.9	PM
		36.7	1.6	38.3	22.9	
		18.0	0.9	18.9	20.0	
Third son	16	47.0	18.9	65.9	2.5	EM

Fig. 2.11 Pedigree chart of family B.



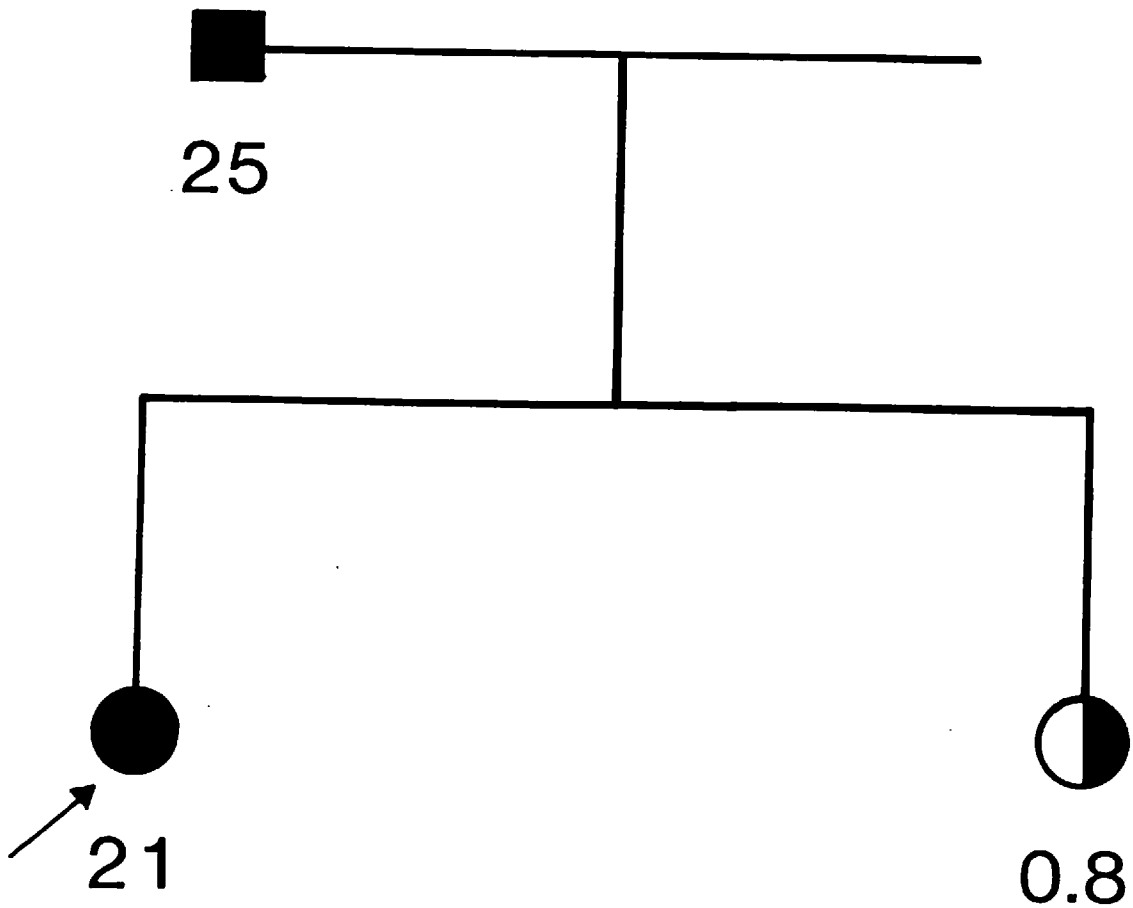
Family C

In this study the proposita was a female with a metabolic ratio of 20.0. Her father was found to be of the PM phenotype. The mother unfortunately could not be investigated as she was on drugs for the treatment of migraine. Her sister was found to be EM. The metabolic ratios and urinary recoveries are shown in Table 2.10 and from which the family pedigree chart was plotted (Fig. 2.12).

Table 2.10 Debrisoquine metabolism in Family C

		% dose in 0-8h urine				
	Age	Debrisoquine	4-hydroxy-deb.	Recovery	Metabolic ratio	Phenotype
Father	50	35.3	1.4	36.7	25.0	PM
First daughter ←	21	59.7 18.1	3.1 0.8	62.8 18.9	19.3 22.6	PM
Second daughter	19	21.8	26.8	48.6	0.8	EM

Fig. 2.12 Pedigree chart of family C.



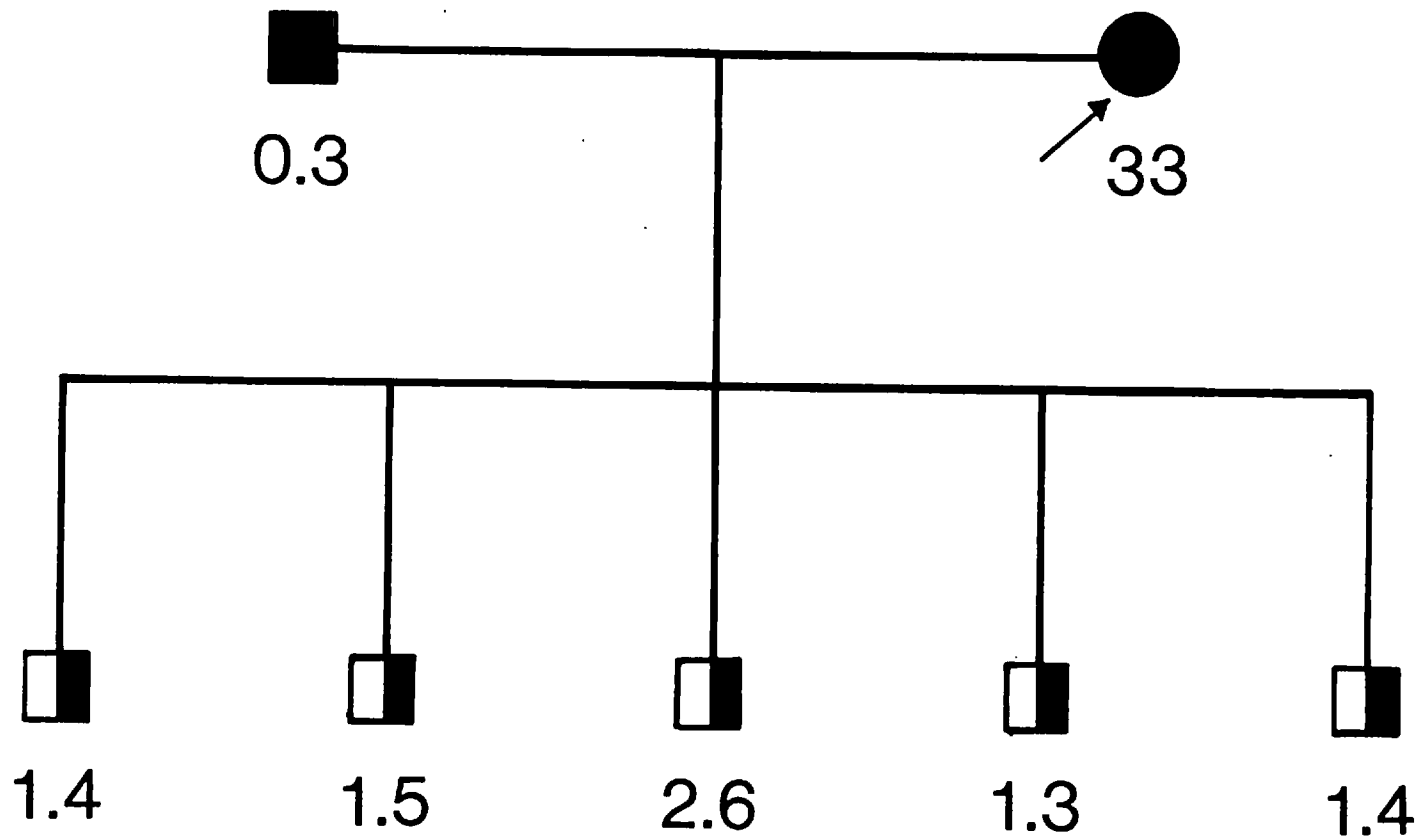
Family D

The proposita was a female with a metabolic ratio of 33.0. The husband was found to be phenotypically EM. All the five sons were found to be phenotypically EM. The metabolic ratios and urinary recoveries of members of this family are represented in Table 2.11. The pedigree chart is drawn in Fig. 2.13.

Table 2.11 Debrisoquine metabolism in Family D

	Age	% dose in 0-8h urine: Debrisoquine	4-hydroxydeb.	Recovery	Metabolic ratio	Phenotype
Father	54	5.4	16.5	21.9	0.3	EM
Mother ←	50	56.1	1.7	57.8	33.0	PM
First son	27	20.9	14.7	35.6	1.4	EM
Second son	21	34.8	23.7	58.5	1.5	EM
Third son	18	24.0	9.3	33.3	2.6	EM
Fourth son	16	32.8	25.4	58.2	1.3	EM
Fifth son	14	24.2	17.3	41.5	1.4	EM

Fig. 2.13 Pedigree chart of family D.



(ii) Liverpool study

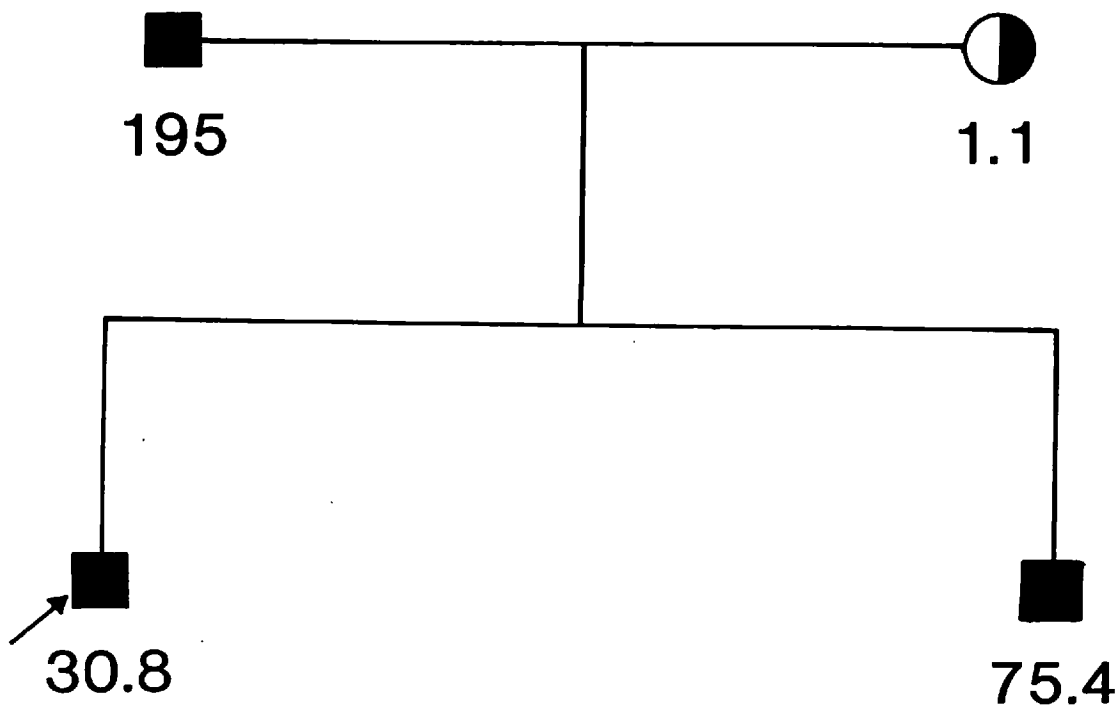
Family E

Table 2.12 shows the metabolic ratios and phenotypes of members of this family. The propositus was a male with a metabolic ratio of 30.0. The father was found to be PM (metabolic ratio > 195); the mother was found to be phenotypically EM (metabolic ratio 1.1). Only one of his brothers was available to be investigated and who was found to be phenotypically PM (metabolic ratio 75.0). The pedigree chart of family E is presented in Fig.2.14.

Table 2.12 Debrisoquine metabolism in Family E

		% dose in 0-8h urine				
	Age	Debrisoquine	4-hydroxy-deb.	Recovery	Metabolic ratio	Phenotype
Father	-	87.7	<0.45	88.2	>195	PM
Mother	-	37.0	32.7	69.7	1.1	EM
First son ←	29	40.0 26.3	1.3 0.9	41.3 27.2	30.8 29.2	PM
Second son	23	90.5	1.2	91.7	75.4	PM

Fig. 2.14 Pedigree chart of family E.



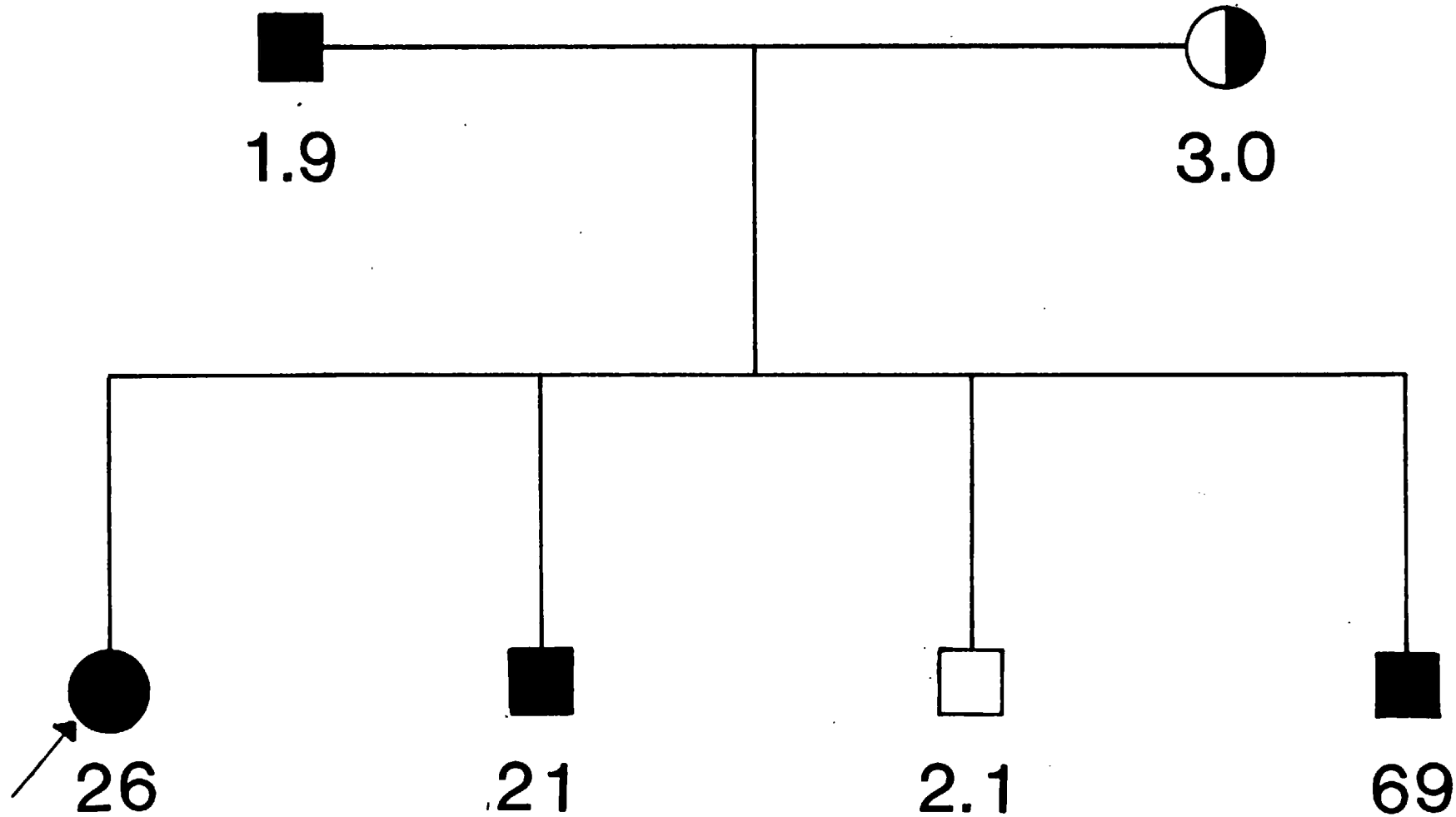
Family F

The metabolic ratios and phenotypes of subjects in family F are shown in Table 2.13. The father of the proposita was found to be phenotypically EM, the mother was also of the EM phenotype. Two brothers were found to be poor metabolizers (PM) and the third brother was EM. The family pedigree chart is presented in Fig. 2.15.

Table 2.13 Debrisoquine metabolism in Family F

		% dose in 0-8h urine				
	Age	Debrisoquine	4-hydroxy-deb.	Recovery	Metabolic ratio	Phenotype
Father	-	14.4	7.7	22.1	1.9	EM
Mother	-	36.5	12.2	48.7	3.0	EM
Daughter ←	20	56.3 18.2	2.2 0.7	58.5 18.9	25.6 26.0	PM
First son	19	71.6	3.4	75.0	21.0	PM
Second son	17	10.0	4.8	14.8	2.1	EM
Third son	15	62.0	0.9	62.9	68.9	PM

Fig. 2.15 Pedigree chart of family F.



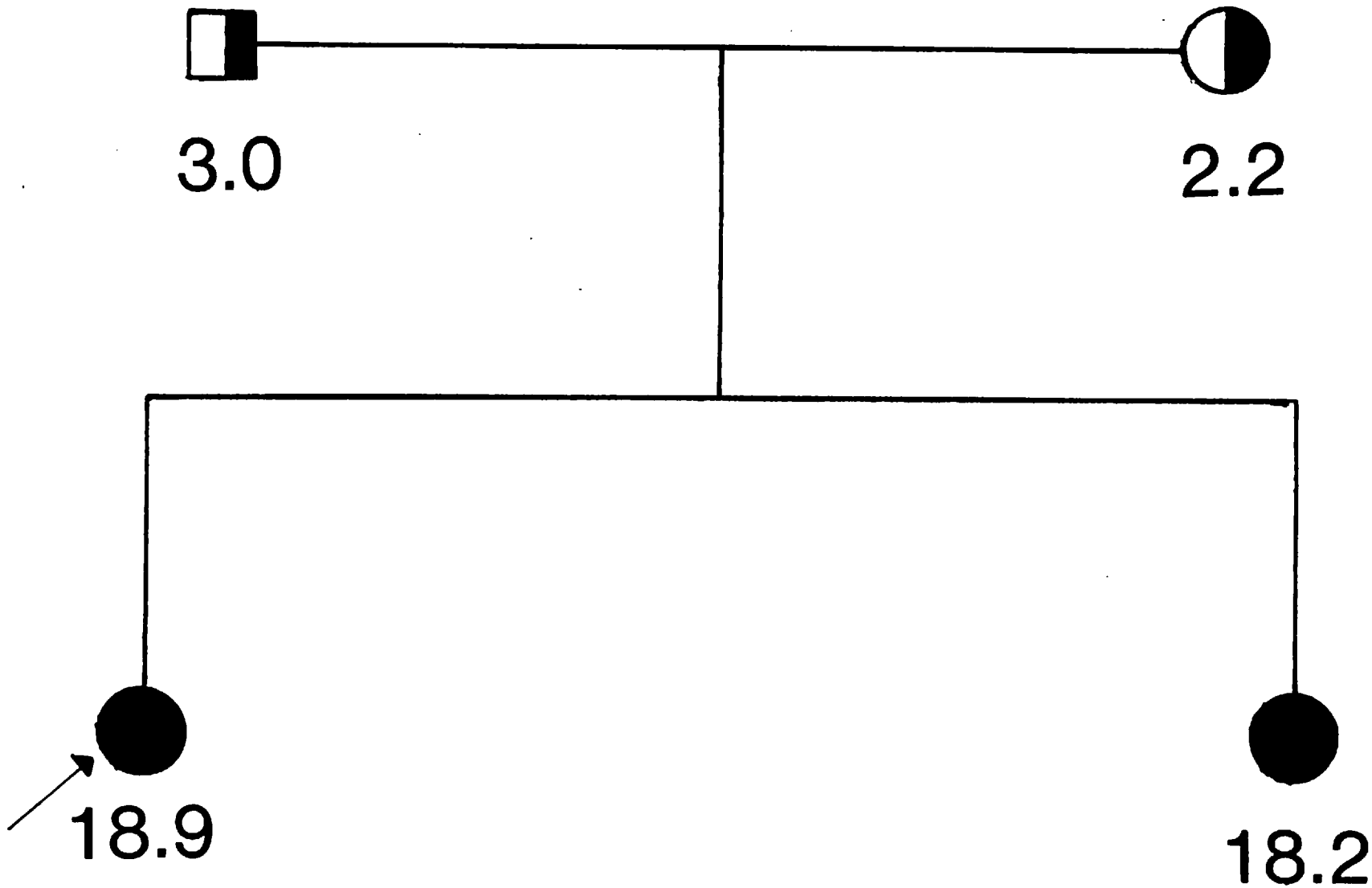
Family G

The proposita was a female (metabolic ratio 38.5). The father was found to be phenotypically EM (metabolic ratio 3.0) as was the mother (metabolic ratio 2.2). Her sister was found to be phenotypically PM (metabolic ratio 18). Table 2.14 shows the metabolic ratios and the predicted phenotypes of members in this family and from which the family pedigree chart is plotted in Fig. 2.16

Table 2.14 Debrisoquine metabolism in Family G

		% dose in 0-8h urine				
	Age	Debrisoquine	4-hydroxy-deb.	Recovery	Metabolic ratio	Phenotype
Father	-	24.2	8.1	32.3	3.0	EM
Mother	-	32.8	14.6	47.4	2.2	EM
First daughter←	20	98.8	1.7	100.5	58.0	PM
		36.0	1.9	37.9	18.9	PM
Second daughter	17	40.0	2.2	42.2	18.2	PM

Fig. 2.16 Pedigree chart of family G.



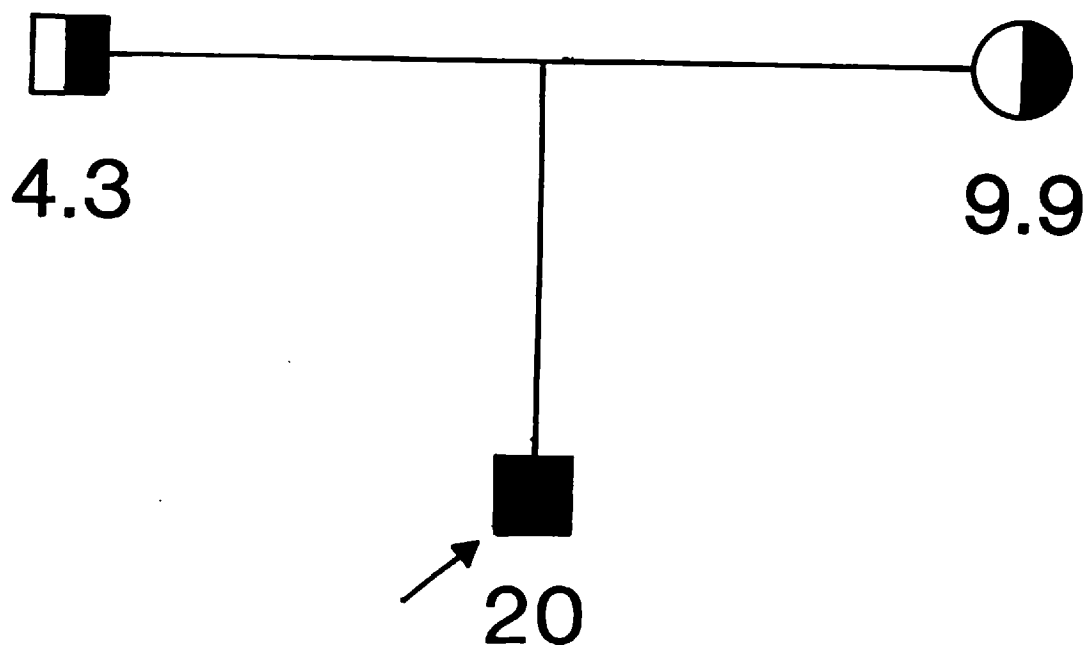
Family H

Results are presented in Table 2.15. The father was phenotypically EM and the mother also belonged to the EM phenotype. The propositus had a brother, but he was not included in the study. The family pedigree chart is shown in Fig. 2.17.

