THE BIOCHEMICAL AND CLINICAL PHARMACOLOGY OF DEBRISOQUINE

by

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To My Mother

and

In Memory of My Father

Abstract

1, The nature and drug therapy of hypertension has been reviewed.

2. The metabolic fate of the antihypertensive drug, debrisoquine, has been studied in rat and man. Oral administration of |^{14}C|debrisoquine was found to result in its rapid excretion in the first day urine of both species (67% in rat; 75% in man). A small proportion of the radioactivity was recovered in the faeces (11-12% in rat; 5-8% in man) over a period of one to three days. The nature of the urinary excretion products was established by reverse isotope dilution and chromatographic and mass spectroscopic techniques.

The drug was found to be extensively metabolized by oxidation in the rat. The metabolites arising from oxidation of the aromatic and heterocyclic rings were identified as 4-, 5-, 6-, 7- and 8- hydroxy-debrisoquine and di-hydroxy-debrisoquine. Two acidic metabolites, resulting from cleavage of the heterocyclic ring were characterized as 2-(guanidinoethyl)-benzoic acid and 2-(guanidinomethyl)-phenylacetic acid.

The metabolic fate of debrisoquine in man was found to be very similar to that in the rat, however, no dihydroxylated metabolite could be detected in the urine of those subjects studied. These metabolic studies revealed the existance of marked inter-individual differences in the extent of oxidative metabolism of the drug.

3. The therapeutic implications of debrisoquine metabolism were investigated in clinical studies with normotensive and hypertensive individuals. It appeared that debrisoquine

itself, and not one of its metabolites, is the hypotensive agent, and that the rate at which debrisoquine is metabolized is a major determinant of responsiveness to the drug.

4. An investigation was undertaken to observe the hypotensive effect of concommitant debrisoquine and hydrochlorothiazide treatment in normotensive subjects and some preliminary results are reported.

Contents

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CHAPTER ONE

INTRODUCTION

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Introductory Remarks

Hypertension is one of the most widespread diseases, afflicting people of all age groups, ethnic origins and socio-economic classes. The pathogenic basis for the elevated blood pressure is unknown in the majority of cases, and is thereby termed "Essential Hypertension". Being basically asymptomatic, it is often neglected, resulting in complications including congestive heart failure, cerebro-vascular disease and renal failure. Epidemiological studies have shown that treatment reduces the high incidence of morbidity and mortality associated with essential hypertension. The development of drugs which act specifically upon various systems to lower the elevated arterial pressure constitute the main form of clinical management. However, these agents provide only palliative therapy and consequently the majority of patients require treatment for the duration of their life-time. Therefore, the necessity for a thorough understanding of the biochemical and clinical pharmacology of these agents is evident.

The introduction to this thesis concerns hypertension, and its drug therapy with emphasis on the use of debrisoquine. However, a more profound understanding of the subject requires a consideration of the various factors involved in the normal physiological control of blood pressure.

Control of Blood Pressure

Arterial blood pressure is regulated by multiple interrelated homeostatic controls including the autonomic nervous system, renal and humoral mechanisms, as well as local myogenic factors. In the discussion of both the pathogenesis and the rationale of treating hypertension, it is important to understand the factors that control the determinants of blood pressure, namely cardiac output and peripheral vascular resistance.

1. Intrinsic Control Mechanism

Local control of blood vessels results partially from inherent myogenic activity, which is responsible for the basal vascular tone in so-called single-unit smooth muscles of precapillary resistance vessels. Bayliss (1902) demonstrated the presence of a positive feedback mechanism, whereby vascular distention brought about by an elevation of arterial pressure augments the tone of vascular smooth muscle. Recent studies on isolated smooth muscle strips, have shown that this is not per se a function of smooth muscle (Sparks and Bohr, 1962). However, the degree of initial stretch very greatly increases the tension developed when either pressor agents (i.e. adrenaline) or electrical stimulation is applied to the muscle. Since normally there is a physiological discharge of vasomotor nerves or some stimulation from pressor agents in the blood, it would appear that the degree of stretch of the vessel walls in the circulation (dependant on transmural blood pressure) is an important factor determining both the tone and the reactivity of their smooth muscle. Suggestive evidence is also accumulating that the level of blood Na^+ is an important controlling

factor of the tone and reactivity of blood vessels and that the contraction of this muscle is accompanied by large transfers of the ion between blood and vascular smooth muscle (Friedman et al., 1963).

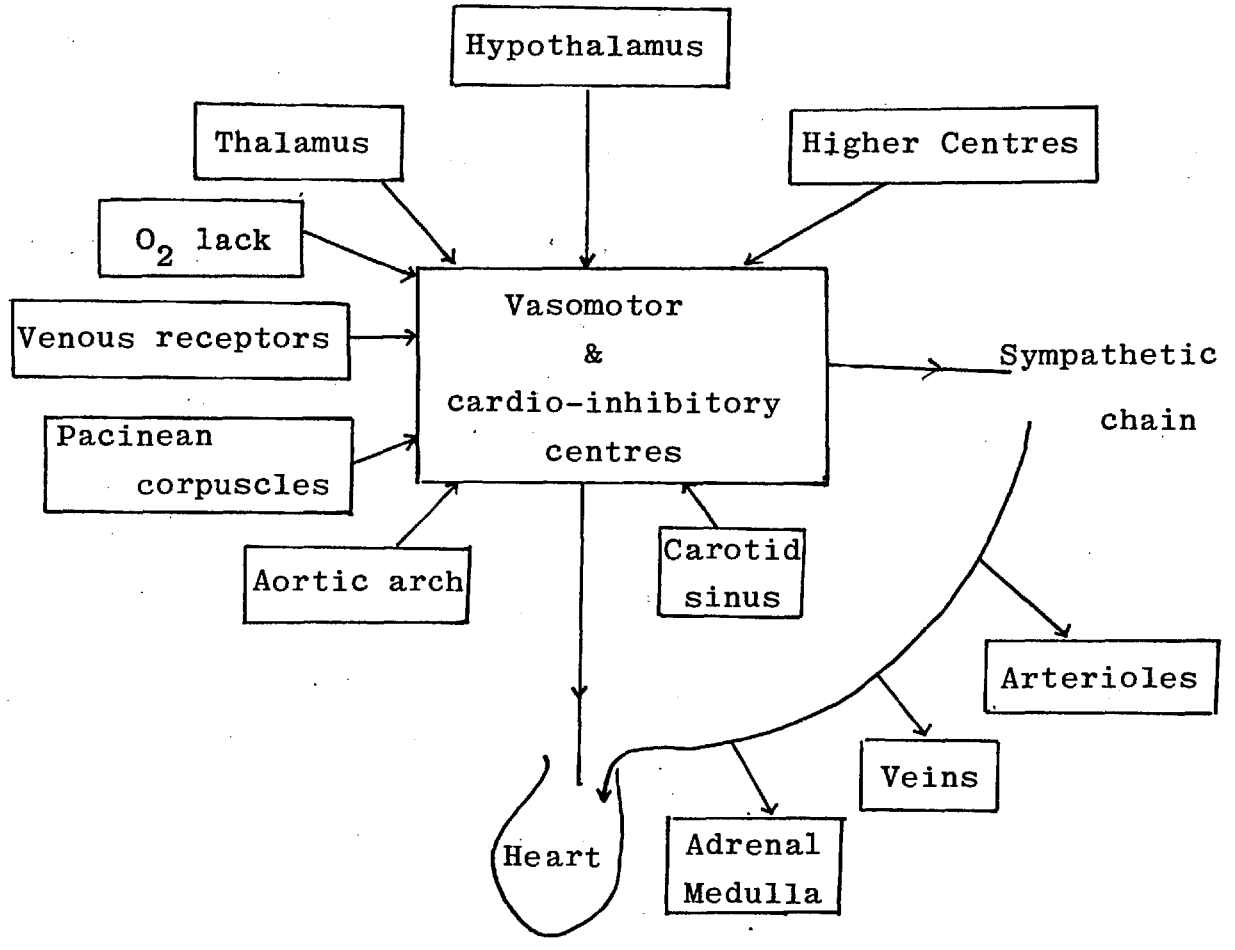
Local autoregulation is achieved, however, by a balance between this positive feedback mechanism and the vasodilator action on smooth muscle of metabolic by-products resulting from tissue activity. The precise mechanism responsible for vasodilatation in metabolically-active tissue is still unknown. Various suggestions have been made, such as release from the muscles of adenosine triphosphate, acetylcholine, histamine, lactic acid and carbon dioxide as well as hydrogen ion concentration (Brobeck, 1973). Low oxygen tension has been postulated as being the dilating factor (Guyton et al., 1973) Oxygen saturation of venous and capillary blood is known to fall drastically during exercise and hence it has been suggested that during muscular activity, skeletal muscles compete with vascular smooth muscles for oxygen, consequently resulting in vasodilatation of the latter.