Table 2.15 Debrisoquine metabolism in Family H

		% dose in 0-8h urine				
	Age	Debrisoquine	4-hydroxy-deb.	Recovery	Metabolic ratio	Phenotype
Father	-	62.2	14.6	76.8	4.3	EM
Mother	-	65.5	6.6	72.1	9.9	EM
Son ←	18	35.2 34.2	1.8 1.7	37.0 35.9	19.6 20.1	PM

Fig. 2.17 Pedigree chart of family H.



Discussion

The population studies of the alicyclic hydroxylation of debrisoquine are suggestive of a genetic polymorphism with a large gene effect. The polymorphism appears to show a bimodal distribution. The first mode is a well defined small hump which comprised subjects with a limited capacity to hydroxylate the drug, excreting in their urine mainly unchanged debrisoquine, hence they were designated "poor metabolizers" and collectively referred to as the PM phenotype. The ability of PM subjects to effect the 4-hydroxylation of the drug is quite variable as they exhibit a wide range of metabolic ratio (20 - 200). This PM phenotype represents about 6 and 8% of the London and Liverpool populations respectively.

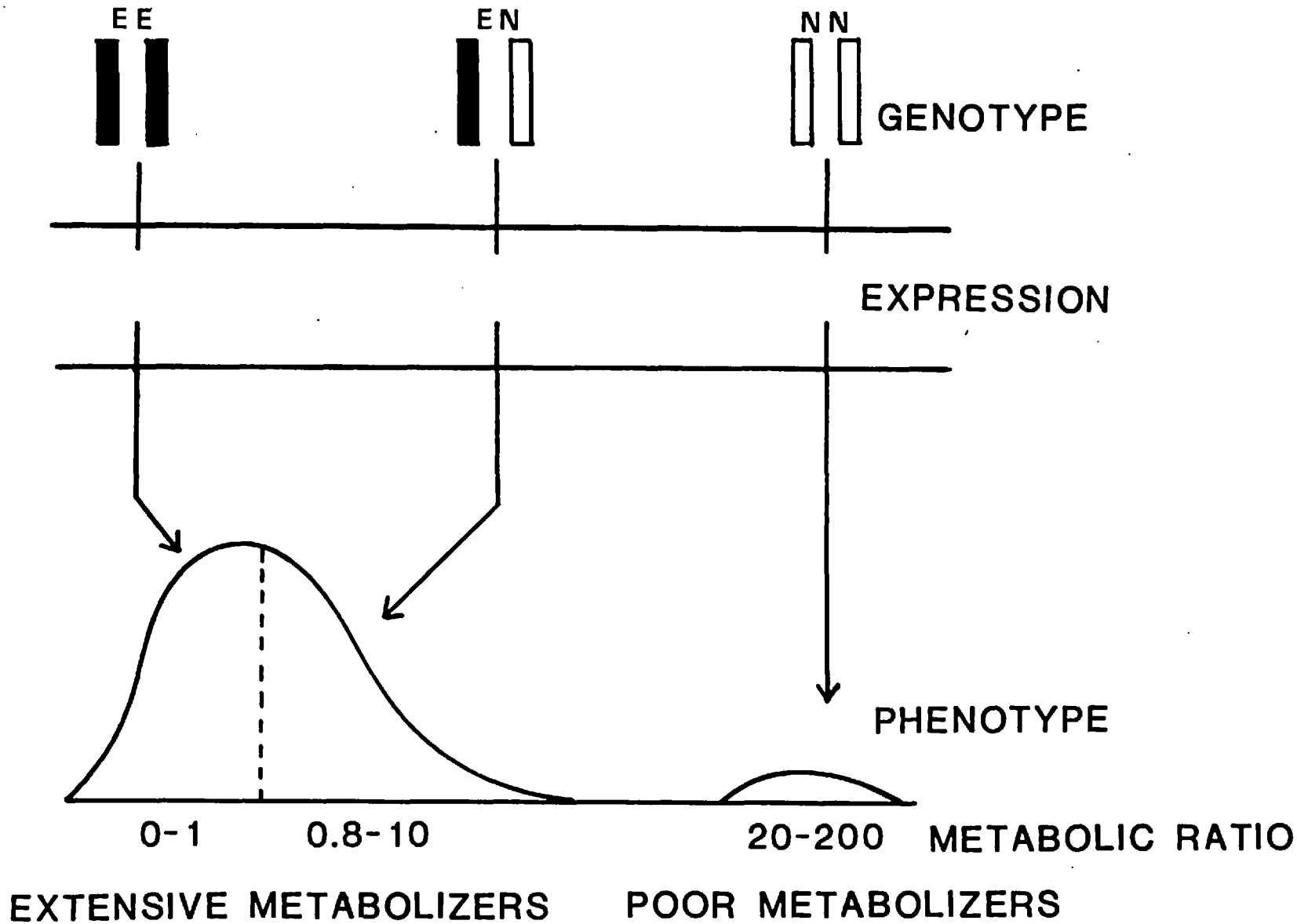
The second mode of the distribution and the largest (about 94 and 92%) includes subjects who metabolize the drug extensively to the 4-hydroxy metabolite and are thus designated "extensive metabolizers". As a group they comprise the EM phenotype. The metabolic ratios of EM phenotypic subjects also showed a wide range of variation from 0.1 to 10.7.

The metabolic ratios seemed to be an individual characteristic as after rechallenging individuals of both the PM and EM phenotypes, little or no change in the metabolic ratio was observed except in the two subjects (2 and 8) in the Liverpool study who showed a significant variation in their metabolic ratio. As it can be seen (Table 2.7), they had excreted, and hence absorbed, differing proportions of the 10 mg dose on each occasion and thus effectively experienced different doses of the

drug. However the phenotype assignment was unaffected by such variation.

The hereditary nature of the defect was confirmed by testing families of the propositi found to be poor metabolizers (PM) during the population study. Familial studies supported the view that the defective alicyclic hydroxylation of debrisoquine is genetically determined, and the 4-hydroxylation is under the control of a single autosomal gene and that a defect at this metabolic step is determined by a pair of mutant alleles. For instance, it can be seen for families F, G and H, that EM parents gave birth to affected (PM) children and also in families A and D affected (PM) parents gave birth to EM offspring. Such family data is inconsistent with the metabolic defect being a dominant trait because in these situations every affected parent should give birth to an affected child, as with the increased resistance to coumarin anticoagulants (**O'Reilly**, 1970). In other words, any affected (PM) child will have at least one of his parents affected (PM). The results however favour a recessive trait not a dominant one since affected offspring and unaffected parents were found (families F, G and H). Because both males and females of varying age were affected (see Appendices I and II) it could be concluded that the defective alicyclic hydroxylation of debrisoquine is under the control of an autosomal locus whose expression is not age-dependent. The possible genotypes and their phenotypic expression could be summarized in the following Fig. 2.18, where it is assumed that normal hydroxylation is controlled by a single gene composed of two alleles

Fig. 2.18 Genotype and Phenotype expression of the debrisoquine polymorphism.



operating on a single autosomal locus. If (E) stands for normal allele and (N) for the mutant or defective allele, the possible genotypes will be EE, EN and NN. EE corresponds to the homozygous EM phenotype, EN for heterozygous EM and NN for homozygous PM phenotype.

At this stage one could say that the mode of the distribution representing EM phenotype is unresolved and comprises the two genotypes, homozygous EE and heterozygous EN extensive metabolizers. The contribution of each genotype is calculated from the Hardy-Weinberg equation (see P 55). The calculated distribution of genotypes was approximately as follows:

London NN, 6%; EN, 37%; EE, 56%.

Liverpool NN, 8%; EN, 41%; EE, 51%.

Having established the mode of inheritance of the defect, it is of interest to know what is the possible mechanism responsible for the defective oxidation, i.e. what single protein is affected. As debrisoquine is mainly metabolized by oxidation (both alicyclic and aromatic rings), and since most oxidation reactions are catalyzed by the mono-oxygenase system located mainly in the liver, it is possible that the defect could be caused by a deficient or altered protein involved in the mono-oxygenase system as rationalized by Hildebrandt & Estabrook (see Fig. 2.7). The possible protein to be involved could be a haemoprotein (cytochrome P-450 or b_5), flavoprotein, a reductase (NADPH cyt P-450 reductase, NADH cyt b_5 reductase) or a more remote protein responsible for proper function or synthesis of these. However,

it is felt that, since such a marked gene effect has been observed with clearly defined characteristics, PM subjects being virtually unable to 4-hydroxylate debrisoquine, the affected protein is probably central to the oxidation mechanism and not peripheral. In other words, NN genotypic subjects are unable to elicit compensatory pathways to 4-hydroxylate debrisoquine, since a central component is missing or virtually so.

Many drugs have been shown to exhibit, as regards their metabolism, the phenomenon of genetic polymorphism. Among these, and well established is the acetylation of isoniazid and its related substrates, where slow inactivators are homozygous for an autosomal defective allele, and rapid inactivators are either homozygous or heterozygous for a normal allele.

Although oxidation reactions are very common, few examples of large gene effects in C-oxidation have been encountered. Kutt (1971) reported the relative inability to para-hydroxylate the anticonvulsant diphenylhydantoin in three families. Kalow et al., (1977) described, in a large pedigree, the occurrence of defective N-hydroxylation of amobarbital[+] and that the defect was recessive. Shahidi (1968) has also described in two members of a Swiss family the inability to deethylate phenacetin, resulting in the increased susceptibility to the methaemoglobinaemic side effects of the drug.

The usual methods used in the study of metabolic pharmacokinetics depends on kinetic parameters, such as

[+]Now there is some evidence that this metabolite may be an N-glucoside (Kalow, 1978).

measuring plasma half lives, rate of drug disappearance from plasma (Whittaker & Evans, 1970). In contrast, the method used here in the elucidation of a debrisoquine polymorphism involved the measurement of the two parameters of the variation, the unchanged drug and the metabolite. Our conviction is that this method is more revealing and might turn out to be a useful tool to the study of pharmacogenetics, enabling us to discover more polymorphisms in drug metabolism in future.

The establishment of a polymorphism with 6-8% defective (PM) subjects and 40% heterozygotes has several important consequences.

Among the questions which emerge include:

a) Do ethnic differences exist in the distribution of phenotypes?

b) What effect has phenotype on debrisoquine responsiveness and therapeutic usage?

c) Is the oxidation of other drugs affected by phenotype?

The following chapters attempt to answer these points.

CHAPTER THREE

Inter-ethnic variability in the frequencies of the (N) allele.

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Introduction

Large inter-individual variations in drug response can arise from multiple genetic and environmental factors affecting drug absorption, distribution, biotransformation, excretion, interaction with receptors or combinations of these. How much is the contribution of each is still a point of controversy. The studies of Vesell (1972) on monozygotic and dizygotic twins of the three drugs namely antipyrine, bishydroxycoumarin and phenylbutazone (see p 51) have suggested that the genetic factors play the major part while the environmental contributions are very small. Fraser et al. (1977) studied the effect of four environmental factors, diet, cigarette smoking, use of steroid contraceptive pills and body weight on antipyrine half life and he found that the four components together constituted 50% of the variation.

However, accumulating evidence suggests that environmental factors can have a significant effect on drug oxidation. A growing list of common environmental agents including dietary factors were found to alter rates of metabolism of many drugs such as Caffeine (Mitoma et al., 1968), Nicotine (Wenzel & Brodie., 1966), 3,4-benzopyrene (Welch et al., 1969), Methylcholanthrene (Welch et al., 1969), Alcohol (Vesell et al., 1971). Cigarette smoking has been found to alter phenacetin metabolism (Beckett & Triggs, 1967; Pantuck et al., 1974) and accelerate antipyrine metabolism (Hart et al., 1976).

Diet has been shown to have a major effect on drug

metabolism in rat (Campell & Hayes, 1974) and man. In man a vegetarian diet was found to prolong antipyrine half life by 50% compared by a non-vegetarian diet (Fraser et al., 1977)

Inter-ethnic variability of allele frequencies

The allele frequencies for the genetic control of the metabolism of some drugs which exhibit the phenomenon of genetic polymorphism were found to show wide differences when estimated in different ethnic groups. Table 3.1 shows the extremes of such variation.

Table 3.1 Inter-ethnic variability of allele frequencies (Evans, 1976)

Polymorphism	Allele for which frequency is quoted	Ethnic group	Frequency
N-acetyl-transferase	Allele controlling slow acetylation	Caucasians and Africans	Approx. 0.75
		Japanese, Koreans	Approx. 0.33
Glucose-6-phosphate dehydrogenase	Allele controlling enzyme deficiency	N. Europeans	About 0.00
		S. Europeans Africans	Up to 0.25
Plasma pseudo-cholinestrase	E_1^a	Caucasians	0.016
		Pacific populations and Africans	0.000
	E_1^f	Europeans	Rare
		Japanese	0.01
	E_1^s	Caucasians	0.003
		Eskimos	0.120
Plasma paraoxonase	N^L	British white	0.7034
		Indians	0.4629
Liver alcohol dehydrogenase	ADH_2^2	Japanese	0.61
		British	0.05
Phenylthiourea tasting	Allele controlling non-tasting	Brazilian Indians	0.111
		Hindu	0.581
Methaemoglobin reductase	Allele controlling enzymic abnormality	Caucasians	Rare
		Eskimos, Alaskan Indians	0.024

Investigative Procedures

The study was extended to various ethnic groups which included, Egyptians, Nigerians, Ghanaians, Gambians and Malaysians.

The protocol was the same as described for the Caucasian study (see Chapter 2) with the exception of the Egyptian study (*vide infra*). Declinax tablets (10 mg) were given orally to volunteers of both sexes and of different age groups (see Appendices) and the 0-8 h urines collected and volume measured. To a sample of urine (5 ml) was added a few drops of 2% Mercuric Chloride Solution as preservative and the urines stored at 0-5^{o}C until they could be transported to London for analysis. Debrisoquine and 4-hydroxy-debrisoquine, and in some cases 5-, 6-, 7- and 8-hydroxy-debrisoquine were determined by gas chromatography. The metabolic ratio was calculated (see Chapter 2) and a histogram which relates number of individuals against metabolic ratios was plotted on a semilog basis.

Egyptian Study

Seventy-three healthy Egyptians, 42 male and 31 female, aged 12-60 years were studied. Thirty of the volunteers were resident in Alexandria and 34 in Aswan Region. A further 9 Egyptians resident in London were studied. The study was made over the period of August - September 1977 (summer) which coincided with the fasting religious month in Islam i.e. "Ramadan". According to this religion people fast (from food and drink) starting from sunrise to sunset. Following sunset they eat a very

large meal, usually consisting of fried chicken and/or lamb, together with rice, bread, vegetables and salad. Drink consisted of diluted natural lemon juice. At the end of the meal subjects were given 10 mg Declinax tablets on a full stomach, and were kept mobile during the following 0-8 h where urine was collected and measured, a preservative added and the samples stored at 0-5^{o}C until they were transported to London to be analysed as described (vide supra).

Results

I. Egyptian Study

(i) Population study

From the study of 64 Egyptians resident in Alexandria and Aswan regions, the mean population recovery measured as debrisoquine and 4-hydroxy-debrisoquine in 0-8 h urine was 16.0 $\pm$ 11.6%. The mean recovery for 33 male subjects studied was 14.8 $\pm$ 8.0% and for 31 female subjects 13.4 $\pm$ 7.5% (see Appendix III).

From the histogram illustrated in Fig. 3.1, the distribution of metabolic ratios in the Egyptian population (73 subjects including 9 subjects resident in London) exhibited a genetic polymorphism, with metabolic ratios ranging from 0.03 - 23.0. Most of the individuals had a metabolic ratio ranging from 0.03 - 1.0 and constituted the EM phenotype. Only one subject was found to have a metabolic ratio of 22.7. Six individuals showed intermediate values between 3.0 - 10.0.

(ii) Family study

Family I: The proposita was a female with a metabolic ratio of 22.7 whose husband had a metabolic ratio of 1.7. There were eight siblings with metabolic ratios ranging from 0.5 - 11.9. Fig. 3.2 represents the family pedigree chart.

Family J: Father and mother were phenotypically EM with metabolic ratios of 0.1 and 0.2 respectively. Two daughters were found to belong to the EM phenotype as their metabolic ratios corresponded to 0.04 and 0.1.

Fig. 3.1 Frequency distribution of the metabolic ratio, urinary debrisoquine/4-hydroxy-debrisoquine in 73 Egyptian volunteers.

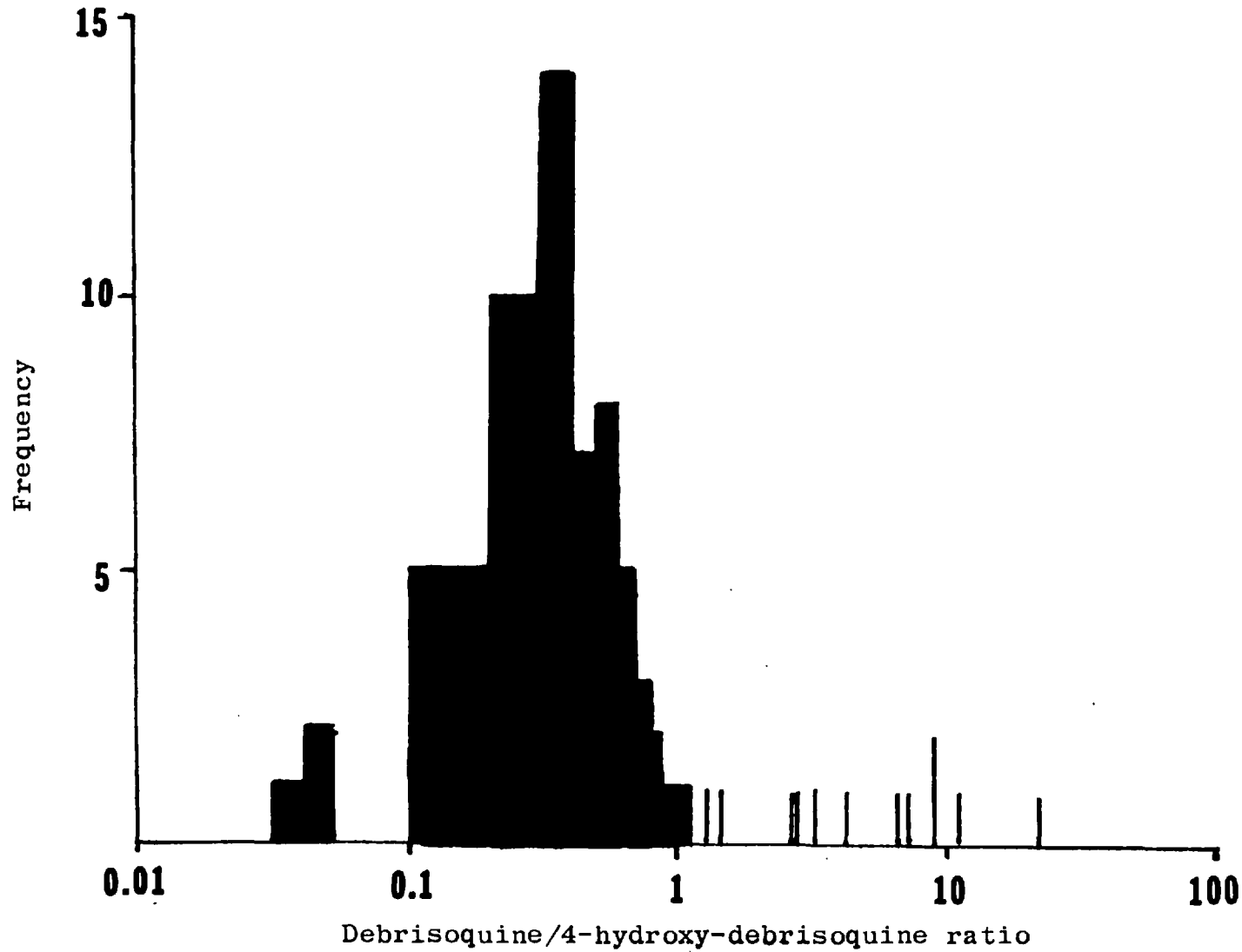
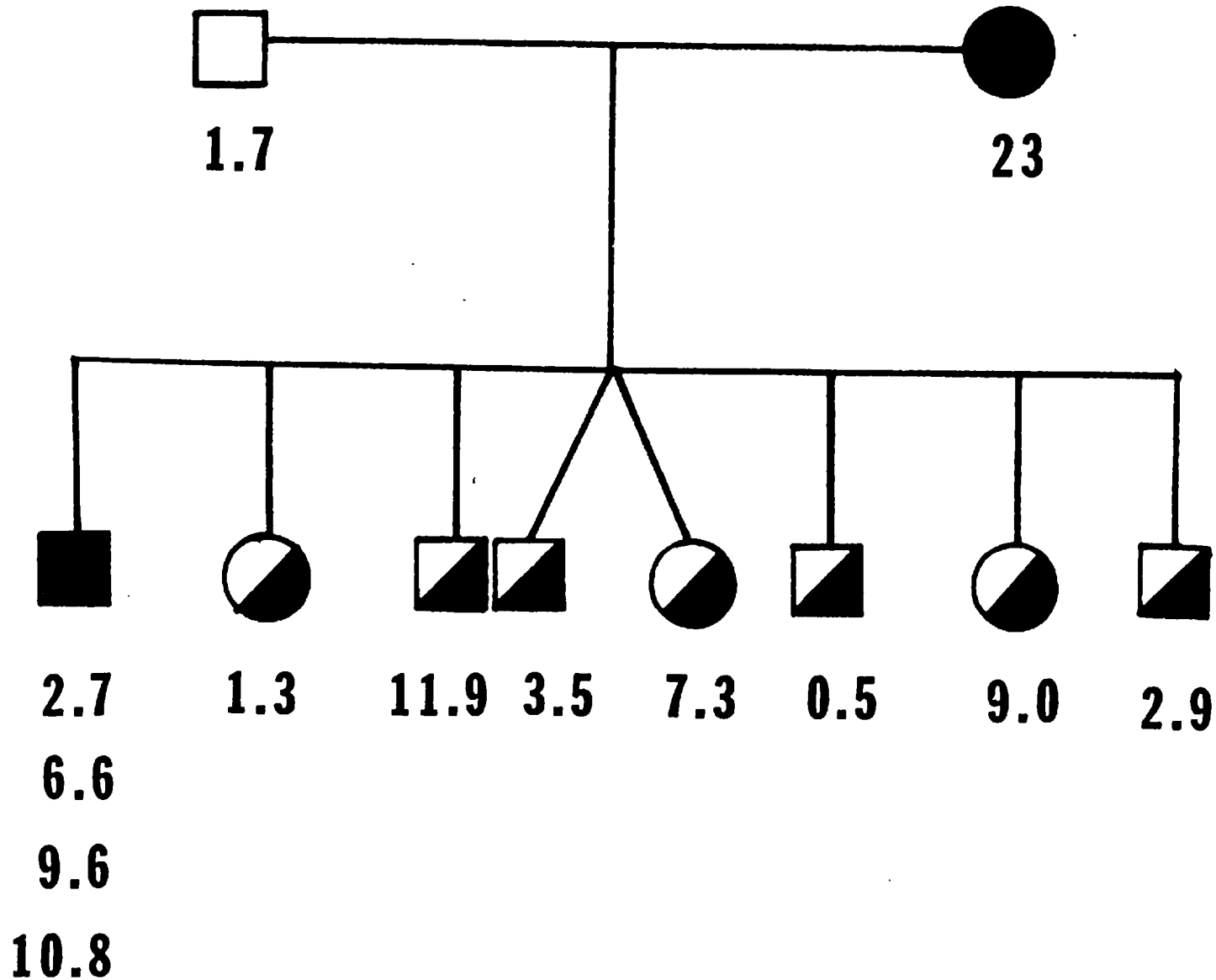


Fig. 3.2 Pedigree Chart of Family I.



The pedigree chart is illustrated in Fig. 3.3.

Family K: Both father and mother belong to EM phenotype with metabolic ratios of 0.5 and 0.03 respectively. Their daughter was found to be a EM with a metabolic ratio of 0.3. From the metabolic ratios the family pedigree chart was plotted in Fig. 3.4.

In an attempt to explain the unusually low recoveries found in the Egyptian population three experiments were performed. Firstly, the phenolic metabolites were measured. However, in no individual did these account for more than 3% of the dose *in toto*. Secondly, it was plausible that the experimental conditions in Egypt (e.g. ambient temperature, fasting) might have influenced events. When 9 Egyptians resident in London were investigated, the mean urinary recovery was 38.1 $\pm$ 14.2% which was significantly higher from the Egyptian recovery ($2P < 0.05$). To further investigate the role played by fasting during Ramadan upon the absorption of the drug, 5 previously phenotyped male Caucasians were fasted for 14-16h. After a large and filling meal comprising meat, salad, bread and cola beverage (the nearest approximation available to the post-fasting evening meal in Egypt), (*vide supra*), subjects were redosed with debrisoquine (see Table 3.2). Urinary recovery of the drug was considerably reduced under such conditions (31.4 $\pm$ 14.7%) and was found to be statistically significantly lower ($2P < 0.05$) than the recoveries previously obtained with these subjects (61.8 $\pm$ 26.0%) indicating that such dietary habits do markedly effect

Fig. 3.3 Pedigree Chart of Family J.