2. Neural Regulatory Mechanisms

Neural mechanisms provide rapid, almost instantaneous regulation of the circulation. All levels of the central nervous system, from the cortex to the spinal cord contribute to the control of the mammalian circulation. However, the main loci of autonomic reflex control are situated in the bulbopontine region of the brain. Studies involving stimulation or ablation of regions in the floor of the IVth ventricle led to the discovery of the "vasomotor centre", which contains specific "pressor" and "depressor" regions (Alexander, 1946). Sympathetic fibres that produce vaso-

constriction, cardio-acceleration and myocardial stimulation arise from the rostral and lateral portions of the vasomotor centre. The medial and slightly more caudal parts transmit inhibitory impulses to the lateral. The dorsal motor nucleus of the vagus, the "cardio-inhibitory centre" is the other important regulatory region and lies lateral to the medullary reticular neurones which modify spinal sympathetic discharge. The dorsal motor vagal nuclear cells are not tonically active except under afferent innervation, since sino-aortic denervation abolishes vagal tone. In this respect, the vagal nuclear cells differ from those of the vasomotor centre whose activity is inherently tonic and is increased by sino-aortic deafferention.

A variety of active afferent fibres act on the vasomotor and cardio-inhibitory centres. These are portrayed schematically in Fig. 1.1.



There is no doubt that the most important autonomic control of the circulation is the carotid sinus aortic arch reflex (Heymans and Neil, 1958). Studies involving cross-circulation techniques (Heymans and De Vleeschhouwer, 1950), led to the clear localization of receptor areas in the aorta and carotid sinus. On the outside curvature of these regions are present the baroreceptors which do not respond directly to pressure but to deformation and stretch, that occur as a result of arterial wall distention due to an increase in blood pressure (Langren, 1952). However, the sino-aortic receptors are by no means the only sensory area involved in the reflex. There are a number of receptors notably in the pacinean corpuscles distributed throughout the visceral circulation, that control local circulation rather than central blood pressure (Heymans and Neil, 1958). Located in the angle of the sinus bifurcation and on the inside curve of the aorta, are the chemoreceptors. These are primarily concerned with the homeostasis of blood gases by control of the respiration. However, strong stimulation of the chemoreceptors has an effect on the circulation.which is probably mediated through connections between the respiratory centre and the vasomotor centre. Both baroreceptors and chemoreceptors of the carotid sinus send impulses to the bulbopintine region via the carotid sinus to join the glossopharyngeal nerve, whereas the aortic receptors excite the depressor brances of the vagus.

Similarly, the hypothalamus can produce powerful excitatory or inhibitory effects on the vasomotor and cardioinhibitory centres. Also portions of the cerebral cortex, such as the motor cortex, and, most notably, the cingulate gyrus, can affect these regions. Emotions can affect the vasomotor centre, however details of the neurophysiology of emotions are not known.

The efferent responses of the central regulatory areas are multiple, but can conveniently be divided into two categories: the cardiac and the peripheral effects (see Fig. 1.1). The cardiac effector mechanism is mainly depressor through the vagal fibres to the heart, in response to the baroreceptor reflex. However, it is also depressor through the inhibition of tonic discharge of the vasoconstrictor nerves in the depressor portion of the vasomotor centre. The mechanism whereby impulses in the cardiac vagal nerves decrease the heart rate is of great historical importance, for it represents the discovery of the first neurotransmitter, acetylcholine (Loewi, 1921). Acetylcholine is liberated at the endings of parasympathetic postganglionic nerves (like the vagus) and is the transmitter in autonomic ganglia.

The non-cardiac effector mechanisms affect the activity of the sympathetic outflow (see Fig. 1.1). This regulates catecholamine secretion from the adrenal medulla and renin release from the juxtaglomerular cells of the kidney (see p. Sympathetic fibres may also increase peripheral vascular resistance in response to a threatened fall in blood pressure. Neural supply of the peripheral vessels consists of constrictor and dilator nerve fibres. All known vasoconstrictor nerves belong to the sympathetic division of the autonomic nervous system and act by releasing noradrenaline at their postsynaptic terminals. The density of sympathetic constrictor innervation varies widely in different parts of the circulation although virtually all arteries and veins, of whatever size, receive some fibres. Small arteries and arterioles in skin, skeletal muscle and the splanchnic bed for example, receive a relatively large number of these fibres and the adrenergic supply of

veins is less dense than that of the corresponding arteries (Ehinger et al., 1966). Regional differences range from a high concentration of fibres in the vessels of the skin to a very sparse innervation in the cerebral vessels. The range of vasomotor control, i.e. the difference between maximal constriction and maximal dilatation, is in most instances directly correlated with the density of innervation. True vasodilator fibres, in which nerve impulses release a transmitter substance that causes relaxation of vascular smooth muscle, are fewer in number and more limited in their distribution than are the constrictors. Nerves of this kind exist in both the sympathetic and parasympathetic systems, and transmission from postganglionic fibre to smooth muscle is cholinergic in both cases. The principal distribution of sympathetic vasodilator fibres is to the vessels of skeletal muscle.

Central neurotransmitters in blood pressure control

Noradrenaline was detected in the mammalian brain by Von Euler as early as 1946, but it was thought to be associated with sympathetic nerves to the cerebral blood vessels. Only since the development of histochemical fluorescence techniques, has it been possible to map catecholamine-containing nerve terminals in the brain with some precision (Falck et al., 1962). Noradrenaline innervation has been found in the Nucleus Tractus Solitarius (Fuxe, 1965), which is one of the primary relays of the baroreceptor fibres (see Fig. 1.2). Firm evidence for the involvement of a central catecholamine mechanism in cardiovascular control was provided by the use of amino acid precursors of catecholamines in combination with selective inhibitors of various steps in their biosynthesis (Henning, 1973). When the enzyme dopa decarboxylase in peripheral tissue is inhibited,

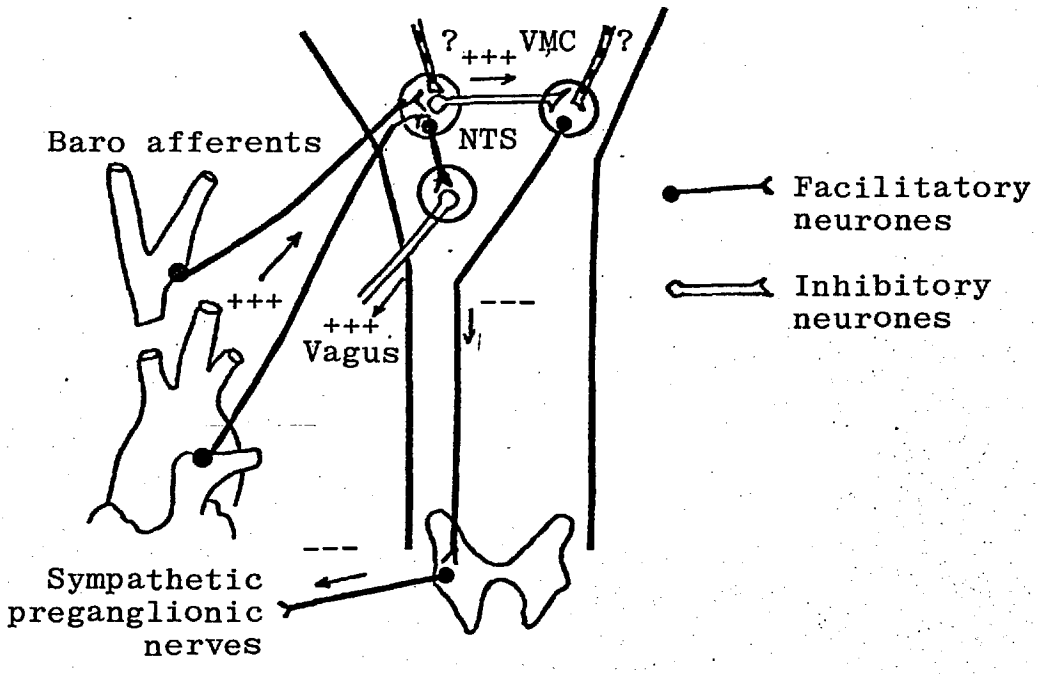


Figure 1.2. Schematic outline of arterial baroreceptor reflex arc showing afferents arising from aortic arch and carotid sinus and terminating in the nucleus of the tractus solitarius in the brain stem. (After Chalmers, 1975).