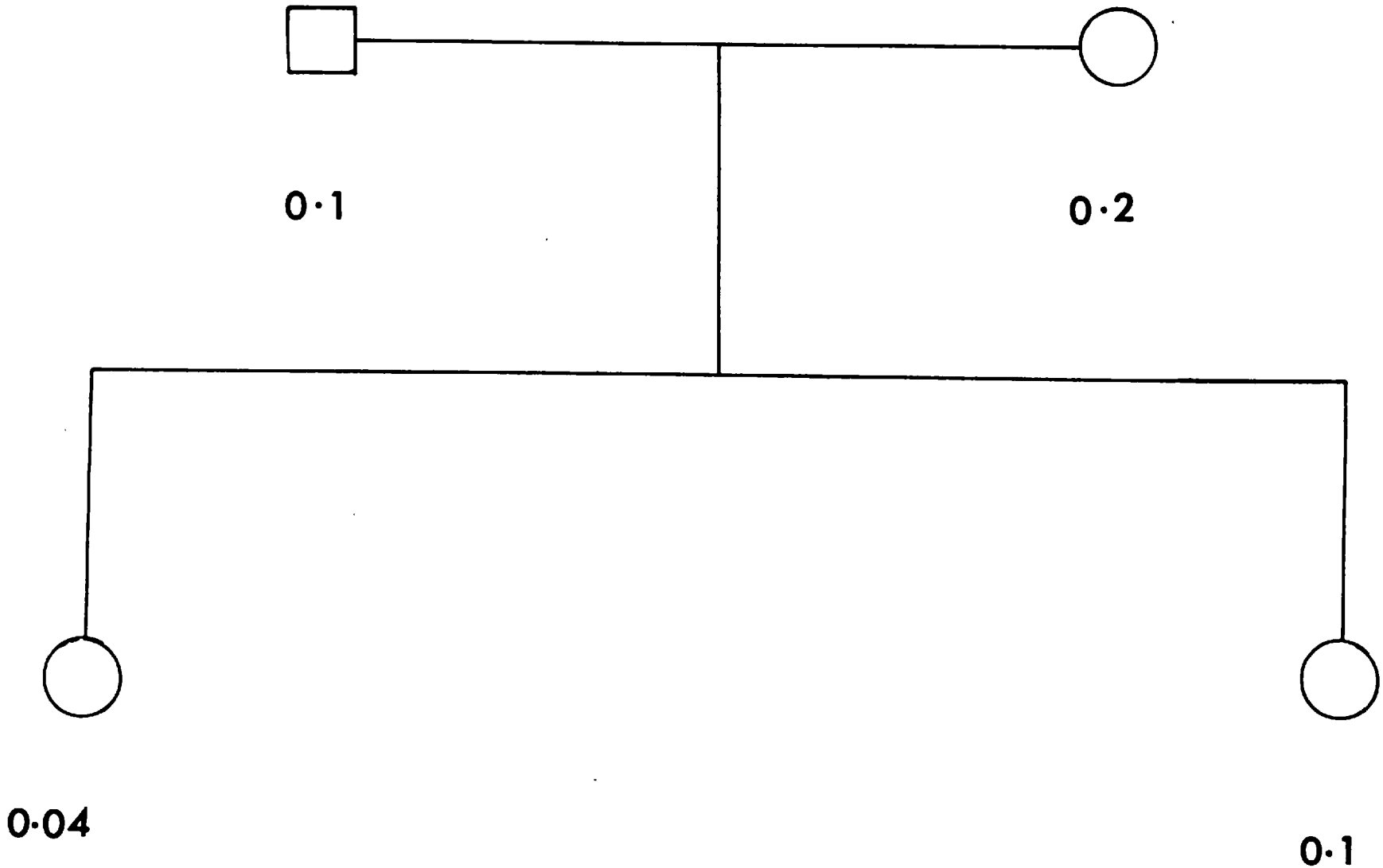


Fig. 3.4 Pedigree Chart of Family K.

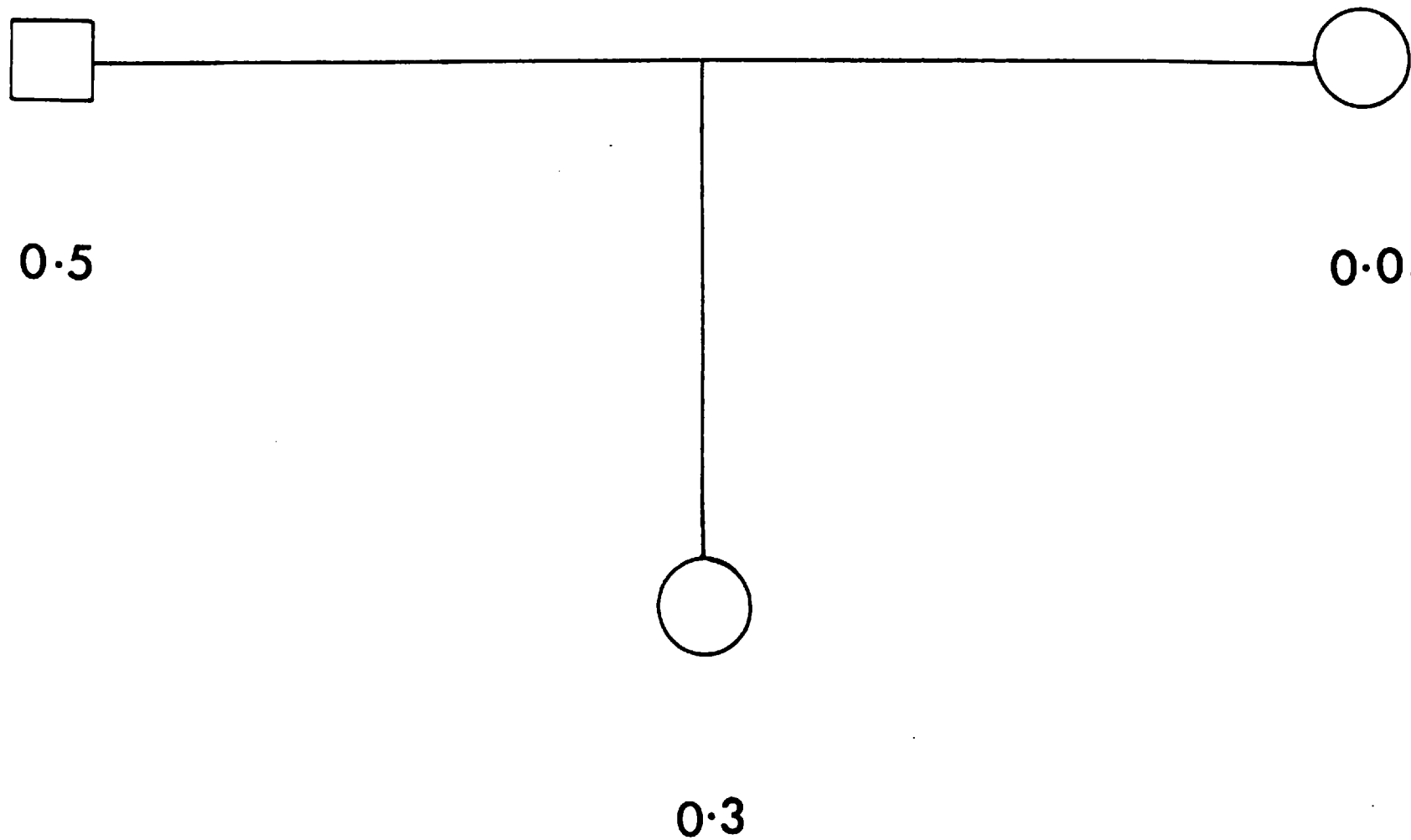


Table 3.2 Urinary recovery and metabolic ratios of 5 Caucasian subjects after 10 mg debrisoquine under normal conditions and on full stomach

Subject	Normal		Full stomach	
	Urinary recovery (0-8 h)	Metabolic ratio	Urinary recovery (0-8 h)	Metabolic ratio
1	41.5,97.1	0.8, 0.6	13.2	0.6
2	90.5,79,7	1.2, 1.4	53.2	1.0
3	73.0,33.6	0.5, 0.5	16.2	0.2
4	43.8	0.7	34.3	0.4
5	35.4	1.0	36.9	2.0
Mean $\pm$ S.D.	61.8 ± 26.0		31.4 ± 14.7	
$2P < 0.05$	(paired student's t-test)			

absorption. However, phenotype assignment was unaffected by such conditions.

II. Nigerian Study

In the 39 Nigerian subjects studied, the mean urinary recovery of debrisoquine and 4-hydroxy-debrisoquine in 0-8 h urine was 40 $\pm$ 18.8% (see Appendix IV).

The histogram plotted in Fig. 3.5 shows a discontinuous variation with metabolic ratios ranging from 0.2 - 40.0. A small group consisting of 5 subjects with metabolic ratios of 30 - 38.0 corresponds to PM phenotype described in the Caucasian study, most subjects having metabolic ratios between 0.2 - 3.0. Five subjects were found to have values intermediate between the other two groups (5 - 18).

III. Ghanaian Study

In 35 subjects studied, mean urinary recovery in 0-8 h urine was 28 $\pm$ 20% (see Appendix V). The distribution of metabolic ratios are shown in Fig. 3.6. Two subjects were found to have a metabolic ratio of 18.2 and 31.6. Most of the observations were found between metabolic ratios of 0.5 - 13.0, with the main variation around metabolic ratio of 1.3 - 1.4.

IV. Gambian Study

In this study 49 subjects were included. The mean urinary recovery in 0-8 h was 25.7 $\pm$ 13.6% (see Appendix VI).

Fig. 3.5 Frequency distribution of the metabolic ratio, urinary debrisoquine/4-hydroxy-debrisoquine in 39 Nigerian volunteers.

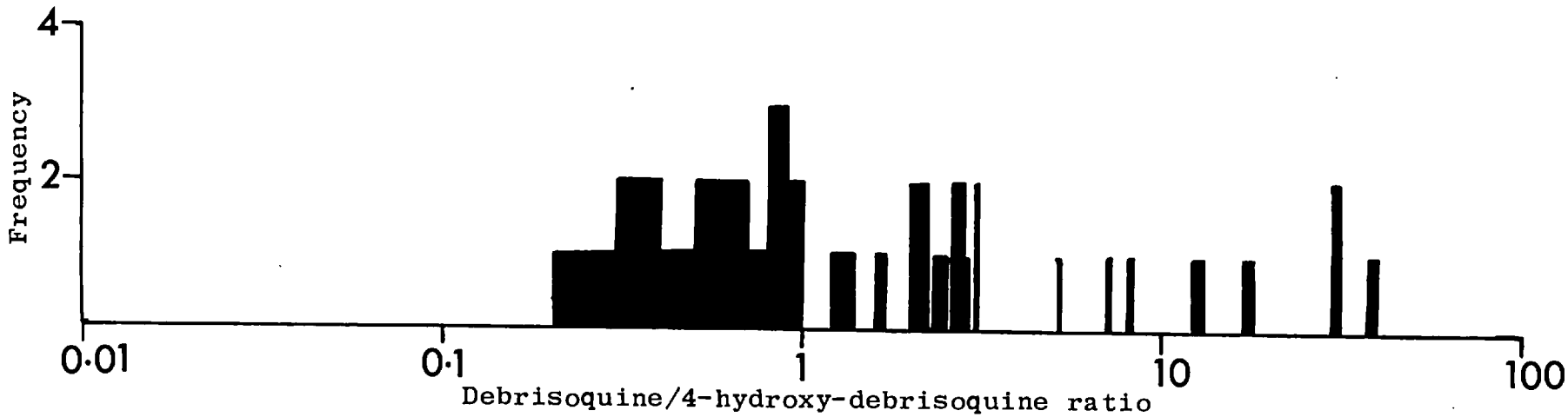
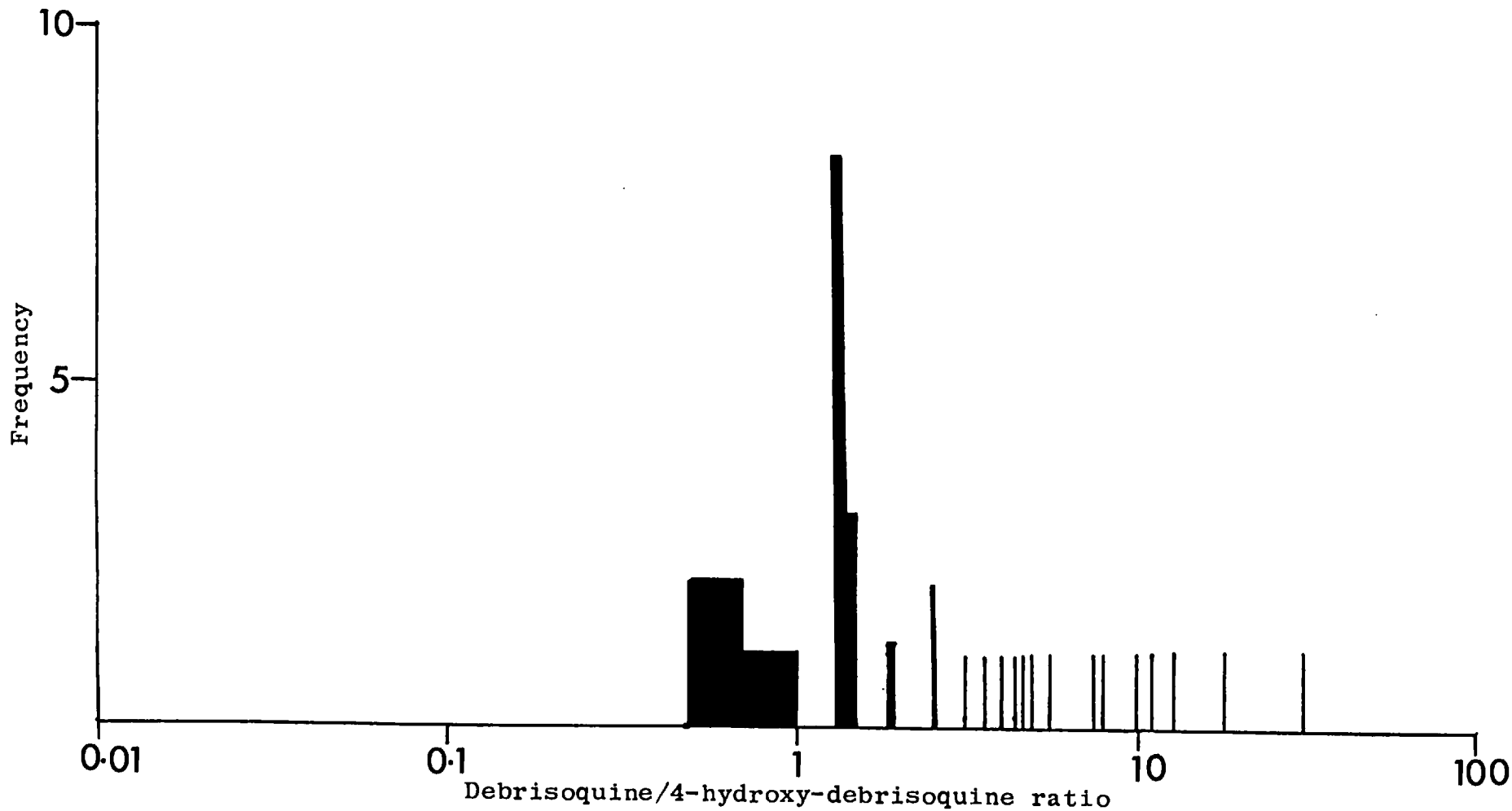


Fig. 3.6 Frequency distribution of the metabolic ratio, urinary debrisoquine/4-hydroxy-debrisoquine in 35 Ghanaian volunteers.



The results were plotted in a histogram which is shown in Fig. 3.7. The distribution was found to be discontinuous. A small group consisting of 6 subjects with metabolic ratios ranging from 22 - >600. A larger group which exhibited a Gaussian distribution included the rest of the observations. The metabolic ratios of subjects in this group ranged from 0.2 - 13.0 with the main variation around 0.7 - 0.8.

V. Malaysian Study

49 Subjects were studied. The mean urinary recovery of the Malaysian population was 23.7 $\pm$ 9.8%.

The metabolic ratios of subjects were plotted in the form of a histogram (see Fig. 3.8). One subject was found to have a metabolic ratio of 28.6. The rest of the observations (48) were normally distributed with a wide range of variation (0.06 - 16.0), with the main variation around 0.5 - 0.6.

Caucasian Study

This includes 194 subjects among Caucasians living in London and Liverpool area, and which were studied previously (see Chapter 2), when grouped together, the distribution of the metabolic ratios were represented in the form of a histogram (Fig. 3.9).

Fig. 3.7 Frequency distribution of the metabolic ratio, urinary debrisoquine /4-hydroxy-debrisoquine in 49 Gambian volunteers.

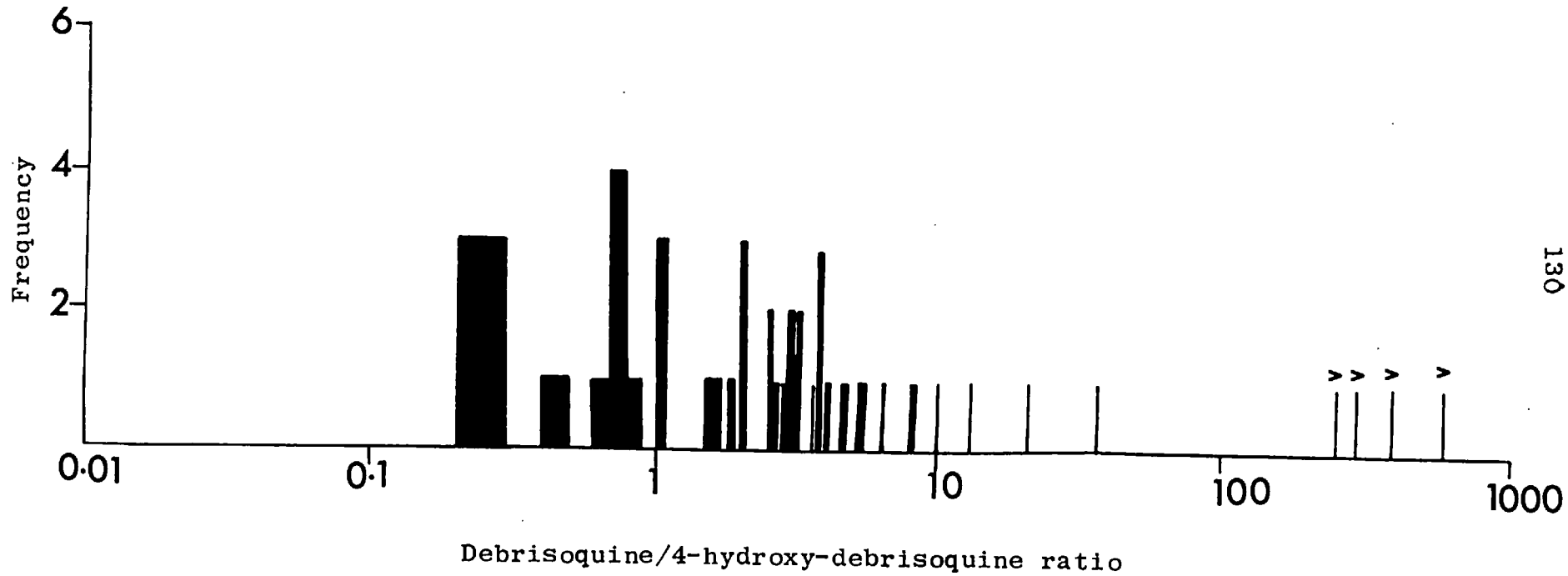


Fig. 3.8 Frequency distribution of the metabolic ratio, urinary debrisoquine /4-hydroxy-debrisoquine in 49 Malaysian volunteers.

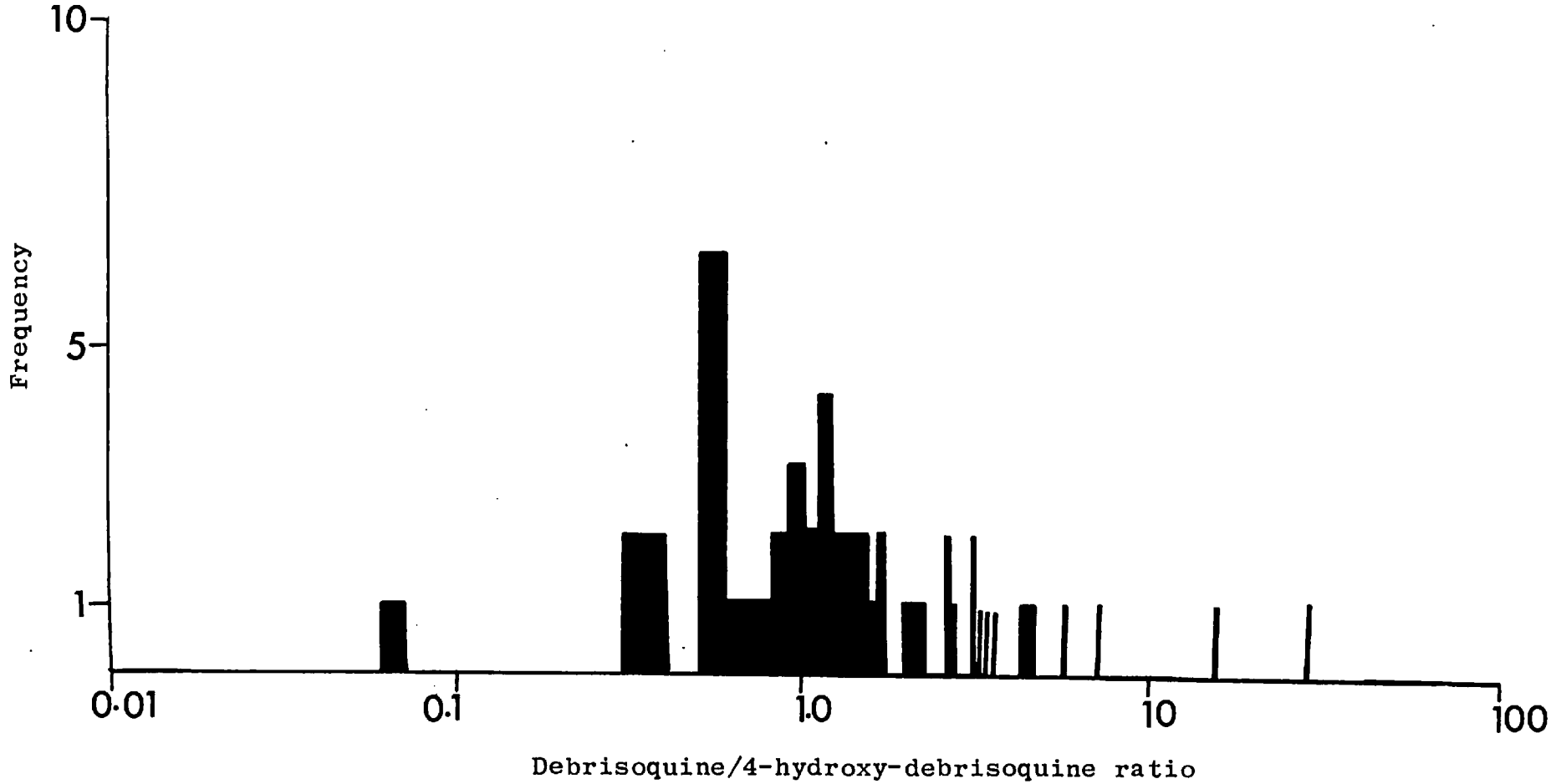
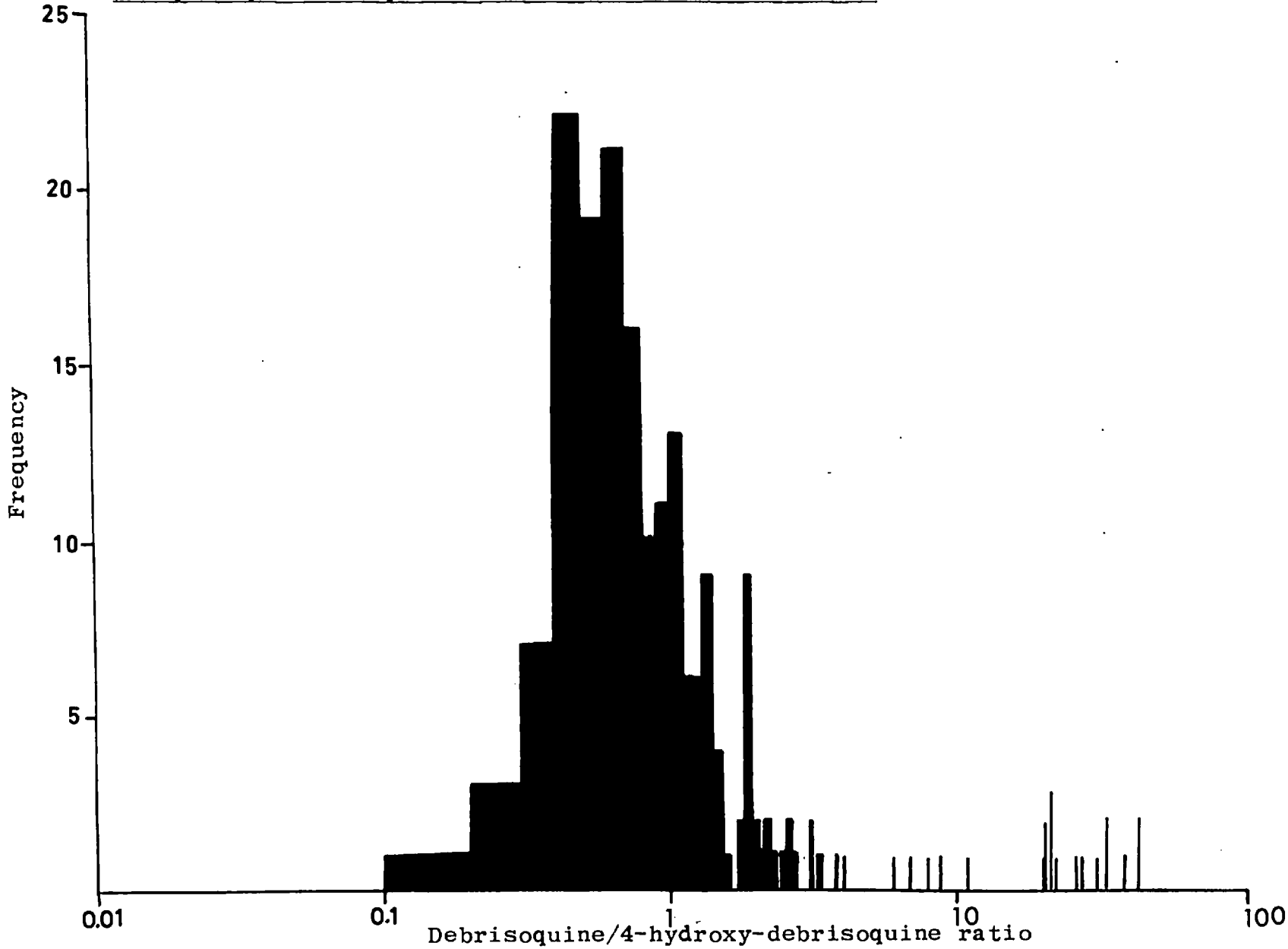


Fig. 3.9 Frequency distribution of the metabolic ratio, urinary debrisoquine /4-hydroxy-debrisoquine in 194 Caucasian volunteers.



Discussion

The allele frequencies (N) and the frequency of the three genotypes for the different ethnic groups were computed using the Hardy-Weinberg equation (p 54) from the data presented in Fig. 3.5 - 3.9 e.g. (see Table 3.3). There was an apparent difference in the allele frequency among different ethnic groups which when tested statistically using the equation $S.E.M._{(q)} = \sqrt{\frac{1-q^2}{4XN}}$ (Emery, 1976) it was only possible to put them in a rank order as shown in Table 3.4. It is clear that Nigerians and Gambians have the highest incidence of poor metabolizers and a large proportion of the population is heterozygote extensive. On the other hand Egyptians and Malaysians are the best hydroxylators of debrisoquine as they showed the lowest incidence of the defective allele (0.12 and 0.14). Also heterozygote carriers represent a smaller proportion of the population. However Caucasians were found to be intermediate (see Table 3.4).

Preliminary drug trials are done on white populations because the main drug houses are largely located in Europe and America. However, since a difference in the extent of metabolism of debrisoquine was found to exist among different ethnic groups as ranked in Table 3.4, testing of new drugs should be extended beyond Caucasians to include different ethnic groups in order to assess properly the therapeutic response and the possible occurrence of side effects in non-Caucasian populations.

Table 3.3 Genotype frequencies among different ethnic groups

	NN (q^2)	% of EN (2Pq)	EE (P^2)	Frequency of N allele (q)
Caucasians (n=194)	7.2	39.3	53.5	0.27
Egyptians (n=73)	1.4	20.7	78.0	0.12
Nigerians (n=39)	12.8	46.0	41.2	0.36
Ghanaians (n=35)	5.7	36.4	57.9	0.24
Gambians (n=49)	12.2	45.5	42.3	0.35
Malaysians (n=49)	2.0	24.5	73.5	0.14

Table 3.4 Frequency of (N) allele among different ethnic groups

Rank	Country	q + S.E.M.*
1	Nigeria	0.36 ± 0.075
2	Gambia	0.35 ± 0.067
3	England	0.27 ± 0.035
4	Ghana	0.24 ± 0.082
5	Malaysia	0.14 ± 0.071
6	Egypt	0.12 ± 0.058

* S.E.M. $_{(q)} = \sqrt{\frac{1-q^2}{4XN}}$ (Emery, 1976)

The Egyptian study has revealed two points of interest. Firstly, the low 8 h urinary recovery of debrisoquine and 4-hydroxy-debrisoquine which proved to be related to the eating habits during fasting in "Ramadan". As the debrisoquine tablet was taken on top of a heavy meal, it is possible that delayed emptying of the stomach might lead to the low absorption of the drug. This is a common finding with many orally administered drugs for example, the absorption of paracetamol is grossly impaired in patients with delayed gastric emptying and pyloric stenosis (Heading et al., 1973).

Secondly, for the first time the three modes of the polymorphism could be characterized from the study of the Egyptian family (Fig. 3.2). Therefore the three genotypes could be defined, although homozygous and heterozygous extensive metabolizers do show some overlap.

EE	0.03 - 0.8
EN	0.5 - 12
NN	> 20

CHAPTER FOUR

Hypotensive response to debrisoquine and hydroxylation phenotype.

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Introduction

Pharmacology of Debrisoquine

Debrisoquine is an adrenergic neurone blocker similar to bretylium, guanethidine and bethanidine. It lowers blood pressure by blocking the transmission of sympathetic nerve impulses at post-ganglionic nerve terminals, thereby decreasing the peripheral vascular resistance. The reduction in post-ganglionic sympathetic transmission is achieved by interference with the physiological release of noradrenaline, without the depletion of major catecholamine stores in cardiovascular tissue and without impairment of cardiac contractile mechanisms (Abrams et al., 1964). There is no evidence that debrisoquine affects the stores of noradrenaline or serotonin in the brain. Debrisoquine does not interfere with parasympathatic activity. It appears to act more on arterioler than on venous tone (Abrams et al., 1964; Pocelinko & Abrams, 1964).

In therapeutic doses debrisoquine inhibits MAO in neurones and in platelets, but not in other tissues (Giachetti & Shore, 1967; Pettinger et al., 1969; Medina et al., 1969).

After the intravenous administration of debrisoquine, a biphasic blood pressure response was observed in human subjects (Kakaviatos et al., 1964). An initial pressor response of 1-4 h duration followed by a prolonged period of hypotension. The initial hypertensive phase was atributable to the release of noradrenaline from physiological stores (Moe et al., 1964). This initial elevation of

blood pressure was also observed after oral administration of large doses of debrisoquine (80 mg or more) accompanied by the excretion of high amounts of VMA (Luria & Freis, 1965).

After a single oral therapeutic dose of debrisoquine, a hypotensive response occurs within a period of 4-10 h and the effect usually lasts for 9-24 h. The lowering of b.p. is more marked in the erect position (Abrams et al., 1964).

Adverse effects

The most frequent and troublesome side effect is orthostatic or postural hypotension, which is in fact an exaggeration of the therapeutic response to the drug. This is usually manifested by, dizziness, light headiness, blurring of vision and fainting on standing (Sommers et al., 1968; Belle et al., 1968; Athanssiadis et al., 1966; Kitchen & Turner, 1966).

Gastrointestinal symptoms such as anorexia, flatulence, eructation, heartburn, periumbilical pain, abdominal discomfort, constipation, vomiting and diarrhoea.

Other symptoms are muscle pain, stiffness and weakness especially after exercise. Failure of ejaculation has also been reported (Heffernan & Carty, 1970).

Acute toxicity

The acute LD50 values for debrisoquine sulphate were determined by four different routes in mice (Moe et al., 1964) as follows:

Route	Dose (mg/kg) ± S.E.M.
Intravenous	31.7 ± 2.5
Subcutanous	136.0 ± 4.0
Intraperitoneal	132.0 ± 3.0
Oral	235.0 ± 4.0

The oral LD50 for the rat is 1580 mg/kg (Toxic substances List, 1973)

Since the hypotensive response to debrisoquine is governed by the rate of uptake of drug into the neurone and, since this uptake obeys first-order kinetics, the response will be determined by the amount of drug made available to these peripheral adrenergic neurones. Clearly, the differences observed in the extent of metabolism of debrisoquine (see preceding chapters) which is genetically determined, may play a large role in controlling blood pressure changes in a particular individual. Thus, the effect of hydroxylation phenotype on the hypotensive response to debrisoquine is to be investigated in accord with the following aims:

Objectives of the present study

1. To establish what difference in response to oral doses of debrisoquine exists, between the two debrisoquine hydroxylation phenotypes.
2. To determine the effect of increasing dosage on response and metabolic ratio within each debrisoquine phenotype.

Investigative Procedures

Healthy normotensive human volunteers previously phenotyped during the population studies, and classified as extensive metabolizers (EM) or poor metabolizers (PM), were included in this study. Subjects were given different oral doses of debrisoquine as its hemisulphate preparation (Declinax, 10-60 mg). Subjects were not investigated more than once per week. PM volunteers were not given debrisoquine above a dose of 20 mg.

All experiments started at 9.00 a.m. when blood pressure (b.p.) of each subject was measured in a quiet room. After lying down for 10 min. both systolic and diastolic b.p. were measured using a random zero sphygmomanometer (Gelman Hawkley, Lancing, Sussex). After standing for 2 min. systolic and diastolic b.p. were remeasured, pulse rates also being recorded. Subjects were then given the appropriate dose of the drug on top of a fat-free breakfast and after emptying their bladders. Blood pressure lying and standing was then measured at hourly intervals for 8 h after dosing during which time subjects were ambulant but did not exert themselves. The mean b.p. was then calculated from diastolic b.p. plus 1/3 pulse pressure (difference between systolic and diastolic b.p.).

Urine was collected during the 8 h of the experiment, pooled and kept at -20^{o}C to be analysed for debrisoquine and 4-hydroxy-debrisoquine as described previously. Metabolic ratios were calculated for the subjects on the various doses to ascertain what effect the phenotype had on metabolic capacity.

Results

Subjects included in this study were among EM and PM individuals who had been phenotyped previously during the population studies. The metabolic ratios and phenotypic assignment are presented in Table 4.1.

Table 4.1 Metabolic ratios and phenotypes of EM and PM subjects (10 mg dose)

Subject	Sex	Metabolic ratio	Phenotype
1 - LW	M	0.6,0.7	EM
2 - TS	M	1.4	EM
3 - TM	M	0.5,0.5	EM
4 - JI	M	0.7	EM
5 - HD	F	23,19	PM
6 - ME	M	20,23,21	PM
7 - RS	M	21,20	PM

(i) 10 mg debrisoquine and b.p. response

None of the subjects, either EM or PM responded to the 10 mg dose of debrisoquine. The standing b.p. remained above the lying b.p. during the 8 h period of the study (see Table 4.2). The mean b.p. both lying and standing is shown in Fig. 4.1.

(ii) 20 mg debrisoquine and b.p. response

The 4 EM subjects (1,2,3 and 4) did not respond to 20 mg dose, as their standing b.p. did not fall below the lying b.p. By comparison the 3 PM subjects (5,6 and 7) showed a significant drop in their standing b.p. 4-6 h

Table 4.2 Blood pressure responses to 10 mg debrisoquine among PM and EM hydroxylation phenotypes

Subject	L.W. (1)	J.I. (4)	R.S. (7)	
Phenotype	EM	EM	PM	
Time after dosing (h)				
0	110/70	120/80	135/85	L
	115/75	115/85	130/85	S
1	110/70	145/100	125/75	L
	115/80	130/100	120/85	S
2	110/60	125/85	107/67	L
	110/70	125/90	125/95	S
3	125/80	125/75	135/80	L
	120/75	110/80	135/95	S
4	-	120/80	125/85	L
		130/80	120/95	S
5	120/75	115/70	135/85	L
	110/85	110/75	130/85	S
6	105/70	125/80	130/80	L
	110/75	115/85	130/85	S
7	110/70	125/75	115/70	L
	110/65	115/85	125/88	S
8	115/70	125/80	125/80	L
	115/70	105/75	120/90	S

L = lying

S = standing

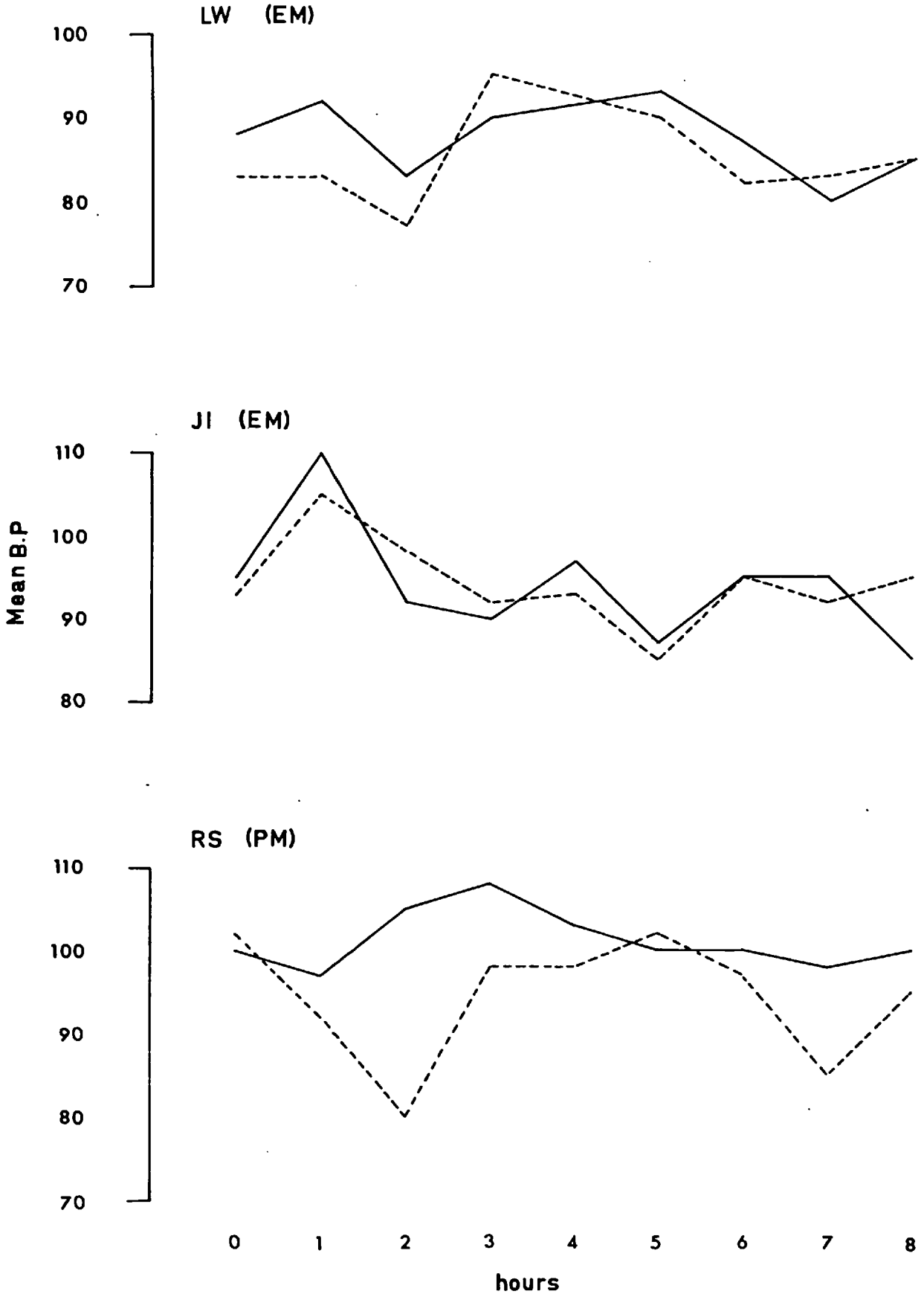


Fig. 4.1 Mean standing and lying b.p. profiles after debrisoquine (10 mg p.o.) in two EM and one PM Subjects.

standing b.p. ——

lying b.p. ----

post dosing (see Table 4.3 and Fig. 4.2). Such lowering of b.p. was accompanied by symptoms of postural hypotension in subjects 5 and 7. This difference when tested for significance using the unpaired two tailed Student t-test showed a statistical significant difference between the mean standing and mean lying b.p. (measured over the 3-7 hour period post dosing) for the two phenotypes ($2P < 0.02$).

(iii) 40 mg debrisoquine and b.p. response

The 4 EM subjects only were tested, as PM subjects showed a response with the 20 mg dose accompanied by the development of symptoms of postural hypotension in two of them. Subjects 1, 3 and 4 did not respond to 40 mg dose, while with subject 2 the standing b.p. dropped below lying b.p. during the period 4-6 h post dosing (see Table 4.4). This subject was shown to be a heterozygote extensive metabolizer from his family study (see Fig.2.13). The mean b.p. of 4 EM subjects are plotted in Fig 4.3.

(iv) 60 mg debrisoquine and b.p. response

This high dose of the drug could not be studied except on 2 EM subjects (2 and 4). Both subjects responded to the drug to various degrees with the development of symptoms commonly associated with postural hypotension of lightheadedness and confusion. Changes in lying and standing b.p. are shown in Table 4.5. In the two subjects the b.p. in both postures rose initially over $1\frac{1}{2}$ h in subject 2 (max. lying 200/95, standing 195/80) and over 4 h in subject 4 (max. lying 135/90, standing 145/100).

Table 4.3 Blood pressure responses to 20(mg) debrisoquine among EM and PM hydroxylation phenotypes

Subject	LW 1	TS 2	TM 3	JI 4	HD 5	ME 6	RS 7	
Phenotype	EM	EM	EM	EM	PM	PM	PM	
Time after dosing (h)								
0	115/65	130/90	120/80	115/80	125/60	120/80	140/85	L
	130/75	130/80	125/100	120/80	110/85	125/105	145/100	S
1	115/75	125/85	135/95	125/75	130/80	110/80	135/100	L
	125/75	115/90	120/100	125/90	110/90	120/100	135/100	S
2	105/70	140/100	130/90	120/80	115/85	115/85	145/100	L
	120/80	140/110	130/100	120/70	120/90	120/100	135/85	S
3	145/85	135/80	130/80	120/70	130/70	125/100	150/90	L
	120/75	140/100	130/95	115/80	120/70	125/100	130/95	S
4	120/65	140/60	120/80	150/90	130/70	130/110	115/110	L
	135/85	120/95	120/100	130/80	110/70	125/100	135/90	S
5	130/80	130/95	120/85	115/70	125/70	140/100	155/110	L
	120/80	130/75	125/100	120/75	100/65	125/95	120/80	S
6	105/65	130/90	120/85	130/60	120/80	125/95	115/80	L
	130/80	120/90	130/100	130/70	100/60	120/90	110/60	S
7	110/65	120/80	140/80	125/80	110/65	130/100	130/95	L
	110/70	120/90	120/85	110/80	90/60	125/95	125/95	S
8	115/70	120/60	130/75	130/80	125/70	125/75	135/100	L
	120/70	130/80	130/100	130/75	125/70	130/100	135/100	S

L = Lying
S = Standing

Fig. 4.2 Mean standing and lying b.p. profiles after debrisoquine (20 mg p.o.) in four EM and three PM Subjects.

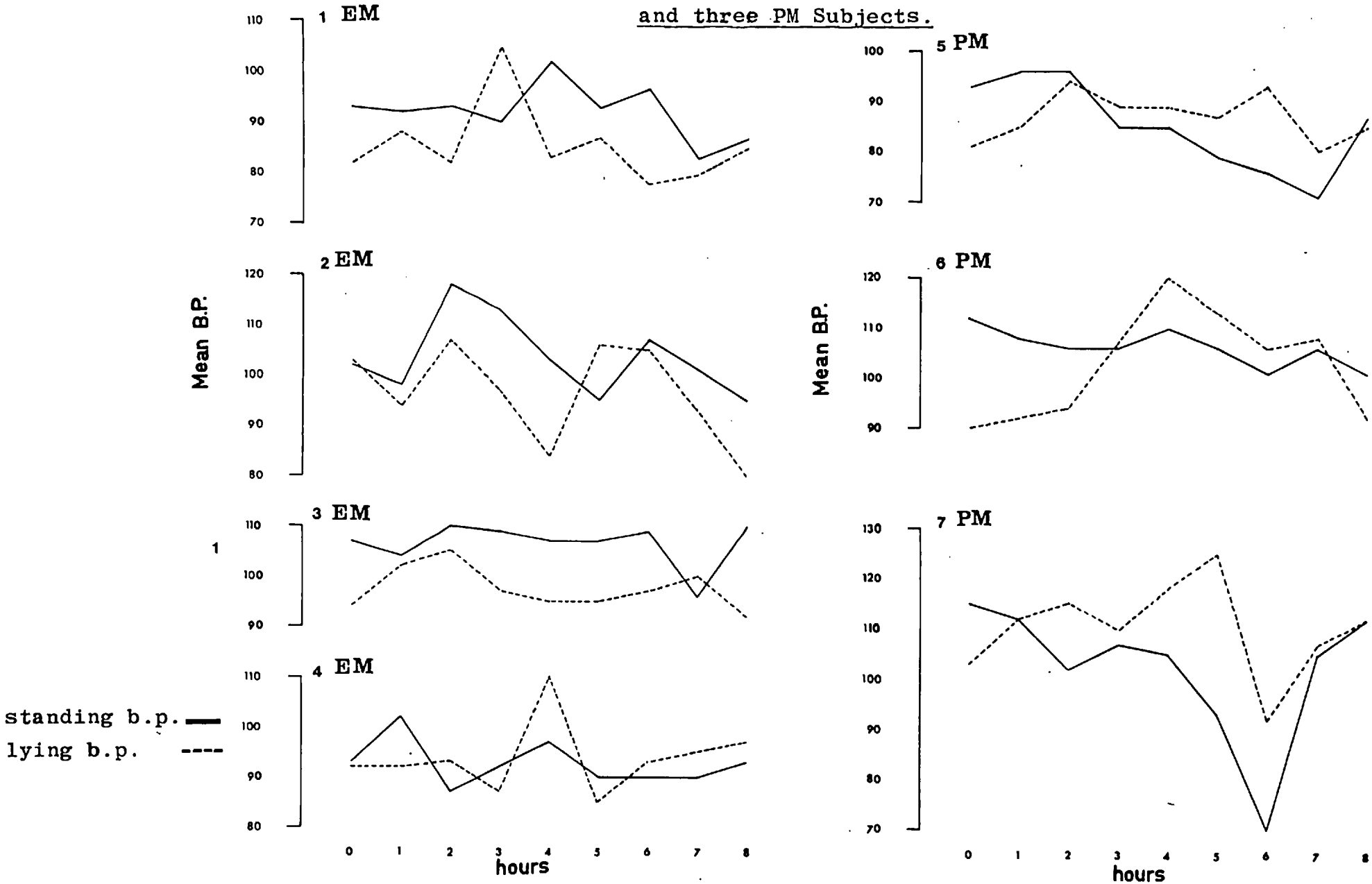


Table 4.4 Blood pressure responses to 40 (mg) debrisoquine among EM hydroxylation phenotype

Subject	LW	TS	TM	JI	
	1	2	3	4	
Time after dosing (h)					
0	105/65	120/55	125/65	120/70	L
	115/85	125/90	125/95	130/100	S
1	110/65	150/60	130/70	130/70	L
	120/70	140/70	125/75	120/80	S
2	115/75	140/80	115/65	120/80	L
	110/80	125/80	110/80	115/80	S
3	120/90	150/80	115/60	115/75	L
	135/95	130/90	125/95	125/95	S
4	120/80	150/100	120/65	120/70	L
	125/95	105/75	120/90	135/95	S
5	130/75	125/70	110/55	140/75	L
	120/80	110/65	115/80	135/90	S
6	120/70	150/100	110/65	125/65	L
	125/85	115/75	115/90	120/90	S
7	120/75	125/55	125/65	125/85	L
	120/80	120/80	120/85	130/90	S
8	110/65	130/70	115/75	120/80	L
	130/90	125/85	130/100	125/95	S

L = Lying, S = Standing

Fig. 4.3 Mean standing and lying b.p. profiles after debrisoquine (40 mg) in four EM Subjects.