L-dopa, (L-3,4-dihydroxyphenylalanine) produces a hypotensive response, accompanied by the accumulation of dopamine in the brain. This is diminished and the hypotensive effect is abolished when dopa decarboxylase is inhibited both centrally and peripherally, demonstrating that the lowering of blood pressure is mediated by an action of dopamine or noradrenaline (or both), formed from L-dopa in the central nervous system (Henning and Rubenson, 1970a,b). These observations lead to the conclusion that activation of central noradrenaline receptor mechanisms mediates a decrease in arterial blood pressure. The nature of these receptors has not been established, but there is indirect evidence to suggest that they conform to

the α-adrenergic type in peripheral tissues; the hypotensive action of L-dopa is antagonized by the α-blockers piperoxan and yohimbine (Schmitt et al., 1972). Further evidence for an α-adrenergic nature of these receptors comes from studies with clonidine (see p.57). An increasing body of evidence points to central components being implicated in the anti-hypertensive action of α-methyldopa and β-adrenergic receptor blocking agents (see p.49 & 54).In summary therefore, it is widely believed that these drugs, through their central α-adrenoreceptor agonistic effect, inhibit central discharge to the sympathetic nerve supply of the heart and blood vessels (van Zweiten, 1973) (see Fig. 1.2).

3. Renal Control Mechanism

The renin-angiotensin-aldosterone system, a part of the inter-related mechanism regulating body water and electrolyte balance is involved in the maintenance of circulatory homeostasis.

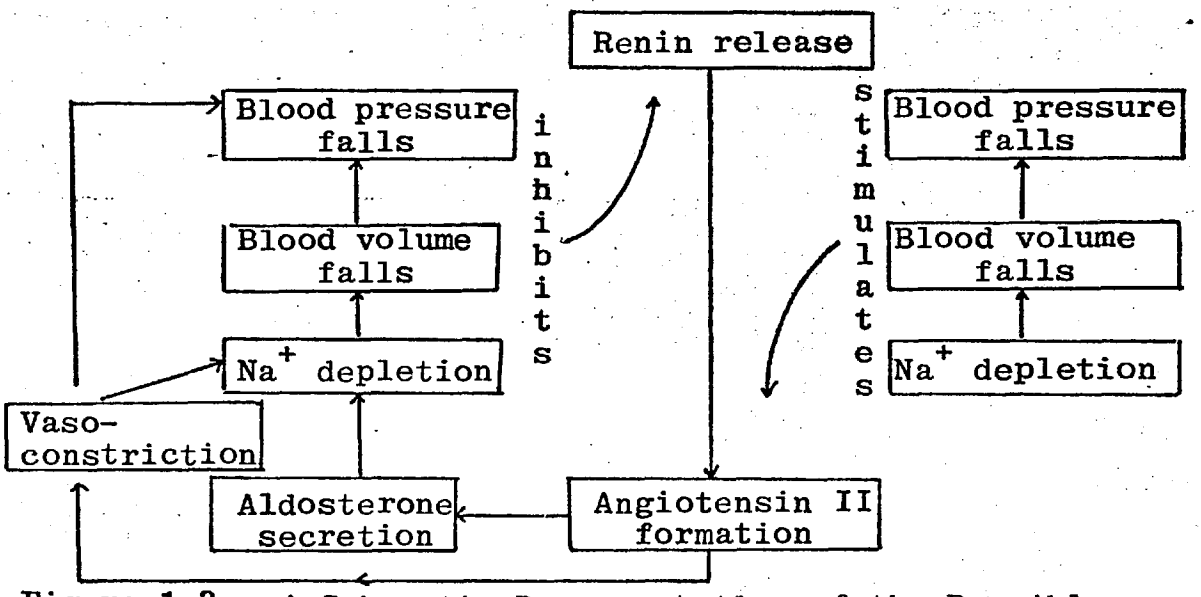


Figure 1.3. A Schematic Representation of the Possible Homeostatic Role of the Renin-Angiotensin-Aldosterone System.

(after Douglas, 1970)

The kidney enzyme renin, is located within granules contained in the juxtaglomerular cells situated predominantly at the site where the afferent arteriole breaks up into the glomerular capillaries (Goormaghtigh, 1945). Renin release is regulated by factors that tend to lower or raise renal perfusion pressure, blood volume and plasma sodium concentration. However, the central nervous system also governs renin release. Studies with isolated perfused kidneys indicate the involvement of β-receptors, since renin release may be stimulated by β-agonists (isoprenaline) and inhibited by β-blocking agents such as propranolol (Peart, 1975). Renin acts on the plasma α-globulin, angiotensinogen, to yield the relatively inactive decapeptide, angiotensin I. This substance is transformed by "converting enzyme" to a highly active octapeptide angiotensin II. Being the most powerful pressor agent known, angiotensin II has immediate peripheral vasoconstrictor effects. There is also evidence to suggest that it can also elicit a systemic rise in blood pressure by central stimulation (Ferrario et al., 1972). The octapeptide is also implicated in aldosterone secretion from the zona glomerulosa of the adrenal cortex. Aldosterone secretion results in sodium retention, expansion of the intravascular volume and increased blood pressure. Thus both the acute and chronic effects of angiotensin II tend to correct the original stimulus to renin release, decreased renal perfusion. The normally functioning renin-angiotensin-aldosterone system is a classical example of a negative feedback regulatory system.

4. The role of circulating vasoactive agents

a) Adrenaline and noradrenaline. These vasoactive catecholamines occupy a major role in vascular reactions.

The central nervous system controls delivery of catecholamines to vessels by way of the bloodstream as well as by way of the sympathetic nerve terminals. However, adrenaline secreted by the adrenal medulla appears to be a much less powerful vascular stimulant than are the impulses in sympathetic vasoconstrictor nerves, when the two are compared at their respective physiological levels (Celander, 1954). Blood vessels differ in their response to catecholamines not merely in sensitivity but in whether they constrict or dilate. A semblance of order has been imposed on a chaos of such observations by Ahlquist's concept of smooth muscle receptors (1948). Receptors that respond to adrenaline and noradrenaline are termed "adrenergic" and are of at least two different pharmacological types, designated "alpha" and "beta". This classification is based entirely on functional characteristics and no structures corresponding to these receptors have been identified morphologically. Experimentally, the receptors are classified by specific agonists and antagonists of the adrenergic system. Vascular smooth muscle that is endowed with α-receptors contracts when these receptors are activated. Smooth muscle cells that possess β-receptors relax on activation of their receptors. In many blood vessels, however, equal distribution of both receptor types are found and here, differences in sensitivity become important. Nevertheless, whatever the basic mechanism of catecholamine action may be, the net effect of intravenously injected noradrenaline is an increase in vascular resistance. The overall response to adrenaline includes its action on the heart as well as blood vessels, the cardiac effects being mediated entirely through

β-receptors. Activation of these receptors enhance myocardial contractility as well as increase heart rate and the velocity of impulse conduction.

b) The plasma kallikrein-kinin system. The occurrence of an endogenous hypotensive substance in man was observed as early as 1908 by Abeloud and Bardier. This was later identified as bradykinin, the active component of the plasma kallikrein-kinin system (Rocha e Silva et al., 1949). Figure 1.4 outlines the postulated sequential interactions that result in bradykinin formation and destruction.

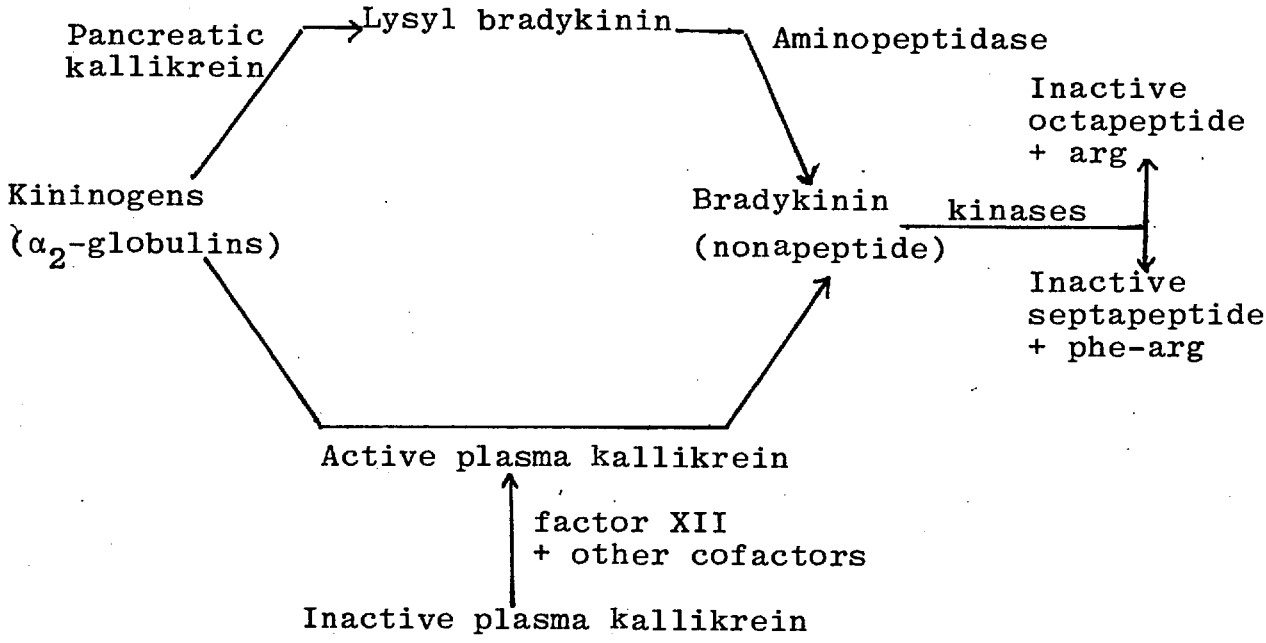


Figure 1.4. Plasma Kallikrein-Kinin System

Although the kinins are amongst the most potent vasodilators, their physiological role in the maintenance of circulatory homeostasis is unknown.

Hypertension Classification, Epidemiology and Prognosis

Classification

The classification of hypertension by Pickering (1968) includes two dissimilar groups (see Table 1.1).

Secondary hypertension describes raised arterial pressure that is merely a manifestation of specific diseases characterized by anatomical and chemical lesions; such disorders include renal and endocrinological diseases as well as pregnancy toxaemia (see Table 1.1). Cure of the underlying condition should cure the hypertension unless irreversible cardiovascular changes are present. The incidence of secondary hypertension is very small (5-10%), and will be ignored for the purposes of this thesis.

In "essential" or "primary" hypertension, the raised pressure and its cardiovascular consequences constitute the disease. This group accounts for over 90% of all cases of hypertension (Jagger and Braunwald, 1974). Since the pathophysiological basis of the disease is unknown, the definition of hypertension has been a subject of conjecture for some time. However, the current belief is that hypertension is an exaggeration of the tendency for blood pressure to rise with age (Brown et al., 1976) and according to Pickering (1968), it is a unique disease distinguished by a quantitative rather than qualitative deviation from the norm.

In the assessment of essential hypertension, it must be remembered that arterial pressure is a continuous variable and that there is no natural division into normotension and hypertension. The dividing line adopted in general practice is quite arbitrary.

Table 1.1. Classification of hypertensions in which both systolic and diastolic pressures are raised. (after Pickering, 1968).

A. Classification of hypertension by kind

I. Secondary hypertension

1. Diseases of the kidney and urinary tract

a. Type I nephritis; in all stages
b. Type II nephritis; in the terminal stage
c. Chronic pyelonephritis, unilateral or bilateral
d. Disease of the renal arteries
e. Polycystic kidney
f. Other congenital disorders of the kidney
g. Renal stone and other lesions destroying the urinary tract.
h. Interstitial nephritis due to analgesics, gout, hypercalcaemia, etc.
i. Diabetes
j. Connective tissue disease, i.e. polyarteritis nodosa, disseminated lupus erythematosus and schleroderma
k. Certain tumours
l. Radiation nephritis
m. Amyloid contracted kidney
n. Hereditary nephritis

2. Contraction of the aorta

3. Phaeochromocytoma

4. Cushing's syndrome

5. Primary aldosteronism

6. Pre-eclamptic toxaemia of pregnancy

7. Post-toxaemic hypertensions

8. Miscellaneous conditions affecting the nervous system.

II. Essential hypertension

B) Classification of hypertension by degree

i. Benign phase

ii. Malignant phase

Epidemiological considerations

Hypertension is generally believed to be one of the most widespread diseases. Determinations of the prevalence of hypertension in populations, depend on many factors of which three are of overwhelming importance: the definition of hypertension used, the circumstances of blood pressure measurement and whether a single value or the mean of several is used, and thirdly, the age, sex and ethnic structure of the population.

For the purpose of obtaining comparable epidemiological data, the World Health Organisation (1962) proposed that all arterial pressure values below 140/90 be considered normal and only those equal to or above 160/95 be taken as hypertensive. However, even those investigators complying with the W.H.O. stipulation, have obtained discrepant results, probably due to other methodological differences. The figures obtained for the occurrence of hypertension in the United States of America vary between 13 and 36% (see Table 1.2).

Sex differences in the distribution of hypertension are also found. Women generally show a lower incidence of the disease than men until middle age, after which the prevalence of hypertension is greater in women (United States National Health Survey, 1964; see Table 1.3).

Epidemiological surveys involving people of different ethnic origins, show a greater predominance of hypertension in American negro populations (United States National Health Survey, 1964; see Table 1.4). A higher mortality is also associated wtih the disease in black people (Paul and Ostfeld, 1965).

Table 1.2. Prevalence of arterial hypertension in the U.S.A.: results obtained from various epidemiological studies (after Jouve and Tassy, 1974).

Survey	% of population
Baldwin County	15.5
National Health Survey	15.2
Alameda County	13.0
Freis et al.	16.0
MacMahon et al.	36.4
Finnerty et al.	24.0
Tecumseh (men)	30.0
Tecumseh (women)	34.0

Table 1.3. Sex differences in the prevalence of hypertension statistics from the United States National Health Survey, 1964

Age	% Men	% Women
18-24 yrs	1.6	1.1
25-34	4.8	3.1
35-44	13.4	8.4
45-54	18.9	18.2
55-64	23.3	31.8
65-74	30.3	49.9
75-79	41.6	45.9

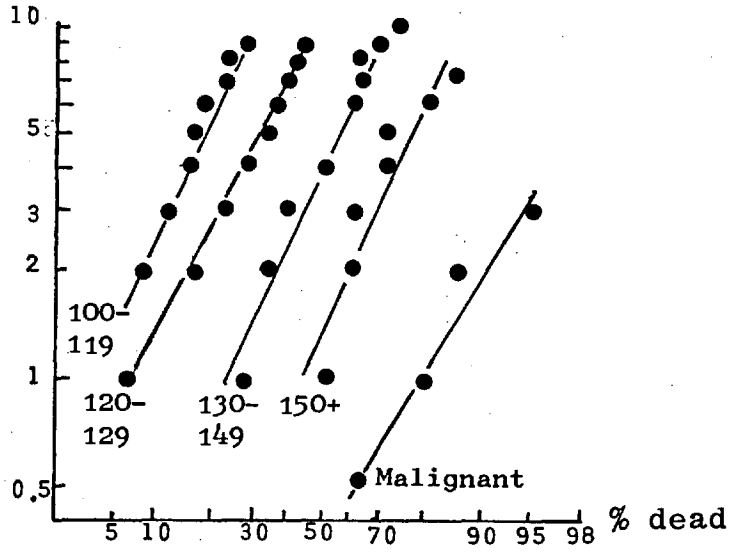
Table 1.4. Ethnic Differences in the Prevalence of Hypertension: Statistics from the United States National Health Survey, 1964.

	Percentage Population	
Age	Caucasian	Negro
Females		
25-34	3.7	12.5
35-44	11.8	26.5
45-54	17.3	30.8
55-64	21.4	44.6
Males		
25-34	2.3	8.5
35-44	6.2	25.6
45-54	15.5	41.9
55-64	31.0	41.0

The consequences of hypertension

Evidence from clinical and epidemiological data, together with actuaries data, has convinced most investigators in this field that raised blood pressure carries an increased risk of cardiovascular morbidity and mortality. Elevated arterial pressure, basically symptomless, leads to conditions such as cerebral haemorrhage, cerebral infarction, myocardial infarction, dissecting aneurysm and renal failure.

Figure 1.5 shows the relationship between arterial pressure at first examination in the clinic and subsequent mortality in Leishman's (1959) untreated patients with essential hypertension.



Diast B.P.	100-119	120-129	130-149	150+	Malignant
Age	47.0	49.5	47.2	49.8	44.3
No.	87	45	38	21	20

Figure 1.5. Data from Leishman's study (1959) shows survival rate estimated by life tables in patients arranged according to their diastolic blood pressure. % dead plotted on probability scale. The age is mean age at which patients were first seen. (after Pickering et al., 1961).