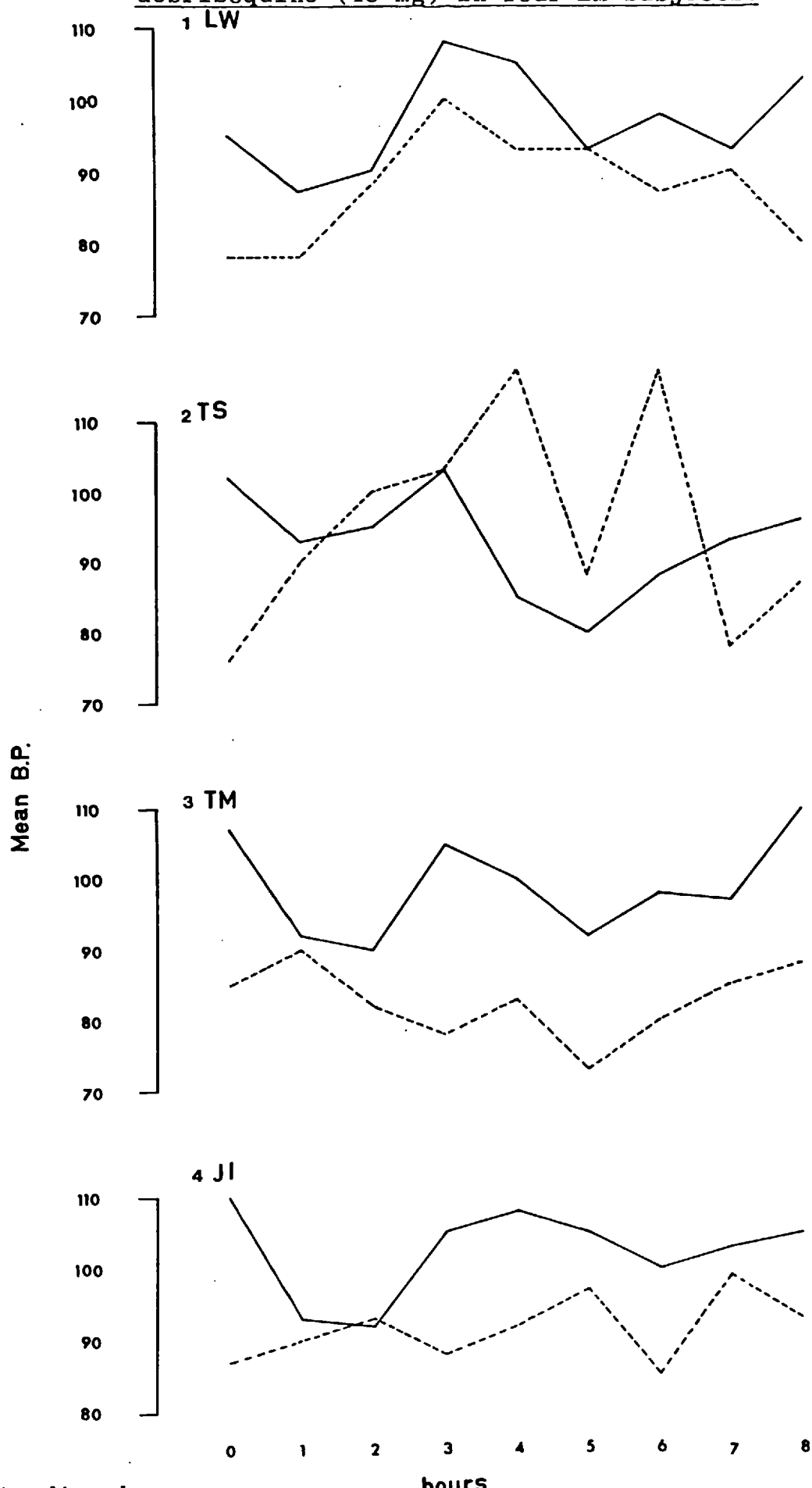


Table 4.5 Blood pressure response to 60 mg debrisoquine among two EM subjects

Subject	T.S. (2)	J.I. (4)
Time after dosing (h)		
0	135/60 125/65	128/68 144/72
½	150/65 145/75	115/65 130/75
1	175/75 175/75	125/55 110/70
1½	200/95 195/80	145/65 130/70
2	172/82 155/70	135/70 130/85
3	155/75 135/75	135/80 125/80
4	140/95 145/75	135/90 145/100
5	155/80 100/55	125/80 135/90
6	150/70 120/50	135/90 105/70
6½	145/75 110/80	140/90 120/80
7	145/75 100/75	125/80 120/85
8	145/65 105/65	130/90 140/100

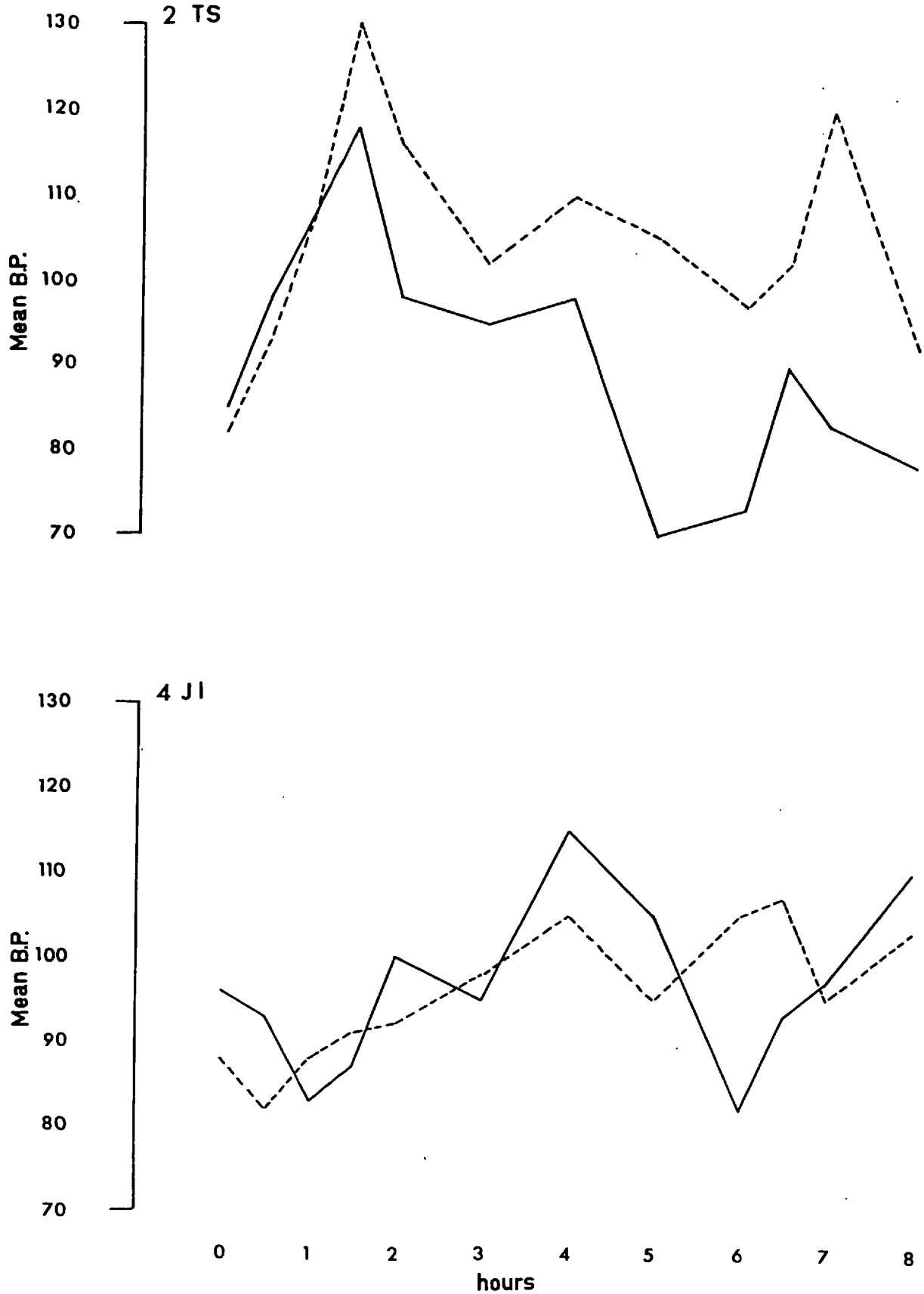


Fig. 4.4 Mean standing and lying b.p. profiles after debrisoquine (60 mg) in two EM Subjects.

standing b.p. ——

lying b.p. ----

This was followed by a fall in b.p. with marked postural hypotension in subject 2 (min. lying 150/70, standing 100/55). Little postural hypotension was measurable in subject 4 (see Fig. 4.4).

Effect of increasing dose on metabolic ratio

These results are summarised in the following table:

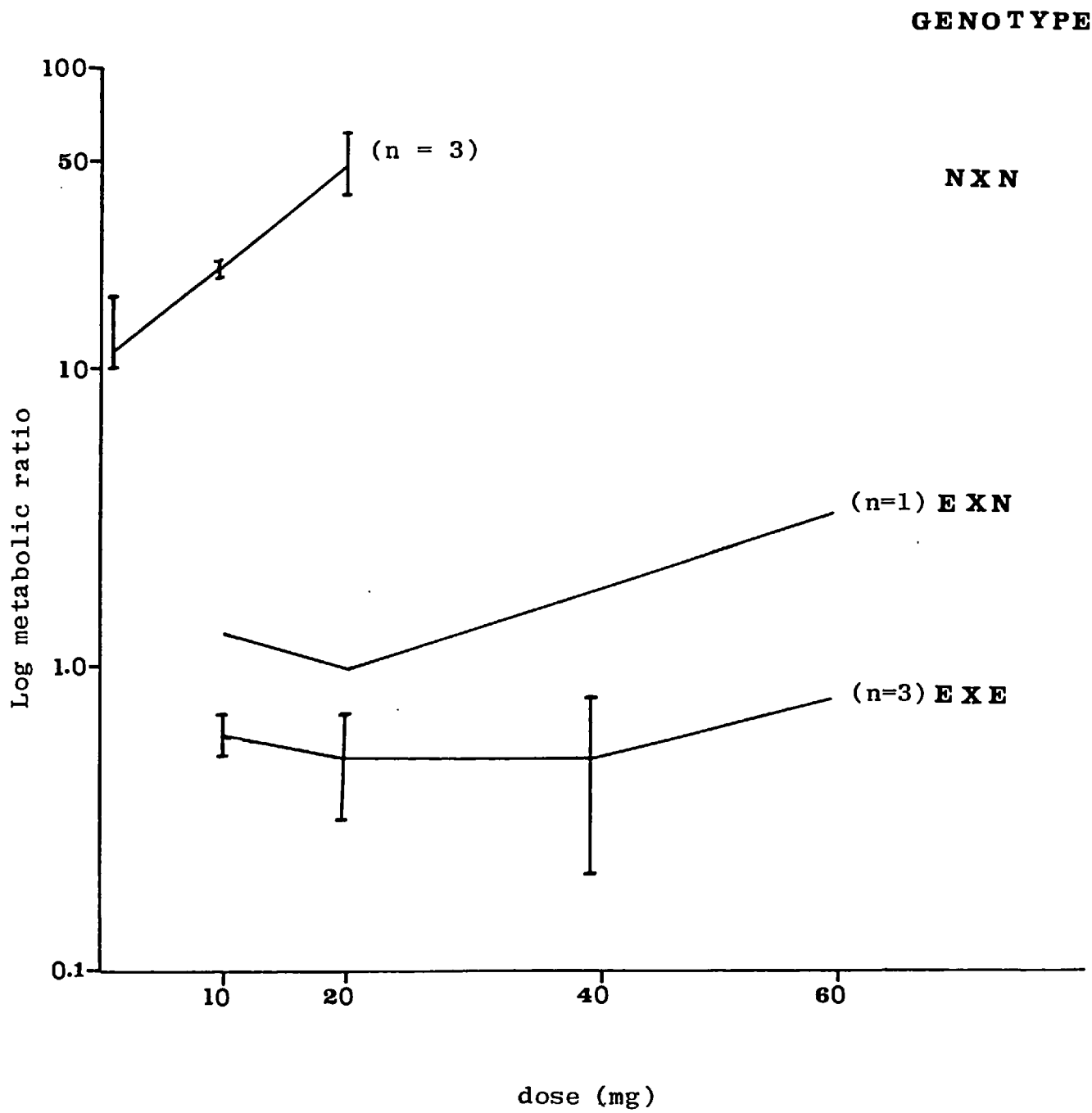
Table 4.6 The metabolic ratios and phenotype assignment of subjects at different doses of debrisoquine (1-60 mg)

Subject	Pheno-type	Geno-type	1 (mg)	10 (mg)	20 (mg)	40 (mg)	60 (mg)
1 - JI	EM	EXE	0.4	0.7	0.7	0.7	0.8
2 - TM	EM	EXE	-	0.6,0.7	0.4	0.6	-
3 - LW	EM	EXE	-	0.5,0.5	0.3	0.2	-
4 - TS	EM	EXN	-	1.4	1.0	1.8	3.3
5 - RS	PM	NxN	17.6	21, 20	56	-	-
6 - ME	PM	NxN	8.8	20,23,21	34	-	-
7 - HD	PM	NxN	8.2	23, 19	52.5	-	-

Metabolic ratios of PM subjects were increased with increment of dosage while in the 4 EM subjects their metabolic ratio showed no significant increase except for subject 4 who was the heterozygote subject previously mentioned.

The relationship between dose and metabolic ratio for the different genotypes is shown in Fig. 4.5.

Fig. 4.5 Relationship between dose and metabolic ratio for the different genotypes.



Discussion

The difference in optimal dose requirement for the onset of response within the three genotypes demonstrated here rationalizes the previously published data. For example, a marked inter-individual variation in debrisoquine optimal dose requirement has been shown to exist among hypertensive patients. A dose ranging from 20 - 400 mg daily was reported in the early studies (Athanssiadis et al., 1966; Kitchen & Turner, 1966) and in a more recent study it was found to vary from 10 - 360 mg daily (Silas et al., 1977).

The cause of this wide inter-individual variation has been studied by many investigators. Angelo et al. (1975) found that inter-individual differences in the amount of unchanged drug excreted in urine is not due to absorption but arose from differences in the ability of individuals to metabolize the drug. Lennard et al. (1976) showed that inter-patient variation in the response to debrisoquine is largely due to differences in the systemic availability of the parent compound, and the urinary recoveries of debrisoquine and its 4-hydroxy-metabolite reflect the plasma levels of these compounds. Furthermore the hypotensive response to debrisoquine was found to be related to the plasma level of unchanged drug (Silas et al., 1977).

It might be possible to identify heterozygotes by studying the debrisoquine metabolic ratio at varying doses (see Fig. 4.5)

It is appreciated however that pre-systemic meta-

bolism is not the sole factor which may determine inter-individual differences in drug response. For example a drug such as bethanidine, also an adrenergic neurone blocker, is not metabolized to any significant degree in man (Shen et al., 1975; Turnbull et al., 1976) yet there are large observable differences in optimal dose requirements for this drug among hypertensive patients (Smirk, 1963).

Having established a difference between optimal dose requirement and hypotensive response, among the three debrisoquine genotypes, it is of importance to study other pathways of C-oxidation, both for debrisoquine and other drugs, to see if there is a relationship between debrisoquine 4-hydroxylation phenotype and the oxidation of other substrates.

CHAPTER FIVE

Polymorphic hydroxylation of other carbon centres.

Contents

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Since it has been established that there exists within the population a metabolic defect in the alicyclic 4-hydroxylation of debrisoquine, involving some 1-13% of persons studied (according to ethnic group), it is of importance to establish whether or not the defect (and its genetic control) is encountered with other drug oxidations. Accordingly, considering the importance and common occurrence of aromatic hydroxylation as a metabolic option for drugs, two selected examples of such a biotransformation have been investigated here.

Firstly, during the course of the previously described investigations of debrisoquine metabolism (Chapters 1-4) limited data was collected on the aromatic hydroxylation of debrisoquine. This has been analysed and presented in this section.

Secondly, the aromatic hydroxylation of guanoxan, an anti-hypertensive drug chemically and pharmacologically related to debrisoquine, has been investigated in panels of EM and PM subjects.

a) Debrisoquine aromatic hydroxylation

As it was shown in Chapter 1 that the oxidative metabolism of debrisoquine involves two main routes namely the alicyclic 4-hydroxylation which is the major pathway and aromatic ring hydroxylation giving rise to the phenolic metabolites 5-, 6-, 7- and 8-hydroxy-debrisoquine (see Fig. 1.1).

Urine samples of 9 EM and 9 PM subjects were analysed for their content of phenolic metabolites using the g.l.c. method described in Chapter 1. The metabolic

ratio of the total phenols was calculated from the ratio of:

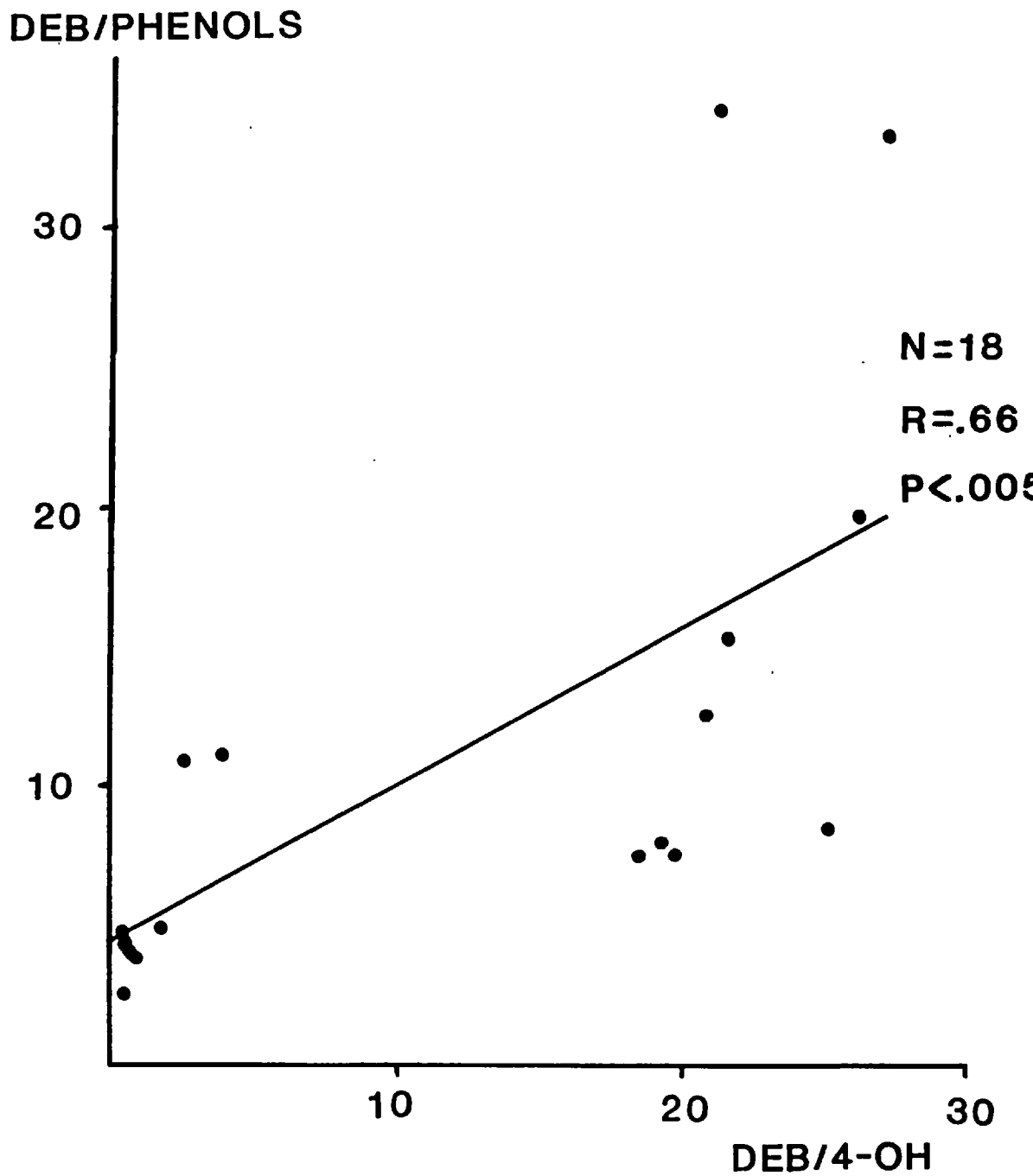
$$\frac{\%\text{ dose excreted in 0-8 h urine as debrisoquine}}{\%\text{ dose excreted in 0-8 h urine as total phenols}}$$

The results were plotted in a graph shown in Fig. 5.1 which relates phenolic ratio (debrisoquine/total phenols) to 4-hydroxy-debrisoquine ratio (debrisoquine/4-hydroxy) and the results were analysed using regression analysis.

Within the group of 18 subjects tested, statistically significant correlations were found between the ability to effect the alicyclic hydroxylation of debrisoquine and the formation of phenolic metabolites. The correlation co-efficient was $r=0.66$, $P < 0.005$. Furthermore, the mean metabolic ratio of total phenols excreted by EM group was 5.6 ± 3.1. Compared to 16.2 ± 10.8 for the PM group and was statistically significantly different ($P < .02$).

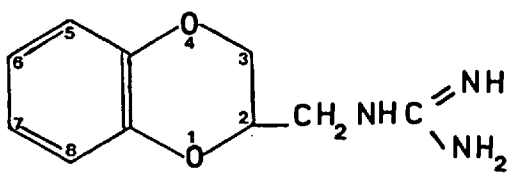
Therefore the aromatic hydroxylation of debrisoquine also exhibits a genetic polymorphism parallel to the alicyclic 4-hydroxylation described in Chapter 2 as judged by the statistical significant difference between the two debrisoquine hydroxylation phenotypes in the extent of total phenol production.

Fig. 5.1 Correlation between total phenol production and 4-hydroxylation in EM and PM phenotypes.



b) Guanoxan aromatic hydroxylation

Guanoxan (2-guanidino-methyl-1,4-benzodioxan sulphate, Envacar, Pfizer) structurally, it combines in one molecule both guanidine and benzodioxan as follows:



"Guanoxan"

The drug is mainly metabolized by aromatic ring hydroxylation at the 6- or 7-positions and excreted mainly in urine as unconjugated 6- and 7-hydroxy-guanoxan. Table 5.1 describes the metabolism of guanoxan in five hypertensive patients. A very interesting observation from this study, which the authors failed to follow up, is the female subject (3) who persistantly excreted the drug unchanged with no evidence of metabolism (Jack et al., 1972).