The relationship is quantitative from the lowest to the highest pressure. In the past, for reasons which may have been ill-founded, systolic blood pressure has not been accepted as a predictor of the risk of cardiovascular disease. However, mortality data from the Framingham study (Kannel, 1974) indicated that systolic blood pressure is at least as reliable as diastolic pressure in the prediction of death and of specific vascular events (see Fig. 1.6).

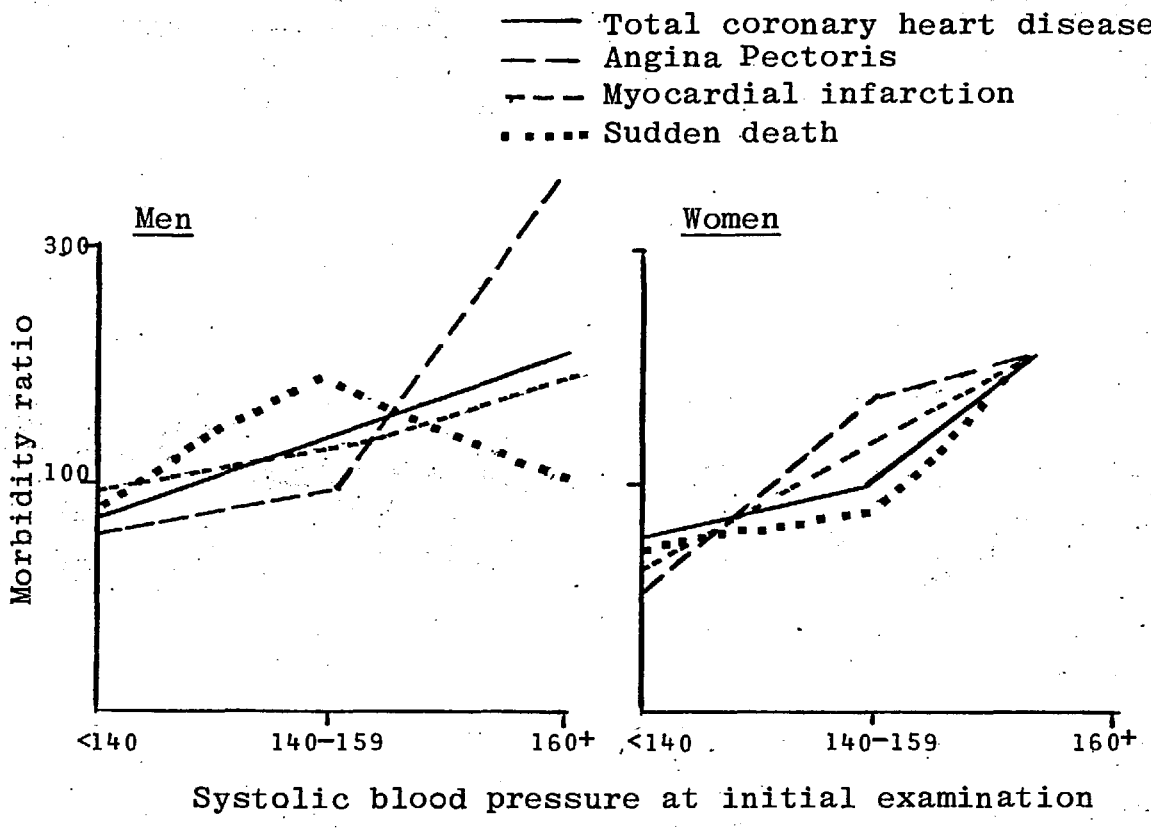


Figure 1.6. Total Risk of Developing Coronary Heart Disease related to Different Levels of Systolic Blood Pressure over an 18 year Follow-Up Period. (after Kannel, 1974).

Low risk subjects in the Framingham study were non-smokers with a low serum cholesterol, normal glucose tolerance and absence of left ventricular hypertrophy on electrocardiogram. In both sexes, the probability of coronary heart disease developing is strongly related to increased levels of blood pressure.

Aetiology of Hypertension

The primary disturbance which promotes hypertension is still unknown. Although blood pressure is determined by both cardiac output and peripheral vascular resistance, it is generally believed that the major haemodynamic alteration in hypertension is an increase in vascular resistance. Several of the aetiological factors which have been proposed as being responsible for the elevation, will be summarized in this section. However, it is unrealistic to assume that any one mechanism may be solely responsible for the pathogenesis of this complex disease.

1. Renal Factors

a) The renin-angiotensin-aldosterone system

Goldblatt (1934) first induced hypertension in laboratory animals by restriction of renal blood flow. Since then, vasoconstrictor substances from the kidney have been suspected of being implicated in initiating the elevation of vascular resistance. Studies in this field have been limited owing to the lack of a specific renin assay, since it has been impossible to isolate the enzyme in pure form so far. Recently, several investigators have employed specific angiotensin receptor blocking agents such as 1-Sar-8-Ala angiotensin II (Pals et al., 1971) and immunological techniques (Christlieb et al., 1969; Bing and Poulson, 1970) to render animals insensitive to the vasoconstrictor. Results obtained have demonstrated that the renin-angiotensin-aldosterone system is involved in some but not all forms of experimental hypertension (Christlieb et al., 1969). Animals rendered hypertensive by restricting blood flow to one kidney and leaving the other kidney intact (known as"two-kidney"

Goldblatt), generally exhibit a lowered blood pressure to angiotensin II blockade (Brunner et al., 1971). This is particularly true during the first few weeks after onset of the hypertensive process. Once the hypertension is established however, blood pressure response to angiotensin II antagonism is much less consistent (Bumpus et al., 1973). In one-kidney Goldblatt animals, rendered hypertensive by restriction of the left renal artery and removal of the contralateral kidney, the induced reduction of blood pressure by blockade is insignificant (Brunner et al., 1971).

In human subjects, estimations of plasma renin activity also show wide variations associated with different forms of renal disease. Several investigators have obtained different results in patients with renal artery stenosis, and here methodology may be important (Mulrow, 1964; Brown et al., 1965; Bath et al., 1968). However, plasma renin activity measurements by immunological estimations of angiotensin I and II, have shown elevated levels of both polypeptides (Boyd et al., 1969). These results were independant of the presence of malignant hypertension, a factor which is known to increase both plasma renin activity and angiotensin (Brown et al., 1966; Boyd et al., 1967). In patients with essential hypertension, there is also a wide scatter of both renin and angiotensin levels (Brown et al., 1965) and there appears to be no significant relationship between diastolic blood pressure and plasma renin concentration (Louis et al., 1974). There is, in fact, a proportion of hypertensive patients having renin levels well below the normal range. This subgroup is reported to show a lower incidence of cardiovascular complications as compared with the rest of the hypertensive population (Brunner et al.,

1972). However, the design of these studies and composition of the patients' samples have been criticized and further studies are required to evaluate this issue.

b) Renal prohypertensive factor

The kidney is thought to possess another prohypertensive mechanism, besides the renin-angiotensin system. Grollman (1970) has described a substance, designated as nephrotensin, which was isolated from renal venous blood of subjects with surgically remediable hypertension, and of animals with acute renal ischaemia hypertension. Nephrotensin is a vaso-constrictor substance different from renin and angiotensin (Grollman, 1970; Grollman and Krishnamurty, 1971). The participation of this substance in human essential hypertension remains to be determined.

c) Renal antihypertensive factors

In addition to the renin-angiotensin system the kidney has been known to possess an antihypertensive mechanism. Extracts of renal medullary tissue have been shown to lower the blood pressure of animals with renal hypertension (Muirhead et al., 1956, 1960, 1970). These extracts have both an acute transient hypotensive action and a slow onset protracted effect.

The acute effect is due to the presence of prostaglandins (Hickler et al., 1964; Daniels et al., 1967). The role of prostaglandins in the pathogenesis of hypertension is of great interest, since it was found that some prostaglandins possess natriuritic as well as vasodilatory activity (McGiff and Itskovitz, 1973). Prostaglandin E_2 (PGE_2) has been suggested as being the primary prostaglandin released from the

kidney (Dunham and Zimmerman, 1970; McGiff et al., 1970). However, it is rapidly metabolized by the lungs (McGiff et al., 1969; Ferriera and Vane, 1967) and therefore could not serve as a general vasodilator. Lee (1967) put forward the concept that A prostaglandins (PGA's), which are more resistant to metabolic degradation may be the circulating antihypertensive hormones. Some degree of controversy exists about the ability of the kidney to form A prostaglandins (McGiff and Itskovitz, 1973). Nonetheless, plasma levels of PGA are found to be lower in hypertensive than normotensive individuals and are virtually absent in the plasma of anephric subjects (Zusman et al., 1973; Lee et al., 1973). Much additional work is required to verify this hypothesis concerning the function of prostaglandins as antihypertensive hormones.

The substance in renal medullary extract responsible for the delayed hypotensive response is a neutral lipid of low molecular weight, known as antihypertensive neutral renomedullary lipid (A.N.R.L.). Given parenterally, it is a powerful antihypertensive agent, reducing the blood pressure of hypertensive animals steadily over 24-48 h (Muirhead et al., 1966, 1967, 1968a). However, in comparable doses it does not alter the blood pressure of normotensive animals (Muirhead et al., 1968b). Thus A.N.R.L. appears to act as a regulator of high pressure.

2. Neural Factors

a) The sympathetic nervous system

The role of the sympathetic nervous system in the development and persistence of high blood pressure, despite the efforts of many investigators, is still in question. The clinical efficacy of adrenergic blocking agents has provided

indirect evidence for the involvement of the sympathetic system in hypertension. Recent studies have attempted to use plasma noradrenaline and dopamine β-hydroxylase as indices of sympathetic tone. However, measurement of noradrenaline has been complicated by methodological difficulties and interpretation of the results must be viewed critically. Studies attempting to relate blood pressure levels to the excretion of noradrenaline have given conflicting results (Nestel and Doyle, 1968), perhaps in part due to rapid metabolism and variable urinary excretion of the catecholamine and its metabolism (Peart, 1966).

Recent investigations using a double isotope derivative assay for noradrenaline (Engleman and Portnoy, 1970) and a radioimmunoassay for dopamine β-hydroxylase (Rush and Geffen, 1972) have shown a correlation between resting diastolic pressure and both plasma noradrenaline and dopamine β-hydroxylase in patients with essential hypertension (Louis et al., 1973; Geffen et al., 1973; De Champlain et al., 1976; Louis et al., 1974). Research in this field at St. Mary's Hospital Medical School (Osikowska and Sever, 1976) involved patients with essentail hypertension, patients with Shy-Drager's syndrome (autonomic failure) and a normal control population. The subjects were investigated after lying and standing when blood pressure and venous samples were taken. Clear differences in plasma noradrenaline levels were observed, which were particularly marked on standing. The levels were significantly lower in Shy-Drager patients and higher in hypertensive patients as compared to the normal population. However, there is also evidence to suggest that excessive sympathetic drive is not the sole factor involved in essential

hypertension, since a substantial proportion of hypertensive patients have been found to have plasma noradrenaline levels in the normal range (Louis et al., 1975). Quite paradoxically, patients with endogenous depression, under observation at the same time, were found to have noradrenaline concentrations significantly higher than the group with essential hypertension, without the same degree of blood pressure elevation. These observations suggest the excess sympathetic activity demonstrated in established essential hypertension, to be acting in the presence of other factors.

b) The baroreceptor reflex

There is substantial evidence to suggest that an alteration of baroreceptor reflex function is associated with hypertension. If this were not the case, bradycardia would be characteristic of most hypertensive syndromes, due to a continued high level of reflex activity. Work done in experimental animals (McCubbin et al., 1956; Aars, 1968; Angell-James, 1973) indicates that although the discharge pattern appears to be normal, there is a re-setting of the receptors to a higher pressure threshold and operating range. Similar re-setting of the whole baroreceptor arc has been seen in man with essential hypertension (Gribbin et al., 1971). A possible explanation for the diminished sensitivity in man, is decreased compliance of the arterial wall in the baro-receptor regions allowing less stretch of receptor per unit rise of blood pressure and thus producing a dampened response (Aars, 1968; Green et al., 1966).

c) Central neurotransmitters

The neurochemical processes underlying central sympathetic mechanisms in relation to arterial hypertension are poorly understood. For ethical reasons, studies have been restricted to various animal models of experimental hypertension, where failure of inhibitory mechanisms is responsible for a higher blood pressure. Of these, the neurogenic type resulting from denervation of the carotid sinus and aortic arch baroreceptors provides the most convincing evidence that central noradrenaline neurones may participate in the origin and maintenance of hypertension. Thus, sino-aortic denervation evokes a pronounced increase in noradrenaline turnover in the thoracocolumbar region of the spinal cord (Chalmers and Wurtman, 1971). This suggests enhanced activity in bulbospinal noradrenaline neurones innervating the sympathetic centres in the lateral horns, which are then responsible for the blood pressure elevation via increased peripheral sympathetic tone (Chalmers and Wurtman, 1971). In addition, treatment with 6-hydroxy-dopamine causes a substantial reduction in spinal and brain stem noradrenaline levels and blocks the blood pressure rise after sino-aortic denervation (Chalmers and Reid, 1972).

3. Anatomical Factors

The morphology and composition of arteriolar walls, the main resistance vessels, are modified in hypertension. Arterioles of hypertensive patients and animals with experimental hypertension contain a significantly greater proportion of water (Tobian and Binion, 1952) and calcium ions (Tobian and Chesley, 1966). The wall is also thickened relative to the lumen (Short, 1966). Furthermore, resistance to flow in hypertensive arterioles is increased (Folkow *et al.*, 1958),

even though the vessels respond normally to vasoconstrictor substances (Redleaf and Tobian, 1958). This observation led to the assumption that elevated smooth muscle tone explains the increased flow resistance. Despite reports of medial hypertrophy in precapillary vessels (Johnson, 1868; Goldblatt, 1938), the possible haemodynamic consequences of structural vascular adaptation have hardly been considered. Instead, it has frequently been proposed that some basic biochemical change of the vascular neuro-effectors may be the main disturbance underlying primary hypertension in both man and rat, making the vascular effector cells generally more sensitive and "reactive" to extrinsic stimuli. However, studies with vessel strips (Spector et al., 1969) and resistance vessels proper (Folkow et al., 1970), of spontaneously hypertensive rats show that the vascular smooth muscle is not supersensitive to noradrenaline. Although most investigators believe the structural changes in the vessel walls are secondary to the increased vascular pressure, some question remains as to whether the functional changes represent primary or secondary events (Bohr, 1974).

4. Plasma Kallikrein-Kinin System

Recently, results of urinary kallikrein estimations in hypertensive animals and patients have revealed the possible involvement of the kallikrein-kinin system in hypertension (Margolius et al., 1972). Although spontaneously hypertensive rats were found to excrete significantly high levels of kallikrein, in rats with renal hypertension and patients with essential hypertension, levels were lower than control values. Additional data suggests that the kallikrein-kinin system may be involved in salt and water excretion by the kidney (Marin-

Grez et al., 1972). The necessity for further studies into the role of this vasodilator and natriuritic system in hypertensive disease is apparent.

5. Genetic Factors

The involvement of genetic factors in hypertension is suggested by the possibility of breeding strains of hypertensive rats (Okamoto, 1969; Smirk and Hall, 1958; Dahl et al., 1962). Data concerning the role of inheritance in man has been of four kinds:

records of single families, studies of family histories in a large series of patients, measurement of blood pressure in twins and measurement of blood pressure in the relatives of patients with essential hypertension.

Records of single families are of little value in a common condition such as essential hypertension. Studies of family histories are also not dependable, since they are indicative of structural arterial disease rather than hypertension, and although the two are related, they are not synonymous (Weitz, 1923; Fishberg, 1939; Bechgaard, 1946). Blood pressure measurements of identical and dizygotic twins, provide important evidence of the extent to which arterial pressure depends on inherited factors. A number of investigators (Stocks, 1930; Varschuer and Zipperlen, 1929; Platt, 1963), have found a greater correlation between members of monozygotic than dizygotic pairs. The results of many such studies are shown in Figure 1.7.