The investigative procedures in this study were the same as described before for debrisoquine phenotyping (Chapter 2). Eight subjects, 4 EM and 4 PM were given orally a 10 mg Envacar tablet containing 10 mg guanoxan sulphate. Urine samples were collected hourly up to 8 h post-dosing to be analysed for its content of guanoxan

Table 5.1 Daily excretion of guanoxan or 6- or 7-hydroxy-guanoxan in urine from hypertensive patients

Patient	Dose (mg/day)	Urine sample (24 h)	guanoxan	6- or 7-hydroxy-guanoxan
1. Male	20	1	0.0	22.5 ± 1.3 S.E.M.
		2	0.0	11.7 ± 0.9 S.E.M.
2. Male	40	1	0.0	22.2 ± 1.0
3. Female	50	1	47.0 ± 4.6	0.0
		2	46.2 ± 2.9	0.0
		3	23.2 ± 1.0	0.0
4. Male	120	1	0.0	46.6 ± 1.3
		2	0.0	46.5 ± 0.3
5. Male	200	1	Traces	27.5 ± 1.3
		2	Traces	53.6 ± 1.7

(From Jack et al., 1972)

and the hydroxylated metabolites by gas chromatography. G.l.c. conditions and derivatization were the same as for debrisoquine and its hydroxylated metabolites except that debrisoquine and 8-hydroxy-debrisoquine were used as internal standards for guanoxan and 6- and 7-hydroxy guanoxan respectively. The metabolic ratio was calculated from the ratio:

$$\frac{\%\text{ dose excreted in 0-8 h urine as unchanged drug}}{\%\text{ dose excreted in 0-8 h urine as hydroxylated metabolites}}$$

From the results shown in Table 5.2 the 4 EM subjects excreted the drug mainly as 6- and 7-hydroxy guanoxan (29.0 $\pm$ 5.0%). A small amount of the dose was excreted as unchanged guanoxan (1.5 $\pm$ 3%). By contrast, the 4 PM subjects excreted the drug mainly unchanged (48.0 $\pm$ 12.4%) with only small amounts of 6- and 7-hydroxy metabolites (6.2 $\pm$ 1.4%). The mean metabolic ratio for the EM phenotype was 0.06 $\pm$ 0.02 and for PM phenotype was 7.8 $\pm$ 0.2.

From these studies it is clear that a single gene effect controlling the alicyclic 4-hydroxylation of debrisoquine extends to other carbon oxidations, such as the aromatic ring hydroxylation of the same compound at 5-, 6-, 7- and 8-positions leading to defective phenol formation. In addition, the aromatic hydroxylation of guanoxan at the 6- and 7-positions is governed by the same gene mechanism. Furthermore, a recent study in this department has shown that the defect extends to the O-de-ethylation of phenacetin (Sloan *et al.*, 1978). Thus-far therefore, an alicyclic, certain aromatic and an

Table 5.2 Hydroxylation of Guanoxan and phenotype

Subject	Sex	Hydroxylation phenotype	% dose excreted in 8 h in urine as: Unchanged guanoxan	Hydroxylated metabolites 6- and 7-hydroxy-guanoxan	Metabolic ratio
1	M	PM	31.0	4.2	7.4
2	M	PM	48.0	6.2	7.8
3	M	PM	60.0	7.6	7.9
4	F	PM	53.0	6.7	7.9
5	M	EM	1.3	36.0	0.04
6	M	EM	1.9	25.0	0.08
7	M	EM	1.2	26.0	0.05
8	M	EM	1.5	29.0	0.05

aliphatic (α-carbon) oxidation have been shown to come under specific control of the same allele pair. There is no reason to suspect that the gene will not therefore extend to many other drugs, even oxidations at non-carbon centres may be affected. The implications of this upon new drug development and drug usage will be discussed in the final chapter.

CHAPTER SIX

General Conclusion

General Conclusion

This investigation has contributed towards the establishment of the cause of the large individual variation in the metabolism of debrisoquine and consequently provides an explanation for the large variation in optimal dose requirement among hypertensive patients.

Debrisoquine is metabolized by oxidation mainly along two pathways. Firstly, alicyclic ring hydroxylation giving rise to 4-hydroxy-debrisoquine which represents the major route of the biotransformation. This metabolite is subject of wide variation (2.5 - 39%). Secondly, by aromatic hydroxylation to 5-, 6-, 7- and 8-hydroxy-debrisoquine. Total phenols account for 2.7 - 14% of the dose.

The alicyclic 4-hydroxylation of debrisoquine is genetically determined. It exhibits a genetic polymorphism which is bimodally distributed among the population. Two phenotypes could be characterised. The extensive metabolizer phenotype (EM) comprises subjects with a great capacity to hydroxylate the drug. The second phenotype comprises subjects with a limited capacity to effect the 4-hydroxylation of the drug hence they are designated poor metabolizers (PM). Family studies suggest that the alicyclic 4-hydroxylation of debrisoquine is under the control of a single autosomal gene and that a defect at this metabolic step is determined by a pair of mutant alleles.

A difference in the defective allele frequency exists among various ethnic groups tested. Using the

Hardy-Weinberg equation to analyse these data it was possible to rank them in order of decreasing gene frequency as shown in Table 3.4 Nigerians and Gambians have the highest mutant gene frequency while Egyptians and Malaysians have the lowest incidence with the English intermediate.

The genotype of the individual is the major determinant of the hypotensive response to the drug. Poor metabolizer subjects are more sensitive to the hypotensive effects of the drug than are extensive metabolizers.

A single gene has been found to control not only the alicyclic oxidation of debrisoquine but also certain aromatic and aliphatic carbon oxidations. There is no foreseeable reason why the gene defect should not extend to other types of non-carbon oxidations such as those found at nitrogen and sulphur centres. This point would provide an interesting area of future research.

The practical and therapeutic implications of this study are manifold. Firstly, the high incidence of the defect among the population, which in the Caucasian study accounted for 7%, is obviously too high and cannot be ignored. Secondly, many of the adverse side effects reported for drugs may turn out to be associated with this gene due to genetically determined metabolic differences. For example, guanoxan itself was withdrawn from the market due to hepatic toxicity, the cause of which is still unknown. It has been shown here that guanoxan metabolism is genetically determined and that EM and PM subjects differ by a factor of around 50. Thus there is a great possibility that a correlation might exist between

guanoxan phenotype and the development of hepatic toxicity. This point needs to be investigated. Thirdly, since carbon oxidation often plays a major role in drug disposition and many oxidative processes may exhibit genetic polymorphism, then surely such factors should be considered during new drug development rather than test the substance in a small number of randomly selected in-house volunteers, as is the present case. It would be more advisable to utilize representative numbers of the two oxidative phenotypes. Methods for phenotyping subjects have been described here. Fourthly, it has been shown that the global distribution of oxidative ability is not homogeneous and thus the prevailing factors should be considered prior to marketing of pharmaceutical agents in non-Caucasian populations.

In conclusion it should be said that the ailing habit of gleaning metabolic data from a combination of Rhesus monkey, dog and rat may eventually become more incredible, since it would appear that even man himself is not good model for man.

APPENDIX

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APPENDIX I

DETAILS OF LONDON CAUCASIANS

Subjects	Age	Sex	% dose as: Debrisoquine	% dose as: 4-hydroxy-deb.	Metabolic ratio
Billington, K.	19	F	17.6	46.2	0.4
Bush, D.E.	20	F	65.0	22.6	2.9
Bewick, M.	20	M	31.8	42.2	0.8
Barber, V.	27	M	29.8	23.3	1.3
Booth, A.C.	19	F	30.2	42.8	0.7
Burton, J.W.	20	M	31.2	33.2	0.9
Bickel, M.	45	M	14.4	36.3	0.4
Bowman, W.J.	20	F	17.7	37.8	0.5
Courtney, M.	27	M	(32.3	45.0	0.7
			(16.6	30.5	0.5
Caldwell, J.*	32	M	39.2	30.6	1.3
Coles, R.J.	20	M	38.0	30.6	1.2
Collins, M.	26	M	28.0	13.1	2.1
Dalgleish, R.	22	M	(28.9	29.5	1.0
			(20.0	23.8	0.8
Dubois, A.	27	M	27.0	24.7	1.1
Dring, G.	40	M	28.9	23.1	1.3
Downer, H.	50	M	12.7	30.9	0.4
Devonshire, H.	21	F	(59.7	3.1	19.3
			18.1	0.8	22.6
Evans, R.N.	20	M	24.3	39.0	0.6
Evason, M.	21	M	(18.0	0.9	20.0
			(56.4	2.7	20.9
			(36.7	1.6	22.9
Evason, J.	16	F	58.2	2.7	21.6
Fennel, J.	29	F	23.6	36.5	0.6

Subjects	Age	Sex	% dose as: Debrisoquine	4-hydroxy-deb.	Metabolic ratio
Frankland, A.W.	19	M	41.3	29.7	1.4
Flannery,	21	M	34.4	32.8	1.0
Grant, R.L.	19	M	16.8	34.9	0.5
Gardiner, G.M.P.	21	M	41.1	38.6	1.1
Getting, R.	20	M	25.5	37.8	0.7
Govier, C.B	20	F	29.0	30.5	1.0
Griffith, B.J.	20	F	53.0	29.0	1.8
Hill, L.N.	20	M	19.8	21.9	0.5
Heley, A.	35	M	56.2	43.5	1.3
Howard,	21	M	14.0	41.6	0.3
Hurst, J.A.	20	F	28.0	35.0	0.8
Isaac, P.A.	21	M	24.0	33.0	0.7
Ioannou, P.	26	M	19.1	63.8	0.3
Iliffe, A.L.	20	F	34.8	44.0	0.8
Knapp, T.	22	F	22.1	36.8	0.6
Kitchen, I.	22	M	15.8	57.9	0.3
Little, P.	25	M	(45.1 (33.4	45.4 46.3	1.0 0.7
Loke, H.T.	22	M	9.8	17.2	0.6
Luckitt, J.K.	20	M	15.9	15.8	1.0
Langdon, D.	21	F	15.2	26.4	0 6
Mehta, R	21	F	28.5	29.5	1.0
Moffatt, J.*	27	M	21.1	56.8	0.4
Mair, C.M.	20	M	27.3	44.3	0.6
Meakin, D.R.	20	M	29.0	16.3	1.8
Mowbray, M.J.	19	M	19.9	33.0	0.6
Mindell, J.	19	M	32.4	21.5	1.5

Subjects	Age	Sex	% dose as: Debrisoquine	% dose as: 4-hydroxy-deb.	Metabolic ratio
Monks, T.	27	M	(41.3 (15.7	55.8 25.8	0.7 0.6
Markiewicz, M.	20	M	51.6	46.0	1.1
Martin, B.*	27	M	38.5	45.7	0.8
McNulty, C.A.M.	20	M	37.4	52.4	0.7
Nash, P.E.	21	F	17.2	42.3	0.4
Notarianni, L.	23	F	7.8	21.4	0.4
O'Horan, P.	20	M	23.0	26.0	0.9
O'Connell, N.J.	19	M	31.7	45.3	0.7
Parker, S.	20	F	46.5	49.0	0.9
Page, J.E.	20	F	31.0	22.3	1.4
Polson, D.W.	19	M	25.6	14.6	1.8
Player, M.H.	24	M	11.6	47.6	0.2
Phillips, J.	19	F	14.7	35.9	0.4
Phillips, D.G.L.	19	M	37.3	16.7	2.2
Patel, B.C.	21	M	9.7	17.7	0.5
Palmer, T.N.	20	M	53.7	29.8	1.8
Parker, R.	23	M	27.5	19.9	1.4
Royle, C.	20	M	17.6	38.3	0.5
Reed, A.	19	M	27.7	42.3	0.7
Ramsden, J.E.	20	F	15.6	26.0	0.6
Richard, B.M.	20	M	26.4	4.4	6.0
Shears, P.	20	M	22.4	25.9	0.9
Sampson, M.P.	20	M	16.3	36.2	0.5
Shorney, N.M.*	20	M	36.7	29.4	1.2
Savage, M.E.	19	M	25.9	59.3	0.4
Sidhu, P.S.	19	M	16.0	39.3	0.4
Stovin, P.H.	19	F	29.3	36.6	0.8

Subjects	Age	Sex	% dose as: Debrisoquine	4-hydroxy-deb.	Metabolic ratio
Shute, P.A.	19	M	24.9	28.5	0.9
Smith, M.R.	19	F	40.3	38.6	1.0
Stock, R.D.	20	M	18.3	42.0	0.4
Smith, R.L.	43	M	(42.7 (45.9 (39.6	2.0 2.5 2.0	21.4 18.5 19.8
Tidy, J.A.	19	M	27.0	35.8	0.8
Turner, S.	27	F	26.6	47.4	0.6
Tai, P.	22	M	27.7	14.8	1.9
Taylor-Roberts	20	M	36.0	31.6	1.1
Ubhi, P.S.	20	M	30.8	47.4	0.6
Wakile, L.	26	M	(24.8 (11.2	48.2 22.4	0.5 0.5
Watson, C.A.	20	M	63.4	20.6	3.1
Wilkinson, A.F.	20	F	10.1	1.3	7.8
Williams, W.W.	20	M	23.0	41.3	0.6
Wood, N.A.	19	F	23.8	5.8	4.0
Kamanyiro, G.	31	M	10.4	8.1	1.3
Idle, J.*	27	M	19.3	24.5	0.8
Sloan, T.*	25	M	20.9	14.5	1.4
Sloan, C.S.*	50	F	56.1	1.7	33.0
Ritchie, J.	22	M	4.9	23.5	0.2
Lawrie, S.	22	M	18.4	5.0	3.7
Hawes, E.	36	M	50.5	1.9	26.4

* Denotes smoker

APPENDIX II

DETAILS OF LIVERPOOL CAUCASIANS

Subjects	Age	Sex	% dose as: Debrisoquine	% dose as: 4-hydroxy-deb.	Metabolic ratio
Austin, C.	20	M	10.4	17.4	0.6
Bell, D.	22	M	10.4	11.9	0.9
Baker, S.F.	40	M	15.3	21.7	0.7
Bentley, C.	60	M	11.5	20.2	0.6
Clare, R.G.*	22	M	7.5	15.4	0.5
Cowie, R.H.*	24	M	8.1	29.8	0.3
Cannon, J.	23	F	9.4	22.3	0.4
Cowie, J.N.*	59	M	12.4	24.4	0.5
Constantine, G.	25	M	14.9	28.8	0.5
Carley, J.	22	F	5.4	5.5	1.0
Cushing, M.	21	M	21.8	18.5	1.2
Donahue, P.	20	M	27.9	27.8	1.0
Deakin, M.	23	M	9.5	18.3	0.5
Desmond, J.	20	M	23.4	37.0	0.6
Donald, M.	23	M	50.4	1.2	42.0
Ellis, L.	22	F	(21.8 (45.7	5.7 12.0	3.8 3.8
Ellis, A.J.*	22	M	14.0	19.2	0.7
Elsworthy, V.	20	F	5.7	15.7	0.4
Finnegan, M.J.	21	M	9.5	18.3	0.5
Flannery, R.B.	24	M	7.7	16.7	0.5
Goodwin, G.W.	23	M	43.7	7.1	6.2
Goodman, C.	25	M	(20.0 (35.7	0.40 0.9	48.8 39.7
Gleave, J.*	24	M	16.6	16.4	1.0

Subjects	Age	Sex	% dose as: Debrisoquine	4-hydroxydeb.	Metabolic ratio
Graham, R	23	M	9.0	23.1	0.4
Gurgen, F.	22	F	11.8	16.2	0.7
Guest, R.	24	M	10.8	19.8	0.6
Gibby, M.	24	M	12.8	17.0	0.8
Greaves, J.	24	M	19.5	22.3	0.9
Halliwell,K.A.*	23	M	59.7	1.8	33.0
Harris, K.	24	M	(34.9 (41.2	1.5 2.2	23.0 18.7
Hastie, K.*	21	M	32.7	16.2	2.0
Hayes, P.	24	F	(98.8 (36.0	1.7 1.9	58.0 18.9
Herbert, M.*	18	M	(35.2 (34.2	1.8 1.7	19.6 20.1
Humphries, L.	25	F	9.9	21.8	0.5
Horner, J.	23	F	(56.0 (8.4	4.4 1.9	12.7 4.4
Hill, E	24	F	5.3	16.6	0.3
Hughes, I.	24	M	14.3	35.7	0.4
Harvey, R.	24	M	5.3	19.7	0.3
Hayhurst, G.	21	M	27.4	13.2	2.1
Halliday, S.E.	22	M	18.9	16.2	1.2
Ingram, S.D.*	24	M	19.7	55.0	0.4
Jackson, R.*	21	M	24.7	23.8	1.0
Jones, H.	21	M	18.5	30.8	0.6
Jones, M.W.	20	M	35.1	37.1	1.0
Kitchen, B.	47	F	18.0	25.4	0.7
Kabo, G.	31	M	15.1	19.5	0.8
Kingston, S.	24	F	(56.3 (18.2	2.2 0.7	25.6 26.0

Subjects	Age	Sex	% dose as: Debrisoquine	% dose as: 4-hydroxy-deb.	Metabolic ratio
Kennedy, R.	22	F	5.9	4.6	1.3
Kurzeja, J.A.	24	M	13.0	19.0	0.7
Lake, P.	21	M	23.1	18.2	1.3
Lambden, K.	27	M	32.8	18.0	1.8
Lane, D.	23	M	20.2	16.7	1.2
Lord, E.R.*	23	M	19.3	27.4	0.7
Lamberton, F.	24	F	7.8	13.0	0.6
Lamb, J.H.	25	M	11.0	19.7	0.6
Keyurangul, V.	24	M	1.5	8.1	0.2
Morris, H.	20	M	16.0	8.8	1.8
Mountford, P.*	21	M	23.1	17.3	1.3
Matthews, S.	18	F	17.3	15.8	1.1
Murphy, D.	24	M	18.7	7.2	2.6
Morris, S.	24	M	19.6	8.0	2.5
Mooney, P.	22	M	22.4	46.7	0.5
Mullet, S.*	23	M	6.1	44.7	0.1
Morton, J.D.	24	M	12.8	13.8	0.9
Monaghan, D.	24	M	18.7	20.2	0.9
Owen, J.H.	20	M	12.0	20.8	0.6
O'Brien, D.	24	M	7.9	15.2	0.5
O'Donnell, J.	21	M	26.3	11.0	2.4
Pierce, P.	21	M	11.3	17.7	0.6
Phillips, D.	23	M	19.8	16.9	1.2
Propper, D.*	24	M	26.6	14.2	1.9
Parry, G.*	23	M	3.8	2.2	1.7
Paige, J.	21	F	10.4	23.5	0.4
Pearce, S.	23	F	6.2	13.6	0.5

Subjects	Age	Sex	% dose as: Debrisoquine	4-hydroxydeb.	Metabolic ratio
Russell, N.S.	20	M	33.3	18.4	1.8
Robinson, R.	24	M	23.6	13.2	1.8
Rowlands, J.*	32	M	14.8	20.0	0.7
Robinson, M.	25	M	20.2	11.3	1.8
Rogers, E.	21	F	8.5	20.7	0.4
Smith, M.N.	29	M	(40.0 (26.3	1.3 0.9	30.8 29.2
Sill, P.R.	24	M	19.2	1.8	10.7
Salt, M.	25	M	15.6	22.8	0.7
Shearer, E.	20	M	7.5	17.4	0.4
Shackleton,	41	M	15.5	14.2	1.1
Somers, J.M.	21	M	13.4	14.4	0.9
Sinton, G.	20	M	7.1	15.6	0.5
Smith, P.	23	F	5.0	3.9	1.3
Thorogood, I.	19	M	25.2	28.9	0.9
Whitecroft, S.	20	F	7.8	8.0	1.0
Walker, C.V.S.	20	M	8.3	21.9	0.4
Whiteside, C.	19	F	28.9	11.7	2.5
Weighill, P.*	20	F	40.8	13.9	2.9
Wells, J.*	24	F	10.2	26.1	0.4
Walker, G.A.*	23	M	15.2	19.7	0.8
Walter, T.	18	M	6.3	17.3	0.4
Whiteworth, J.	20	M	4.2	13.3	0.3
Wilson, D.	21	M	26.2	45.2	0.6

* Denotes smoker

APPENDIX III

Debrisoquine Metabolism in Egyptian Subjects

Subject	Age	Sex	% dose as: Debrisoquine	4-hydroxy-deb.	Recovery (0-8h)	Metabolic ratio
1	28	F	4.5	17.3	21.8	0.3
2	15	M	2.1	0.5	2.6	4.2
3*	35	M	2.6	5.1	7.7	0.5
4*	50	M	5.7	11.3	17.0	0.5
5*	38	M	1.3	10.0	11.3	0.1
6	50	M	5.3	7.0	12.3	0.8
7*	54	M	0.8	8.8	9.6	0.1
8	13	F	5.8	19.6	25.4	0.3
9	40	F	5.0	17.8	22.8	0.3
10	22	M	4.7	12.5	17.2	0.4
11	41	M	3.7	13.0	16.7	0.3
12	16	M	4.4	6.9	11.3	0.6
13*	40	M	8.4	18.5	26.9	0.5
14	20	F	5.0	9.3	14.3	0.5
15	35	F	2.2	11.5	13.7	0.2
16	22	M	1.4	8.9	10.3	0.2
17	17	F	1.0	4.8	5.8	0.2
18	20	F	17.5	9.6	27.1	1.8
19	14	M	7.8	11.6	19.4	0.7
20	29	F	3.4	18.0	21.4	0.2
21*	40	M	4.5	11.3	15.8	0.4
22	17	F	1.4	4.3	5.7	0.3
23	14	M	0.5	1.5	2.0	0.3
24	15	F	3.2	6.3	9.5	0.5
25	17	M	1.4	3.8	5.2	0.4

Subject	Age	Sex	% dose as: Debrisoquine	4-hydroxy-deb.	Recovery (0-8h)	Metabolic ratio
26	14	M	7.3	5.0	12.3	1.5
27	13	M	4.0	12.0	16.0	0.3
28*	40	M	3.8	10.0	13.8	0.4
29	17	M	2.3	8.3	10.6	0.3
30*	30	M	3.7	22.4	26.1	0.2
31	17	M	5.7	8.2	13.9	0.7
32	35	F	0.2	6.2	6.4	0.03
33	27	F	5.4	9.3	14.7	0.6
34	17	F	4.4	11.9	16.3	0.4
35	29	F	1.7	5.3	7.0	0.3
36	12	F	2.7	17.5	20.2	0.2
37	45	F	0.8	3.3	4.1	0.2
38	12	F	5.1	19.7	24.8	0.3
39	14	M	2.3	2.3	4.6	1.0
40	50	F	13.6	0.6	14.2	22.7
41*	60	M	2.0	1.2	3.2	1.7
42	22	M	20.6	2.3	22.9	9.0
43	19	M	4.7	8.6	13.3	0.5
44	27	M	19.0	1.6	20.6	11.9
45	22	F	8.7	1.2	9.9	7.3
46	12	M	2.5	5.3	7.8	0.5
47	14	M	13.0	4.5	17.5	2.9
48	30	F	5.4	4.1	9.5	1.3
49	18	F	11.9	3.4	15.3	3.5
50	18	F	4.6	6.5	11.1	0.7
51	40	F	0.6	15.0	15.6	0.04
52	12	F	8.3	9.2	17.5	0.9

Subject	Age	Sex	% dose as: Debrisoquine	4-hydroxy-deb.	Recovery (0-8h)	Metabolic ratio
53	22	F	0.8	3.6	4.4	0.2
54*	45	M	2.3	13.2	15.5	0.2
55	14	F	0.8	6.3	7.1	0.1
56	13	F	6.6	19.6	26.2	0.3
57	75	F	0.3	2.0	2.3	0.2
58	22	F	1.3	3.6	4.9	0.4
59	19	M	7.1	20.0	27.1	0.4
60	15	M	14.5	24.5	39.0	0.6
61	17	M	6.6	12.8	19.4	0.5
62	35	F	0.8	9.9	10.7	0.1
63*	40	M	4.5	13.9	18.4	0.3
64	27	F	0.25	5.7	6.0	0.04
65	35	F	28.6	50.4	79.0	0.6
66*	36	M	(12.5	7.0	19.5	2.7
			(27.0	4.1	31.1	6.6
			(32.4	3.0	35.4	10.8
			(36.3	3.8	40.1	9.6
67	30	F	10.4	21.1	31.5	0.5
68*	36	F	44.2	4.7	48.9	9.4
69	32	F	20.6	19.2	39.8	1.1
70*	27	M	13.4	16.1	29.5	0.8
71*	40	M	Lo.5	17.9	28.4	0.6
72*	47	M	9.1	29.5	38.6	0.3
73*	34	M	3.9	29.8	33.7	0.1