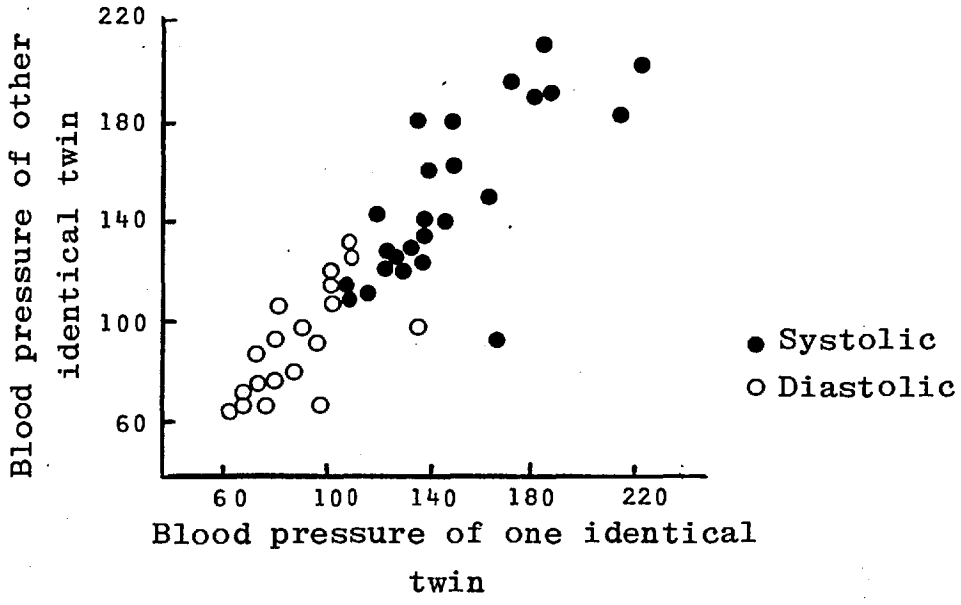


Figure 1.7. Comparison of the Blood Pressures of a Series of Idential Twins. (after Smirk, 1957)

Early investigations involving measurement of blood pressure in relatives of patients with essential hypertension showed a prevalence of high blood pressure in this population as compared with control subjects of similar age (Weitz, 1923; Ayman, 1934). Sobye (1948) conducted an extensive study whereby he measured blood pressure in 2023 relatives of 186 patients with nephrosclerosis, associated with malignant essential hypertension. He observed a disposition to hypertension in one generation in 22.6%, in two generations in 61.3%, in three generations in 10.2%, and in four generations in 0.5% of the families investigated.

These early investigations are, however, inadequate in their control studies, particularly on relatives of subjects without hypertension. Moreover, the assumption is made that there is a dividing line between normal blood pressure and hypertension. Therefore, although it was possible to postulate that inheritance plays an important role in the genesis of

hypertension, no quantitative data could be obtained. This information was first provided by the St. Mary's survey (Hamilton et al., 1954). Appropriate control subjects were studied, but the patients were selected on the basis of their arterial pressure either because they were above or below a certain arbitrary value. Results showed a quantitative resemblance between first degree relatives in the hypertension series, suggesting that arterial pressure is inherited, like height, as a graded character. That this applies to the population at large was shown by the Miall Oldham surveys (1955, 1958, 1963), which employed strictly random selection methods. These workers estimated the contribution of inheritance as opposed to environment in arterial pressure determination, and found it to be one-third to two-thirds of the whole.

The nature of inheritance of hypertension is polemic. Platt (1959) provided evidence to support a single Mendelian gene hypothesis. However, since that time, no specific biochemical fault corresponding to a single gene defect could be found. Moreover, taking into consideration the dependance of arterial pressure on a number of factors such as vascular resistance, cardiac output and central mechanisms, it is unlikely to be determined by only one gene. Hence, hypertension may be regarded as a classical example of polygenic inheritance (Pickering, 1968).

6. Age

Population studies in South Wales (Miall and Oldham, 1963) reveal the tendency for arterial pressure to rise with age. Figure 1.8 shows the mean value obtained for each group of males

and females. In each of the approximately 3,000 subjects, the measurements were repeated four years later. It is noted that the curve for diastolic pressure tends to flatten after the age of 50 in males and 70 in females, and later to turn downwards. It is tempting to presume that this may be related to survival of those with lower blood pressures in old age (Record and Whitfield, 1964).

Figure 1.8. Mean systolic and diastolic pressures in the Rhondda Fach and Vale of Glamorgan populations, based on the findings in two surveys. The area of each square is inversely proportional to the standard error of the mean and so indicates the weight to be attached to each mean (After Miall and Oldham, 1963).

7. Environmental Factors

A number of environmental factors have emerged from population studies as influencing arterial pressure.

a) Weight and obesity

There is a definite correlation between blood pressure and the relation of body weight to height (Symonds, 1923; Short and Johnson, 1939; Boynton and Todd, 1948). Results of an extensive study involving approximately 68,000 subjects indicate that for every 10 kg increment in weight, systolic pressure rises by 2 mm Hg and diastolic pressure by 3 mm Hg. Reduction in weight by dieting may produce considerable falls in arterial pressure when this is initially high (Terry, 1923; Preble, 1923). Similar observations were made during the starvation in the last great war in Europe (Lups and Francke, 1947).

b) Salt intake

Gross restriction in salt intake is an accepted therapy for essential hypertension. It has, therefore, been postulated that intake of salt may participate in the pathogenesis of essential hypertension. Menelly and Dahl (1961) have been able to induce hypertension in certain rat strains by feeding increasing amounts of sodium chloride. Studies in man, however, have shown contradictory findings. Dahl and Love (1954, 1957) presented evidence that individuals who took additional salt with their food, had higher blood pressures than those who did not, their conclusions being based on the proportion of men having hypertension (B.P. 140/90 or over). The studies were repeated by Miall (1959) who found no systematic difference between males having a high salt intake and a low salt intake.

c) Psychological factors

There is some belief that essential hypertension is a psychosomatic disease, however, this is a highly contraversial hypothesis. In trying to assess the evidence for a possible

relationship between hypertension and a specific personality type, it should be borne in mind that arterial pressure is very dependant on the emotional state of the subject at the time of measurement. Emotional situations, such as public speaking, are accompanied by considerably greater increases in blood pressure than physical exercise (Taggart, 1975). Harris et al. (1953) investigated the personalities of undergraduate women with low and high blood pressure. They found the latter were less able to handle stressful situations without becoming emotionally upset, and that therefore they are more likely to be subject to the autonomic accompaniments of emotion, including repetitive rises in blood pressure. Hence such repetitive rises may be one condition in the network of events leading to fixed hypertension. However, other investigators have found that the role of psychological stress is very similar in renal and essential hypertension (Ostfeld and Lebovits, 1959). Hence they concluded that personality factors are not aetiologically related to essential hypertension, serving only transiently to influence the level of blood pressure.

Recently a more scientific approach to this area, has involved work on animals. Moderately sustained hypertension has been produced experimentally in rats subjected to subthreshold stimulation in the "defence" area of the hypothalamus (Folkow and Rubinstein, 1966). In a more elegant experiment involving a tightly structured colony of mice (Henry et al., 1967) experimental crowding was found to alter the social pattern so that one of the male mice was forced to become "dominant". This dominant mouse assumed a role of patrolling the females in their nesting boxes and kept out

the other males. The dominant mouse developed hypertension.

d) Alcohol and smoking

Edwards et al. (1959) observed that in the seventh decade of life, blood pressures were higher in subjects who had drunk alcohol. They found the highest pressures in men who had not smoked but who had drunk regularly, and the lowest in smokers who did not drink. Dawber et al. (1967) also found a greater rise in heavy drinkers. Blood pressures are generally lower in smokers than in non-smokers, and highest in ex-smokers in males (Miall and Oldham, 1958; Edwards et al., 1959; Karvonen et al., 1959).

In summary therefore, perusal of the literature has shown that there is no single causative mechanism in the initiation and maintenance of high blood pressure. Interaction of various factors have been proposed as being responsible. Autonomic overactivity is thought to raise the blood pressure initially, which then produces generalized vascular changes that maintain the hypertension (Folkow, 1975). Alternatively, the initial elevation in pressure by autonomic overactivity is believed to be sustained by the kidney (Ledingham, 1971). Both genetic and environmental influences are apparent in hypertension; one may operate through the kidney and the other through the autonomic nervous system.

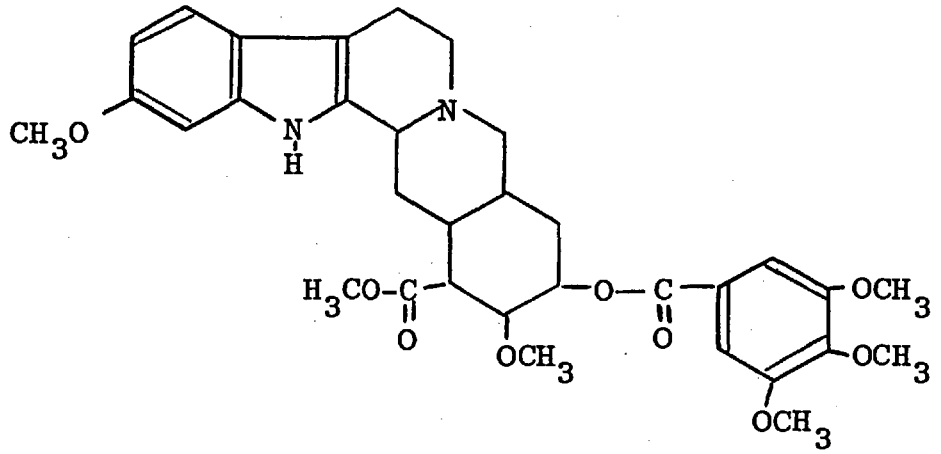
Drug Therapy of Hypertension

Although great progress in the treatment of hypertension has unquestionably been made in recent years, the ultimate therapy remains to be discovered. The available spectrum of compounds that act on various systems to ameliorate the symptoms of this disease (raised arterial pressure), contribute a great deal to its treatment. Results of controlled therapeutic trials by the Veterans Administration (1967, 1970) have shown that treatment is associated with a statistically significant reduction in morbidity and mortality in those patients with a diastolic pressure of 115 mm Hg or above. However, the beneficial effect of treatment in subjects with diastolic pressures between 90 and 114 mm Hg, was found to be less conspicuous.