* Denotes smoker

APPENDIX IV

Debrisoquine Metabolism in Nigerian Subjects

Subject	Age	Sex	% dose as: Debrisoquine	4-hydroxy-deb.	Recovery (0-8h)	Metabolic ratio
2	-	M	14.3	15.8	30.1	0.9
3	-	M	40.3	30.9	71.4	1.3
4	21	M	(14.9 (14.3	4.7 6.2	19.6 20.5	3.2 2.3
5	26	M	7.6	17.6	25.2	0.4
7	24	M	83.5	32.0	115.5	2.6
10	22	M	23.5	2.6	26.1	9.0
11	22	M	18.2	11.6	29.8	1.6
12	21	M	24.8	31.5	56.3	0.8
13	24	M	24.4	10.0	34.4	2.4
14	24	M	83.0	2.7	85.7	30.7
15	25	M	15.2	26.0	41.2	0.6
16	26	M	33.0	1.1	34.1	30
17	24	M	5.4	28.7	34.1	0.2
18	24	M	53.7	9.0	62.7	6.0
19	22	M	35.4	16.5	51.9	2.1
20	23	M	7.7	8.8	16.5	0.9
21	23	M	7.6	14.5	22.1	0.5
23	27	M	20.5	1.2	21.7	17.1
24	21	M	20.0	40.0	60.0	0.5
26	23	M	78.8	2.1	80.9	37.5
27	27	M	7.8	2.1	31.1	0.3
28	24	M	10.2	37.4	47.6	2.7
29	24	M	34.4	0.9	35.5	38.0
30	24	M	29.5	24.4	53.9	1.2

Subject	Age	Sex	% dose as: Debrisoquine	4-hydroxydeb.	Recovery (0-8h)	Metabolic ratio
32	24	M	11.5	2.1	13.6	5.5
33	24	M	10.2	3.9	14.1	2.6
34	23	M	52.4	17.3	69.7	3.0
35	22	F	43.5	14.7	58.2	3.0
36	24	M	18.2	24.1	42.3	0.8
38	23	M	22.9	11.7	34.6	2.0
39	22	M	35.0	12.5	47.5	2.8
40	23	M	30.6	2.4	33.0	12.8
41	21	M	14.4	18.03	32.4	0.8
42	25	M	32.8	3.9	36.7	8.4
44	21	M	15.8	5.8	21.6	2.7
45	21	M	21.7	29.3	51.0	0.7
46	23	F	21.4	10.3	31.7	2.1
47	21	F	37.0	5.2	42.2	7.1
48	23	M	16.0	25.2	41.2	0.6

APPENDIX V

Debrisoquine Metabolism in Ghanaian Subjects

Subject	Age	Sex	% dose as: Debrisoquine	% dose as: 4-hydroxy-deb.	Recovery (0-8h)	Metabolic ratio
A*	21	M	50.4	6.2	56.6	8.0
B	22	M	18.8	1.7	20.5	11.1
C	25	M	26.3	5.7	32.0	4.6
D	22	M	6.8	1.4	8.2	4.9
1	22	F	50.4	37.9	88.3	1.3
2	22	M	7.4	11.9	19.3	0.6
3	23	M	3.7	2.8	6.5	1.3
6	24	M	7.8	10.0	17.8	0.8
8	22	F	10.3	11.3	21.6	0.9
10	21	M	6.0	11.5	17.5	0.5
11	23	M	11.5	8.8	20.3	1.3
19	22	M	17.1	12.2	29.3	1.4
21	21	F	20.4	15.2	35.6	1.3
25*	24	M	16.5	4.1	20.6	4.0
27	23	M	4.0	3.0	7.0	1.3
28	22	M	15.1	4.8	19.9	3.1
30	23	M	24.8	2.4	27.2	10.3
31	22	M	11.3	8.6	19.9	1.3
44	23	F	36.0	2.7	38.7	13.3
45	23	M	3.4	6.4	9.8	0.5
46	22	M	18.0	4.1	22.1	4.4
49	20	M	9.7	7.4	17.1	1.3
50	21	M	7.9	6.0	13.9	1.3
51	21	M	15.9	4.4	20.3	3.6

Subject	Age	Sex	% dose as: Debrisoquine	4-hydroxy-deb.	Recovery (0-8h)	Metabolic ratio
52	24	M	43.6	31.2	74.8	1.4
53	22	M	49.5	12.4	61.9	4.0
56	24	M	23.6	3.1	26.7	7.6
59	22	M	5.5	7.6	13.1	0.7
61	22	M	11.1	17.3	28.4	0.6
64	23	M	10.8	4.1	14.9	2.6
66	23	M	56.9	1.8	58.7	31.6

* Denotes smoker

APPENDIX VI

Debrisoquine Metabolism in Gambian Subjects

Subject	Age	Sex	% dose as: Debrisoquine	4-hydroxy-deb.	Recovery (0-8h)	Metabolic ratio	Tribe
1*	72	M	32.9	10.7	43.6	3.1	M
2	55	M	21.1	10.6	31.7	2.0	M
3	72	M	21.1	1.0	22.1	21.1	J
4	42	M	21.7	1.6	23.3	13.6	M
5*	44	M	15.9	6.3	22.2	2.5	J
6*	72	M	21.6	8.8	30.4	2.5	M
7*	58	M	14.0	18.3	32.3	0.8	M
8	37	M	30.2	<0.01	30.3	>300	M
9*	44	M	23.3	7.3	23.6	3.2	M
10	51	M	47.0	10.4	57.4	4.5	F
11*	51	M	23.8	5.1	28.9	4.7	M
12	61	M	49.4	4.8	54.2	10.3	M
13*	44	M	20.9	7.3	28.2	2.9	M
14*	43	M	8.7	3.3	12.0	2.6	M
15*	31	M	3.9	1.3	5.2	3.0	M
16	25	M	5.5	23.2	28.7	0.2	J
17*	51	M	25.2	12.3	37.5	2.0	M
18*	41	M	15.4	21.0	36.4	0.7	M
19	60	M	8.0	5.2	13.2	1.5	M
20	71	M	17.4	9.7	27.1	1.8	M
22*	21	M	51.1	12.9	64.0	4.0	M
24	29	F	15.4	8.8	24.2	1.8	M
25	26	F	12.8	<0.05	12.85	>250	J
26	43	F	3.9	6.8	10.7	0.6	F

Subject	Age	Sex	% dose as: Debrisoquine	4-hydroxy-deb.	Recovery (0-8h)	Metabolic ratio	Tribe
27	60	F	2.0	8.7	10.7	0.2	S
28	29	F	6.4	17.3	23.7	0.4	M
29*	53	F	13.8	4.6	18.4	3.0	M
30*	46	M	5.0	1.3	6.3	3.8	J
31*	51	F	9.3	1.1	10.4	8.5	M
32*	65	F	16.6	3.0	19.6	5.5	J
33	46	F	11.5	17.3	28.8	0.7	S
34	45	F	6.7	10.2	16.9	0.7	F
35	31	F	19.8	5.2	25.0	3.8	M
36	51	F	40 9	7.7	48.6	5.3	M
37	61	F	4.2	19.9	24.1	0.2	M
38	61	F	10.1	6.2	16.3	1.6	M
39	51	F	10.0	9.9	19.9	1.0	M
40	51	F	27.0	13.2	40.2	2.0	J
41*	45	F	15.6	15.4	31.0	1.0	M
42	23	F	4.6	6.3	10.9	0.7	M
43	85	F	30.2	<0.05	30.25	>600	M
44	49	F	6.8	1.8	8.6	3.8	M
45	46	F	41.0	<0.1	41.1	>400	M
46*	71	M	31.0	0.8	31.8	38.8	J
47*	24	M	17.6	4.9	22.5	3.6	M
48	31	M	8.3	1.3	9.6	6.4	M
49	35	F	1.1	0.4	1.5	2.8	M
50*	33	F	11.7	11.4	23.1	1.0	M

Tribes: Mandinka = M Jolla = J
Fulla = F Serer = S

* Denotes smoker

APPENDIX VII

Debrisoquine Metabolism in Malaysian Subjects

Subject	Age	Sex	% dose as: Debrisoquine	% dose as: 4-hydroxy-deb.	Recovery (0-8h)	Metabolic ratio
1	21	M	19.8	12.9	32.7	1.5
2	31	M	11.2	9.5	20.7	1.2
3	24	F	27.9	7.8	35.7	3.6
4	24	M	13.30	12.8	26.1	1.0
5	25	M	4.50	4.10	8.6	1.1
6	35	M	23.0	5.5	28.5	4.2
7	22	M	7.6	16.7	24.3	0.5
8	20	M	24.4	7.7	32.1	3.2
9	30	M	5.8	20.2	26.0	0.3
10	30	M	4.8	9.5	14.3	0.5
11	27	F	18.1	4.2	22.3	4.3
12	23	F	17.3	12.8	30.1	1.4
13	23	M	2.2	7.7	9.9	0.3
14	22	M	16.0	13.9	29.9	1.3
15	21	M	20.6	1.3	21.9	15.8
16	22	F	0.6	9.6	10.2	0.06
17	20	F	1.5	1.4	2.9	1.1
18	21	M	14.8	7.0	21.8	2.1
19	22	M	11.7	14.7	26.4	0.8
20	20	M	14.9	11.9	26.8	1.3
21	21	M	11.8	23.3	35.1	0.5
22	24	M	19.6	17.3	36.9	1.1
23	21	M	24.2	8.1	32.3	3.0
24	21	M	22.0	16.7	38.7	1.3

Subject	Age	Sex	% dose as: Debrisoquine	% dose as: 4-hydroxy-deb.	Recovery (0-8h)	Metabolic ratio
25	20	M	12.6	12.4	25.0	1.0
26	22	M	17.7	8.9	26.6	2.0
27	20	M	11.3	8.0	19.3	1.4
28	18	M	18.2	11.4	29.6	1.6
29	20	M	9.9	11.3	21.2	0.9
30	21	M	43.5	5.2	49.7	7.0
31	21	F	8.4	9.6	18.0	0.9
32	20	M	9.1	3.6	12.7	2.5
33	21	M	40.0	1.4	41.4	28.6
34	26	F	9.1	10.4	19.5	0.9
36	39	M	10.5	13.2	23.7	0.8
37	21	M	8.3	11.8	20.1	0.7
38	24	F	3.1	0.9	4.0	3.4
39	25	F	11.8	18.5	30.3	0.6
40	22	F	1.6	3.3	4.9	0.5
41	28	M	4.8	9.5	14.3	0.5
42	24	F	18.9	6.4	25.3	3.0
43	22	M	19.0	7.7	26.7	2.5
44	22	M	11.5	10.6	22.1	1.1
45	23	M	16.5	8.9	25.4	1.9
46	29	M	15.5	2.7	18.2	5.7
47	32	M	17.6	10.7	28.3	1.6
48	23	M	9.1	16.7	25.8	0.5
49	24	M	8.2	3.1	11.3	2.6

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HYPOTENSIVE RESPONSE TO DEBRISOQUINE AND HYDROXYLATION PHENOTYPE

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SUMMARY

Seven human volunteers with normal blood pressure, who had been phenotyped according to their ability to effect the 4-hydroxylation of the antihypertensive drug debrisoquine, each took orally a 20mg tablet of the drug. Lying and standing blood pressures were measured at hourly intervals up to 8h post-dosing. The four extensive metabolizers (EM phenotype) showed little or no response to the drug, whilst the three non-metabolizers (NM phenotype) demonstrated a statistically significant orthostatic hypotensive effect compared to the EM group. Dose-dependent metabolism was also observed with the NM subjects. It is concluded that the genetic factor which controls the metabolism of debrisoquine is also largely responsible for the differences in response seen between the two debrisoquine hydroxylator phenotypes.

There occur marked inter-individual differences in the debrisoquine optimal dose requirement among hypertensives. In early studies (1,2) the daily dose was found to range from 20 to 400mg, the average being about 70mg while in a more recent study (3) of 120 hypertensives it was found to vary from 10-360mg. Subsequent studies showed that these inter-individual differences were not related to variations in absorption but arose from differences in ability to metabolize the drug (4). A positive correlation was shown to exist between hypotensive response to debrisoquine and the amount of unchanged drug excreted in the urine. These observations were confirmed and extended in a study of hypertensive subjects and furthermore it was shown that interpatient differences in response were related to plasma levels (3).

The drug undergoes oxidative metabolism to give a series of hydroxylated products, namely, 4-,5-,6-,7- and 8-hydroxydebrisoquine, the major pathway being the conversion to the 4-hydroxy derivative (5). Recently, it has been shown that this metabolic pathway, which is a key determinant of the response to the drug, is mainly under genetic control (6). There exists two distinct phenotypes in the population as regards ability to effect this reaction. One group, designated EM (extensive metabolizer), is characterised by an ability to extensively convert debrisoquine to 4-hydroxydebrisoquine as judged by a ratio of urinary excretion of $\frac{\text{unchanged drug}}{\text{4-hydroxydebrisoquine}}$ of less than 8 and a second phenotype, designated NM (non-metabolizers), showing a metabolic ratio

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of >21(6). In this study (6) the incidence of the NM phenotype was about 4% though this might be an underestimate (7).

Because of the early studies linking hypotensive response with extent of metabolism we considered it of interest to investigate the two in individuals who had been phenotyped as regards hydroxylation status. In this communication it will be shown that the NM phenotype is more responsive to a single oral dose of 20mg of debrisoquine, in terms of development of postural hypotension than the EM phenotype, and that this is related to the relative inability of the NM phenotype to effect oxidative conversion of the drug to its 4-hydroxy derivative.

INVESTIGATIONS

Seven normotensive subjects were selected from the 94 previously investigated. Three of these have been designated NM phenotype in this study and the remaining four, EM phenotype.

Each subject took a light fat-free breakfast before taking the drug and refrained from taking other drugs or alcohol during the investigation. The lying and standing b.p. was measured by an observer who knew the phenotypes using a random zero sphygmomanometer to reduce operator bias: subjects remained in a supine position in a quiet room for 5 min. and both systolic and diastolic b.ps. were recorded. After standing for 2 min. the systolic and diastolic b.ps. were redetermined. Pulse rates were also recorded. Each subject then took orally a single tablet of Declinax, containing 25.6mg of debrisoquine sulphate, equivalent to 20mg debrisoquine base. Lying and standing b.p. values were then measured for each subject at hourly intervals for 8h after dosing. Mean b.p. was calculated from the diagnostic pressure plus one-third of the difference between systolic and diastolic b.p. values. Observed differences between the mean standing and mean lying b.p. (over the 3-7h period post-dosing) for both groups were tested statistically for significance using an unpaired two-tailed students t-test. Urine samples were collected over the 8h period, combined for each subject and an aliquot used for estimation of debrisoquine and 4-hydroxydebrisoquine by the method described previously. The results allow calculation of the metabolic ratio and assignment of phenotype.

RESULTS AND DISCUSSION

Phenotype Status

The hydroxylation phenotype status of the seven subjects as judged by the ratio of $\frac{\text{urinary debrisoquine}}{\text{4-hydroxydebrisoquine}}$ is shown in Table 1. The results previously obtained for phenotyping with a 10mg dose are also shown. With the 20mg dose the same phenotype assignments were made as previously with the 10mg dose. An important difference in the metabolic ratio at the two dose levels was however observed which is worthy of comment. The observed metabolic ratios for subjects 1 to 4 (designated EM) were little changed when the dose was increased from 10mg to 20mg and for three of the subjects showed a decrease. By contrast, for the three NM subjects there was a marked increase in the metabolic ratio with the larger dose of debrisoquine. This would suggest that the limited 4-hydroxylation capacity of the NM phenotypes is readily saturable. This would indicate another point of difference in the handling of debrisoquine between NM and EM phenotypes since the drug will show dose-dependent metabolism pharmacokinetics in the former phenotype at dose levels when this is not seen in the EM phenotype.

TABLE 1

Hydroxylation Phenotypes of Subjects

Subject	Sex	Age(yrs)	Metabolic Ratio (20mg)	Metabolic Ratio (10mg*)	Assigned phenotype
1	M	24	0.4	0.6,0.7	EM
2	M	26	1.0	1.4	EM
3	M	27	0.3	0.5,0.5	EM
4	M	27	0.7	0.7	EM
5	F	20	53	23,19	NM
6	M	21	34	20,23,21	NM
7	M	43	56	21,20	NM

* Results of previous study

Blood Pressure Response

Table 2 shows the actual hourly lying and standing b.p. values of the seven subjects before and after the dose of debrisoquine. This data is represented in Fig.1 in which the mean lying and standing b.p. has been plotted against time. From these profiles it can be seen that subjects 1-4 (EM phenotype), compared to the three NM phenotypes showed no orthostatic hypotensive response to the 20mg dose of debrisoquine. In the latter group the drug induced an orthostatic hypotension as shown by the trend of the b.p. profiles and by the symptoms elicited in two of the subjects. Using an unpaired two-tailed students t-test there was a statistically significant trend in the difference between the mean standing and mean lying b.p. (measured over the 3-7h period post-dosing) for the two phenotypes ($2P < 0.02$). This indicates that the degree of post-ganglionic sympathetic blockade induced by the drug is greater in the NM than in the EM phenotype. Two of the NM phenotypes (subjects 5 and 7) complained of symptoms of postural hypotension (giddyness and faintness on standing) over the period 4-6h after taking the drug but these disappeared by 8h. By contrast the four EM subjects were symptom free over the entire period.

These studies indicate a direct relationship between hydroxylation phenotype and the development of postural hypotension to a single oral dose of 20mg of debrisoquine. Clearly, persons of NM phenotype are more sensitive to the hypotensive effects of the drug than those of EM phenotype and this may be attributed to their genetically determined inability to effect 4-hydroxylation of the drug.

These findings have a practical bearing upon the therapeutic use of debrisoquine. Our initial studies suggest an incidence of about 4% for NM phenotype. Because this subject is much more sensitive to the hypotensive effects of debrisoquine it suggested that in commencing therapy with this drug one should begin with small doses (10-20mg) and increase cautiously to avoid overdosing of a NM phenotype patient. Alternatively, a phenotype determination can be readily carried out using the procedure described previously (6).

TABLE 2

Blood Pressure Responses to Debrisoquine among NM and EM Hydroxylation Phenotypes

Subject	1	2	3	4	5	6	7	
Phenotype	EM	EM	EM	EM	NM	NM	NM	
Time after dosing (h)								
0	120/80	130/90	115/65	115/80	125/60	120/80	140/85	L
	125/100	130/80	130/75	120/80	110/85	125/105	145/100	S
1	135/95	125/85	115/75	125/75	130/80	110/80	135/100	L
	120/100	115/90	125/75	125/90	110/90	120/100	135/100	S
2	130/90	140/100	105/70	120/80	115/85	115/85	145/100	L
	130/100	140/110	120/80	120/70	120/90	120/100	135/85	S
3	130/80	135/80	145/85	120/70	130/70	125/100	150/90	L
	130/95	140/100	120/75	115/80	120/70	125/100	130/95	S
4	120/80	140/60	120/65	150/90	130/70	130/110	155/110	L
	120/100	120/95	135/85	130/80	110/70	125/100	135/90	S
5	120/85	130/95	130/80	115/70	125/70	140/100	155/110	L
	125/100	130/75	120/80	120/75	100/65	125/95	120/80	S
6	120/85	130/90	105/65	130/60	120/80	125/95	115/80	L
	130/100	120/90	130/80	130/70	100/60	120/90	110/60	S
7	140/80	120/80	110/65	125/80	110/65	130/100	130/95	L
	120/85	120/90	110/70	110/80	90/60	125/95	125/95	S
8	130/75	120/60	115/70	130/80	125/70	125/75	135/100	L
	130/100	130/80	120/70	130/75	125/70	130/100	135/100	S

L = Lying, S = Standing

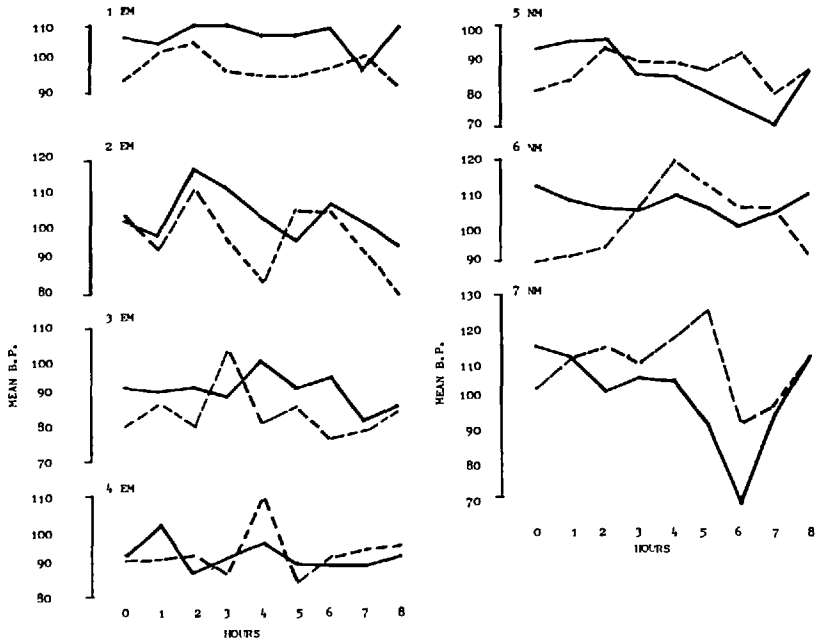

FIG. 1

Mean standing and lying b.p. profiles after debrisoquine (20mg p.o.) in four EM and three NM subjects. Solid line represents standing b.p. and broken line the lying b.p.

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Defective alicyclic hydroxylation of debrisoquine in man

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Previous studies have established the qualitative aspects of the metabolism of debrisoquine, namely, that it is metabolized by oxidation to 4-, 5-, 6-, 7- and 8- hydroxy-debrisoquine (Allen, East, Francis & Haigh, 1976; Angelo, Dring, Lancaster & Smith, 1976). However, the quantitative aspects of these metabolic conversions are largely lacking and this becomes of importance in view of reports that some individuals are relatively unable to metabolize debrisoquine (Angelo, Dring, Lancaster, Latham & Smith, 1975; Angelo *et al.*, 1976; Silas, Lennard, Tucker, Smith, Malcolm & Marten, 1977). In these cases it is not known whether or not the defective hydroxylation is specific to one or more positions of the molecule or is general to all. Accordingly, we have made a quantitative study of the formation of the 4-, 5-, 6-, 7- and 8-hydroxy derivatives of debrisoquine in five subjects whose metabolic status, as far as ability to metabolize the drug was concerned, had been established by prior study. Three of the subjects had been typed as extensive metabolizers (EM) and two as poor-metabolizers (PM).

The three EM subjects were given ^{14}C-labelled debrisoquine orally (40 mg) and the urine collected for 24 h for analysis. The two PM subjects were given the unlabelled drug orally (10 mg) and the urine collected for 8 h. Debrisoquine and 4-hydroxy-debrisoquine were determined by e.c.g.c. after derivatization with hexafluoroacetylacetone (Erdtmansky & Goehl, 1975). The phenolic derivatives (5-, 6-, 7- and 8-hydroxy-debrisoquine) were determined following conversion to their trimethylsilyl ethers.

The EM subjects excreted mainly unchanged debrisoquine (28–45% of dose) with 4-hydroxy-debrisoquine (30–39%) as the main oxidation product. Small quantities of the four phenolic metabolites were also eliminated (total 2.7–13.7%) (see Table 1). A good correspondence was obtained between ^{14}C recovery (67–71%) and total drug as measured by e.c.g.c. (70–80%). By contrast, the two PM subjects excreted, as expected, most of the dose of debrisoquine as unchanged drug. The amounts of phenolic metabolites produced were similar to those found for the three EM subjects. There was however a dramatic difference in the amount of 4-hydroxy-debrisoquine formation between EM and PM subjects.

It is concluded that the major metabolic difference between EM and PM subjects is that the latter are relatively defective in their ability to specifically effect the 4-hydroxylation of debrisoquine.

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Table 1 Metabolites of debrisoquine in man, expressed as a percentage of the dose

	Human subjects				
		EM		*PM*	
Compound	*J.I.*	*A.L.*	*R.L.*	*R.S.*	*M.E.*
Debrisoquine	27.7	26.5	45.0	39.6	56.4
4-Hydroxy-debrisoquine	37.0	38.8	29.8	2.0	2.7
5-Hydroxy-debrisoquine	1.3	2.3	0.5	0.2	0.1
6-Hydroxy-debrisoquine	1.0	3.6	0.7	0.9	0.8
7-Hydroxy-debrisoquine	1.9	6.3	1.2	4.0	3.5
8-Hydroxy-debrisoquine	0.6	1.5	0.3	0.2	0.1
Total phenols	4.8	13.7	2.7	5.3	4.5
Urinary recovery:					
(a) by e.c.g.c.	69.5	79.7	77.5	46.9	63.6
(b) by ^{14}C	66.8	70.4	70.6	—	—

A population and familial study of the defective hydroxylation of debrisoquine

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The previous communication (Angelo, Dring, Idle, Lancaster, Mahgoub & Smith, 1977) established that certain individuals are characterized by a relative inability to effect a specific metabolic hydroxylation of debrisoquine, namely, to convert it to 4-hydroxy-debrisoquine. It was considered of interest to investigate the incidence of this particular hydroxylation defect in a larger sample of the population. Accordingly, debrisoquine (10 mg) was administered orally to 94 student and staff volunteers at St. Mary's and the urine collected for 8 h and analyzed for unchanged debrisoquine and its 4-hydroxy derivative by e.c.g.c. as described previously (Erdtmansky & Goehl, 1975). Ninety one of the subjects were typed as extensive metabolizers (EM) and three as poor metabolizers (PM). For the purpose of constructing a frequency distribution histogram it was found convenient to express the findings in terms of the following metabolic ratio:

$$\frac{\%\text{ dose excreted as unchanged debrisoquine}}{\%\text{ dose excreted as 4-hydroxy-debrisoquine}}$$

The frequency distribution histogram (Figure 1) revealed two distinct population groups with the EM group exhibiting a metabolic ratio of 0–8 and the PM group with this ratio >21. No subjects were found to have ratios in the range 9–20. Subsequent rechallenging of 10 EM subjects and the 3 PM subjects with debrisoquine resulted in no change in the observed ratio. Investigation of the families of the 3 PM subjects suggests that the defective alicyclic hydroxylation of debrisoquine is an inherited trait.

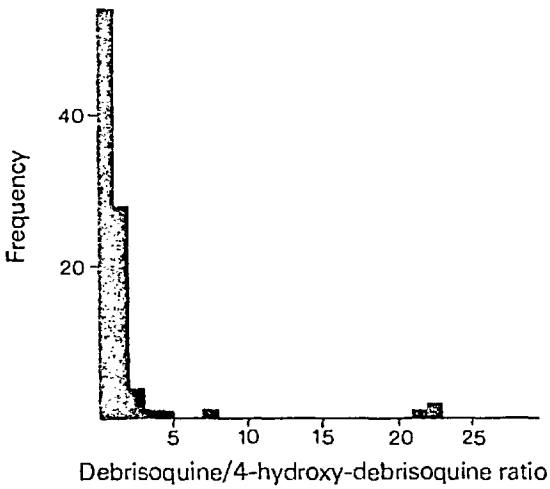

Figure 1 Frequency distribution histogram of the debrisoquine metabolic ratio in 94 subjects.

We are grateful to Professor D. Price-Evans, Department of Medicine, University of Liverpool, for his advice on this study.

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Polymorphism of carbon oxidation of drugs and clinical implications

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Summary and conclusions

Eight volunteers previously phenotyped for their ability to hydroxylate debrisoquine (four extensive metabolisers (EM), four poor metabolisers (PM)) were investigated for their metabolic handling of guanoxan and phenacetin. All three drugs are oxidised at carbon centres. Oxidative dealkylation of phenacetin was determined by measuring the rate of formation of paracetamol.

The EM subjects excreted mostly metabolites of guanoxan (mean 29% of dose), whereas the PM group excreted large amounts of unchanged drug (48% of dose). The rate of formation of paracetamol was noticeably slower in the PM group, and, when analysed by minimum estimates of apparent first-order rate constants, the difference between the two phenotypes was significant. Thus the hydroxylation defect shown for debrisoquine metabolism carries over to the oxidative metabolism of phenacetin and guanoxan.

Some 5% of the population are genetically defective hydroxylators of drugs. Thus methods for evaluating the metabolism of new drugs in respect of usage and side effects need to be revised.

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BMJ/368/78

Introduction

Drug metabolism is a major determinant of the therapeutic and toxic responses to many drugs. Metabolism of a drug, however, may be highly variable, and this is often the source of major interpatient variations in response to drugs. A person's metabolic capacity depends on the interaction of various genetic, environmental, physiological, and possibly pathological factors. Some drug metabolism reactions are mainly under genetic control and exhibit polymorphism in the population. Examples are the polymorphic acetylation of various amino compounds such as sulphonamides and hydrazines. The therapeutic implications of polymorphic acetylation of drugs have been discussed.[1 2] The hydrolysis of certain ester drugs such as succinylcholine (suxamethonium) and paroxon is also mainly under genetic control and exhibits polymorphism.[3 4]

The most common metabolic reaction in the metabolism of drugs is oxidation at a carbon centre: this may be aliphatic, alicyclic, or aromatic. Despite the prevalence of carbon oxidation in drug metabolism no examples of polymorphism of such reactions have been described, though several have a genetic component, which in some cases has been quantified.[5 6] We have reported the occurrence in Caucasians of a polymorphism in respect of the carbon oxidation of the antihypertensive drug debrisoquine.[7] Some people (about 5% of the population) have a reduced ability to effect the alicyclic hydroxylation of this drug and, furthermore, are more sensitive to its hypotensive effects.[8] Apparently this 4-hydroxylation of debrisoquine is under the control of the alleles at a single autosomal locus and defective alicyclic hydroxylation is a recessive character.[7]

Because of the importance of carbon oxidation as a determinant of drug response it became necessary to know whether the alleles responsible for the polymorphic metabolism of debrisoquine also control the oxidation of other drugs. We therefore examined the oxidative metabolism of two model substrates—namely, phenacetin and guanoxan—in subjects previously phenotyped for their ability to hydroxylate debrisoquine. Phenacetin, guanoxan, and debrisoquine each undergo three different types of carbon oxidation—namely, oxidative dealkylation, aromatic hydroxylation, and alicyclic hydroxylation.

Investigation and results

GUANOXAN

Guanoxan (2-guanidinomethyl-1, 4-benzodioxan) is an antihypertensive drug chemically and pharmacologically related to debrisoquine and guanethidine. Jack *et al* showed that it undergoes oxidative metabolism to give phenolic metabolites.[9] Their study included a woman who excreted only the unchanged drug with no evidence of phenolic metabolite formation. Apparently she was not further investigated.

We studied the metabolism and elimination of guanoxan in a panel of eight volunteers, four of whom were phenotyped as extensive metabolisers (EM) and four as poor metabolisers (PM) in respect of debrisoquine 4-hydroxylation. Each received a single tablet of Envacar containing 10 mg guanoxan sulphate. Urine samples were collected hourly for up to eight hours after administration. These samples were then analysed for guanoxan and its phenolic metabolites by gas chromatography using a modification of an assay for guanidine drugs.[10]

Table I shows the pattern of urinary elimination of guanoxan by the eight volunteers. The metabolic ratio was calculated as: % dose

TABLE 1—*Hydroxylation of guanoxan and phenotypes of eight volunteers*

Subject	Sex	Hydroxylation phenotype*	Characteristic and % of dose excreted in urine in 8 hours		Metabolic ratio
			Unchanged	Hydroxylated metabolites†	
1	M	PM	31·0	4·2	7·4
2	M	PM	48·0	6·2	7·7
3	M	PM	60·0	7·6	7·9
4	F	PM	53·0	6·7	7·9
5	M	EM	1·3	36·0	0·04
6	M	EM	1·9	25·0	0·08
7	M	EM	1·2	26·0	0·05
8	M	EM	1·5	29·0	0·05

*Determined by metabolic ratio for 4-hydroxylation of debrisoquine.[7] PM = Poor metabolisers. EM = Extensive metabolisers.
†Consisted of about equal amounts of 6- and 7-hydroxyguanoxan.

excreted as unchanged guanoxan/% dose excreted as hydroxylated metabolites. There was a pronounced difference between the two phenotypes in the metabolic disposition of guanoxan. The four EM subjects excreted the drug mostly as 6- and 7-hydroxyguanoxan (mean 29·0±SD 5·0% of dose) with only small amounts of the unchanged drug (1·5±3·0%). In contrast, the PM group excreted large amounts of the unchanged drug (48·0±12·4%) with only small amounts of the metabolites (6·2±1·4%). The mean metabolic ratio for the EM phenotype was 0·06±0·02, and for the PM phenotype 7·8±0·2.

The pharmacogenetics of hydroxylation of guanoxan and debrisoquine in these subjects appeared to be the same. The results also show that the aromatic hydroxylation of guanoxan is polymorphic and that there are at least two phenotypes, one of which is characterised by a reduced ability to metabolise the drug.

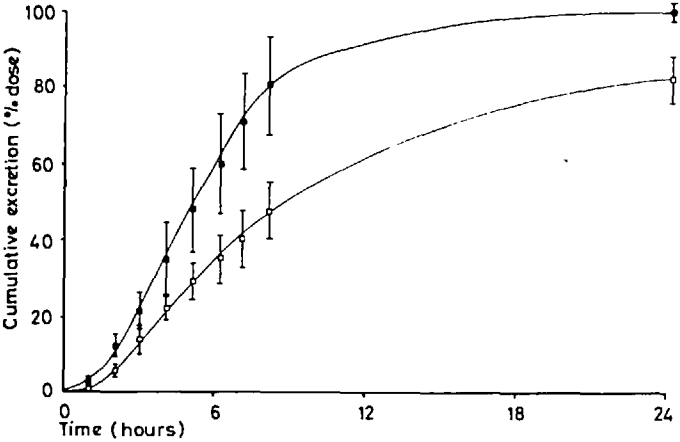
PHENACETIN

Phenacetin is metabolised along several pathways, the main one being oxidative de-ethylation to paracetamol, which is excreted in a conjugated form.[11] The rate of oxidative dealkylation of phenacetin taken by mouth was investigated in the same panel of volunteers by measuring the rate of formation of paracetamol. This was assessed by determining the rate of elimination of paracetamol conjugates by colorimetry.

Each subject took a gelatine capsule containing 500 mg phenacetin.

Urine samples were collected hourly for eight hours, an 8-24-hour collection then being made and the samples bulked. The samples were then analysed for paracetamol by colorimetry after enzymic deconjugation.[12]

The figure shows the rate of formation and elimination of paracetamol in the EM and PM subjects. The rate of formation in the PM phenotype group was noticeably slower than in the EM group.



Cumulative urinary excretion of paracetamol in extensive metabolisers (■) and poor metabolisers (□) after single oral dose of 500 mg phenacetin.

To qualify this difference minimum estimates of the apparent first-order rate constants for the formation of paracetamol ($K_{F\ paracetamol}$) were calculated from the urinary data (table II).[13,14] The mean value of the rate constant for the PM phenotype was $0{\cdot}09 \pm SD\ 0{\cdot}024$, and for the EM phenotype $0{\cdot}277 \pm 0{\cdot}064$. This difference was significant ($P < 0{\cdot}005$). Thus the rate of oxidative de-ethylation of phenacetin in the EM subjects was about three times faster than in the PM subjects.

Discussion

We have shown that the genetically determined hydroxylation polymorphism described for debrisoquine also controls the oxidative metabolism of guanoxan and phenacetin. Thus the same genetic defect appears to influence metabolic oxidative processes at such dissimilar carbon centres as the aromatic 6- and 7-carbon centres of guanoxan, the alicyclic 4-carbon centre of debrisoquine, and the aliphatic α-carbon centre of phenacetin. Hence the defect apparently influences some component common to the processes concerned in the oxidation of

several dissimilar centres. Whether the defect influences oxidation at non-carbon functions in drugs, such as nitrogen and

TABLE II—*De-ethylation of phenacetin and hydroxylation phenotypes of eight volunteers*

Subject:	1	2	3	4	5	6	7	8
Hydroxylation phenotype ..	PM	PM	PM	PM	EM	EM	EM	EM
K_F paracetamol/hour* ..	0·07	0·09	0·13	0·09	0·36	0·25	0·21	0·30

*First-order rate constant for formation of paracetamol—see text.
PM = Poor metabolisers. EM = Extensive metabolisers.

sulphur centres, is unknown and is under investigation, but it certainly seems plausible.

The main implication of our findings is that the metabolism of a whole range of drugs that are metabolised by oxidation at carbon centres may be defective in some people. The question therefore arises of the possible consequences of this. The first point to consider is the size of the group. Our studies with debrisoquine metabolism suggest that among Caucasians the prevalence of the defect is about 5%. Thus a town with a population of about 200 000 would be expected to include about 10 000 people defective in their capacity to metabolise drugs. Furthermore, a GP with a list of, say, 3000 might expect to have around 150 defective hydroxylators. Clearly the prevalence of the defect is too high to be ignored. The second question is whether the defect alters people's responsiveness to drugs. Little information on this is available at present, but existing data suggest that it does. We, for example, showed that people with an oxidation defect for debrisoquine are much more sensitive to the hypotensive effects of this drug than are extensive metabolisers.[8] Some of the poor metabolisers reported severe side effects when given therapeutic doses of the drug. Shahidi studied the pedigree of two sisters with a faulty de-ethylation of phenacetin similar to that described by us and found that they were exceptionally sensitive to the methaemoglobin-producing effects of the drug.[15] In our view many of the side effects reported for drugs will be found to have their origins in a genetically determined impaired ability to oxidise drugs.

The third point to consider is the practical implications of these findings in the development of new drugs and the use of drugs. So far as metabolic studies on man are concerned contemporary practice is singularly ineffective in detecting interindividual variations in drug metabolism. This is because the number of subjects studied is usually small (three to six) and therefore rarely permits the detection of pronounced intersubject variations. Also, current practice in using pharmacokinetic analyses of drug disposition may disguise important aspects of intersubject variation in handling drugs. This is because of the common use of hybrid pharmacokinetic variables—for example,

plasma elimination $t_{\frac{1}{2}}$, whole-body clearance rates, etc—without defining their major components—for example, metabolism—which may show pronounced interindividual variation without this necessarily being clearly evident when subjects are evaluated in terms of hybrid variables. This is exemplified in the case of debrisoquine itself. The first-order rate constant for the overall elimination of debrisoquine (K) did not differ significantly between the extensive and poor metabolising phenotypes—EM (n=4): $K=0{\cdot}20\pm0{\cdot}04$/h; PM (n=4): $K=0{\cdot}16\pm0{\cdot}13$/h—whereas the respective rates of formation of the pharmacologically inactive 4-hydroxy metabolite (K_F) did differ significantly—EM: $K_F=0{\cdot}05\pm0{\cdot}02$/h; PM: $K_F=0{\cdot}0015\pm0{\cdot}0007$/h; $P<0{\cdot}025$.

In our view the present practice for the metabolic evaluation of new drugs needs to be revised. It is important to define the metabolic variability that may be encountered for a particular drug and to evaluate its implications in terms of drug use and possible side effects. The problems of such an approach are not insuperable. We advocate that information of this sort would be best obtained by studying drug disposition and effects in panels of subjects previously phenotyped for oxidation status. Techniques for ascertaining this have been described.[7]

TPS is in receipt of an MRC studentship. AM is grateful to the EAR Educational Bureau for a scholarship. We thank the Wellcome Trust for a grant for interdisciplinary research, and Pfizer Ltd for the samples of guanoxan and metabolites.

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POLYMORPHIC HYDROXYLATION OF DEBRISOQUINE IN MAN

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Summary Debrisoquine and its primary metabolite, 4-hydroxydebrisoquine, were measured in the urine of 94 volunteers after a single oral dose of 10 mg debrisoquine. The ratio between excreted debrisoquine and its metabolite was bimorphically distributed in the study population. Family studies supported the view that alicyclic 4-hydroxylation of debrisoquine is controlled by a single autosomal gene and that a defect in this metabolic step is caused by a recessive allele.

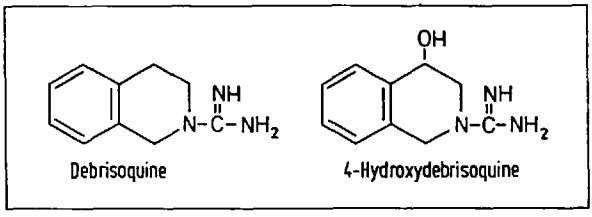

Fig. 1—Chemical structure of debrisoquine and its major metabolite, 4-hydroxydebrisoquine.

Introduction

HUMAN beings vary widely in their hypotensive response to the adrenergic-blocking drug debrisoquine ('Declinax')—a phenomenon seen in both normal volunteers[1] and hypertensive patients.[1-3] A relationship has been demonstrated between the fall in standing systolic blood-pressure and the amount of unchanged drug excreted in the urine.[1-3] A positive correlation has been found between drug concentration in plasma and the observed fall in blood-pressure.[3] The authors concluded that the availability of the unchanged drug at its site of action was the key factor in determining the response of an individual to debrisoquine.[3] Since the drug is well

absorbed,[1] such differences in availability must reflect an interindividual variation in its rate of metabolism. We have shown[4] that some people have a defective ability to convert debrisoquine to its major metabolite, 4-hydroxydebrisoquine (fig. 1), the excretion of the less prominent phenolic metabolites 5-,6-,7-, and 8-hydroxydebrisoquine showing no such variation.

We report here that the alicyclic 4-hydroxylation of debrisoquine, the process which removes most of the drug from the systemic circulation, is expressed as two apparent phenotypes which we have designated extensive metaboliser (E.M.) and non-metaboliser (N.M.). Examination of the families of 3 N.M. subjects suggests that defective 4-hydroxylation of debrisoquine is an inherited trait.

Population Study

94 healthy staff and students from St. Mary's Hospital Medical School each took debrisoquine orally (declinax, 10 mg

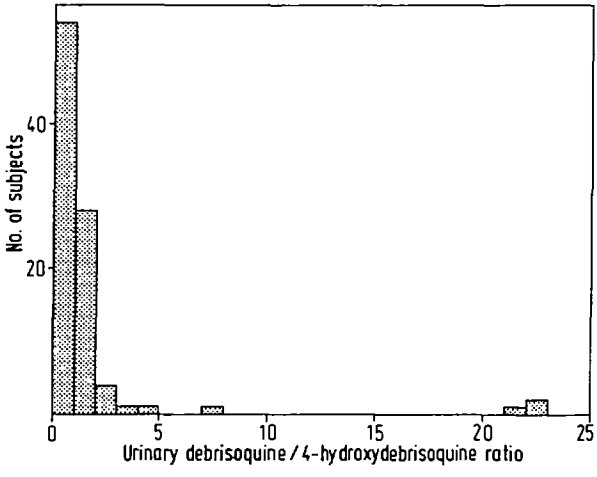

Fig. 2—Frequency distribution of the ratio, urinary debrisoquine/4-hydroxydebrisoquine, in 94 human volunteers.

tab.) in the morning after voiding their bladders. Urine was collected up to 8 hours, during which time subjects were allowed unlimited food and drink. Neither other medicaments nor alcohol were allowed during the trial. Urines were stored at −20°C and then analysed by electron-capture gas-chromatography for debrisoquine and 4-hydroxydebrisoquine after their derivatisation with hexafluoroacetylacetone.[5]

Family Studies

Family A were husband and wife with three sons aged 17–21, one of whom had been designated N.M. in the population study. Family B comprised a widowed grandmother with a daughter and two sons, one of whom appeared as N.M. phenotype and had a wife and three sons aged 9–14. Family

METABOLIC RATIOS (DUPLICATE VALUES)* IN 6 EXTENSIVE METABOLISERS AND THE 3 NON-METABOLISERS

Subject no.	% Dose excreted in 8 h as		Metabolic ratio
	Debrisoquine	4-Hydroxydebrisoquine	
Extensive metabolisers:			
1	15·7	25·8	0·6
	41·3	55·8	0·7
2	16·6	30·5	0·5
	32·3	45·0	0·7
3	28·9	29·5	1·0
	20·0	23·8	0·8
4	45·1	45·4	1·0
	33·4	46·3	0·7
5	28·6	18·7	1·5
	10·4	8·1	1·3
6	24·8	48·2	0·5
	11·2	22·4	0·5
Non-metabolisers:			
7	42·7	2·0	21·4
	39·6	2·0	19·8
8	18·1	0·8	22·6
	59·7	3·1	19·3
9	36·7	1·6	22·9
	18·0	0·9	20·0
	56·4	2·7	20·9

*Triplicate values shown for subject 9.

C were two sisters (one known to be N.M.) and their father. The mother was unable to take part in the study because she was receiving drug treatment for migraine. All subjects over 14 years were given the drug as described previously. The two children aged 9 and 12 were given 5 mg and 7·5 mg respectively. Urines were collected and analysed for drug and metabolite as described above.

Results and Discussion

Fig. 2 shows the frequency distribution of the following ratio:

$$\frac{\%\text{ dose excreted in 8 h as unchanged debrisoquine}}{\%\text{ dose excreted in 8 h as 4-hydroxydebrisoquine}}$$

This histogram reveals two distinct populations, the E.M. group (91 subjects) showing a metabolic ratio of 0–8 and the N.M. group (3 subjects) with a ratio above 21. No subject had a ratio in the range 9–20. 6 E.M. and the 3 N.M. subjects showed no change in the metabolic ratio when rechallenged with debrisoquine (see table), showing that this ratio is an individual characteristic. Such a bimodal distribution indicates a single-gene mechanism controlling the metabolism of the drug.[6] The relatively uncommon N.M. phenotype is assumed to be due to homozygosity of an autosomal recessive allele (nn). (A similar mechanism produces polymorphic acetylation of isoniazid.[7]) This assumption that defective debrisoquine metabolism is an autosomal recessive trait seems justified by the fact that no subjects with a metabolic ratio in the intermediate range (9–20) were detected in the population study. The E.M. phenotype could be either the dominant homozygote (ee) or the heterozygote (en).

The frequency of the 4-hydroxylation defect (nn) in this population was about 3% (3 of 94). Neither sex, race, nor smoking habits were found to affect this aspect of debrisoquine metabolism.

Investigations of the families of the 3 N.M. subjects confirmed our inference that incomplete alicyclic 4-hydroxylation of debrisoquine is an autosomal recessive trait. Fig. 3 shows the metabolic ratios for the 3 families and the probable genotypes derived from the available data. In family A father and two sons were N.M. (nn), and the mother and third son were E.M. Since the mother must carry the n allele for two sons to be nn and must also carry the e allele for the third son and herself to be extensive metabolisers, then she herself can only be heterozygous (en). The third son must also be heterozygous, having inherited the n allele from his father. In family B we have no direct knowledge of the grandfather's metabolic status, but information about his widow and children indicates that he was a non-metaboliser (nn) or a heterozygous extensive metaboliser (en). All three grandsons are extensive metabolisers, and their mother could therefore be either ee or en. Likewise, family C was incomplete, and it would have been interesting to study the mother, to confirm that she is a heterozygote (en).

The observed defective alicyclic 4-hydroxylation of the antihypertensive drug debrisoquine was thus found to have a genetic basis. It is suggested that this meta-

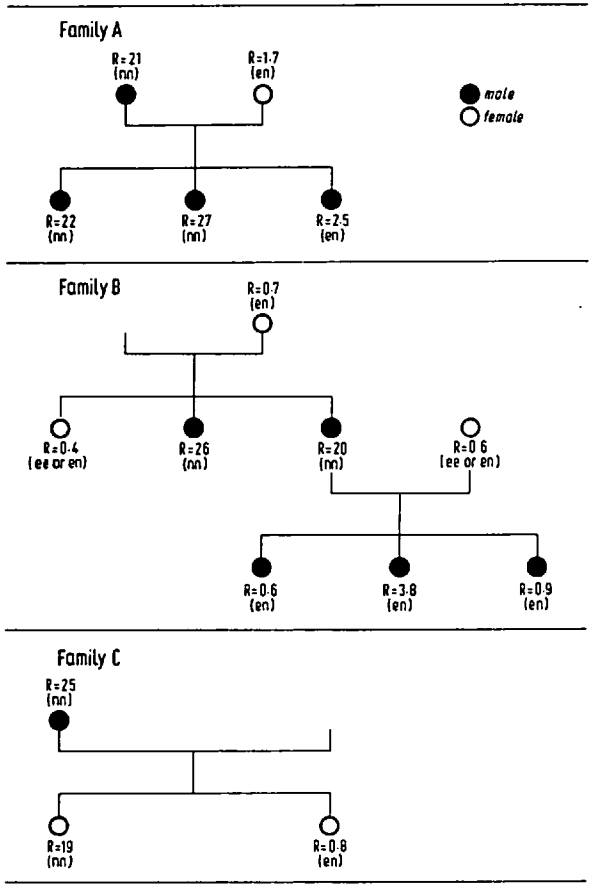

Fig. 3—Metabolic ratios (R) in 3 families and possible genotypes.
e=normal allele, n=mutant gene (probably recessive) for defective 4-hydroxylation of debrisoquine.

bolic reaction, which removes the pharmacologically active agent from the circulation, is under the control of a single autosomal gene, and that defective 4-hydroxylation is caused by a recessive allele. The alicyclic 4-hydroxylation of debrisoquine thus exhibits genetic polymorphism.

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