A brief review of the various types of antihypertensive agents will be given in this section.

Reserpine

Reserpine is the principle alkaloid of Rauwolfia Serpentina, a climbing shrub of the family Apocynaceae. Since very ancient times, crude extracts of the plant, which is indigenous to India and neighbouring countries, have been recommended by native doctors in the treatment of hypertension.



The alkaloid exhibits a complex pattern of pharmacological activity, mainly attributed to its central and peripheral depletion of catecholamines and 5-hydroxytryptamine (Bein, 1956). However, its action on the central nervous system, does not appear to be of major importance in the reduction of sympathetic outflow (Iggo and Vogt, 1960; Macleod, 1972). Reduction in blood pressure by reserpine, may involve a decrease in peripheral resistance which is most marked in the skin. Nevertheless, the antihypertensive effect of chronically administered reserpine is associated with a reduced cardiac output (Cohen et al., 1968).

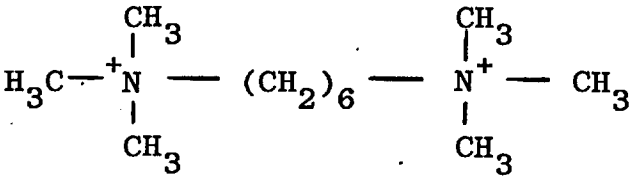
Adverse effects:- Reserpine produces a considerable incidence of nightmares and psychic depression (Quetsch et al., 1959). Carcinoma of the breast has also been associated with chronic reserpine treatment (Boston Collaborative Drug Surveillance Program, 1974).

Metabolic fate:- Reserpine is well absorbed from the gastro-intestinal tract and binds to sites involved with the storage of biogenic amines. Human subjects dosed orally with [^{3}H]-reserpine, excrete 8% of the dose in urine and 60% in the faeces within 4 days. The faecal radioactivity corresponds mainly to unchanged drug, whereas the urinary fraction is composed of some reserpine and two of its hydrolysis products, trimethoxybenzoic acid and methylreserpate (Maas et al., 1969).

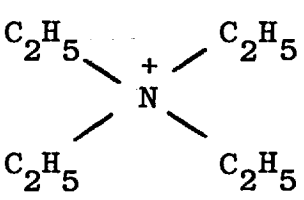
Ganglion blocking drugs

The discovery of the "nicotine paralysing" action of tetraethylammonium (Marshall, 1913; Burn and Dale, 1915) led to the development of a series of polymethylene bistrimethyl-ammonium (methonium) compounds having ganglion blocking properties

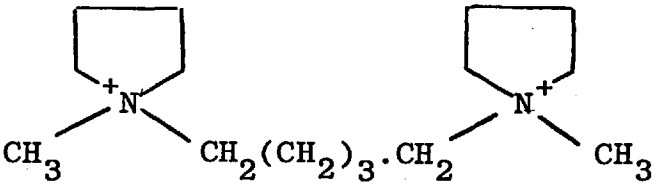
(Paton and Zaimis, 1948, 1949; Barlow and Ing, 1948).



Hexamethonium



Tetraethylammonium



Pentolinium

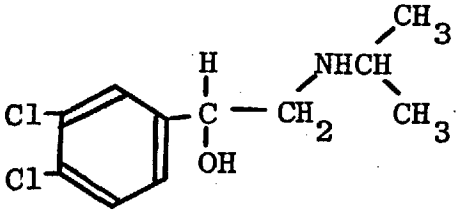
These drugs block transmission by stabilizing the post-synaptic membrane against the action of acetylcholine liberated from presynaptic nerve endings. They do not modify the conduction of impulses in preganglionic or postganglionic fibres and do not prevent the release of acetylcholine by preganglionic impulses (Paton and Zaimis, 1952). The hypotensive action of ganglion blocking drugs is achieved mainly by blockade of sympathetic ganglia which interupts adrenergic control of arterioles and results in vasodilatation. Decreased cardiac output also results due to diminished venous return.

Adverse effects:- The variety of adverse effects caused by these methonium compounds outweigh by far their therapeutic efficacy. These include visual disturbances, difficulty in micturition, and constipation (Paton and Zaimis, 1952). Marked postural hypotension is often observed, which may be so severe as to cause faintness or collapse (Ball, 1950).

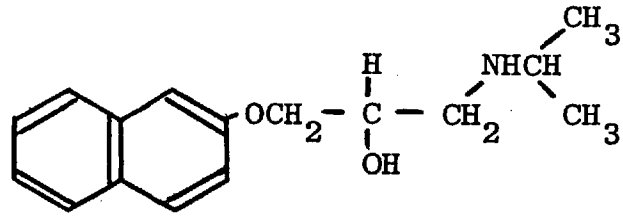
Metabolic fate:- The depression of propulsive movements of the gastrointestinal tract by methonium compounds and the inability of these compounds to penetrate cell membranes results in their incomplete and erratic absorption (Milne and Oleesky, 1951). Intravenous administration of methonium compounds in man and animals shows that they are excreted mainly unchanged by the kidneys. No demethylation or any other metabolic degradation products could be found (Zaimis, 1950).

β-Adrenergic blocking drugs

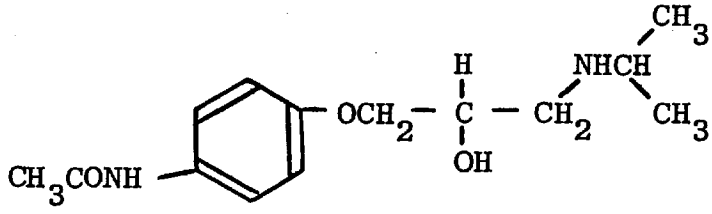
Since the discovery of the β-blocking properties of dichloroisoprenaline (Powell and Slater, 1958), a series of similar compounds have been synthesized. These are all derivatives of the β-agonist, isoprenaline and differ in potency, β-selectivity and membrane stabilizing actions.



Dichloroisoprenaline



Propranolol



Practolol

The side chain of these compounds with an isopropyl-substituted secondary amine, appears to favour interaction with β-receptors. The nature of substituents on the aromatic ring determines whether the effect will be predominantly activation or blockade.

The aliphatic hydroxyl seems to be essential for activity, it gives the molecule optical activity. The (-) forms of these compounds are more active than the (+) forms.

β-Adrenergic antagonists decrease heart rate and cardiac output. Although they have minimal direct effect on the peripheral vasculature, peripheral resistance is increased as a result of compensatory sympathetic reflexes. The exact mechanism responsible for their antihypertensive effect is not clear, but one likely explanation is that there is a gradual loss of reflex increase in peripheral vascular resistance in response to the reduced cardiac output (Tarazi and Dustan, 1972).

β-Blockers are also thought to reduce cardiac output by an effect not specifically related to β-receptor blockade, since this effect is gradual in onset and produced by both isomers of propranolol, whereas β-receptor blockade is immediate and stereospecific for the laevo form (Laverty, 1973). A mode of action involving the central nervous system has been proposed. Intracerebroventricular studies of propranolol in animals, by a number of investigators (Day and Roach,1973; Dollery et al., 1973; Ram and Garvey, 1973) have led to the view that its hypotensive activity is centrally mediated and depends on the integrity of noradrenergic neurones. Blockade of β-adrenoreceptors in the brain is claimed to cause hypotension and bradycardia (Day and Roach, 1973). However, it is noteworthy that practolol, the cardioselective β-blocker (Bayer et al., 1972), which does not cross the blood-brain barrier, lowers blood pressure very effectively in man (Esler and Nestel, 1973; Wood et al., 1973). There is also increasing evidence that the drug reduces plasma renin (Hansson, 1973;

Bühler et al., 1973; Bravo et al., 1974). However, reduction of plasma renin activity is not necessary for the antihypertensive effect of β-adrenergic blocking drugs (Stokes et al., 1974).

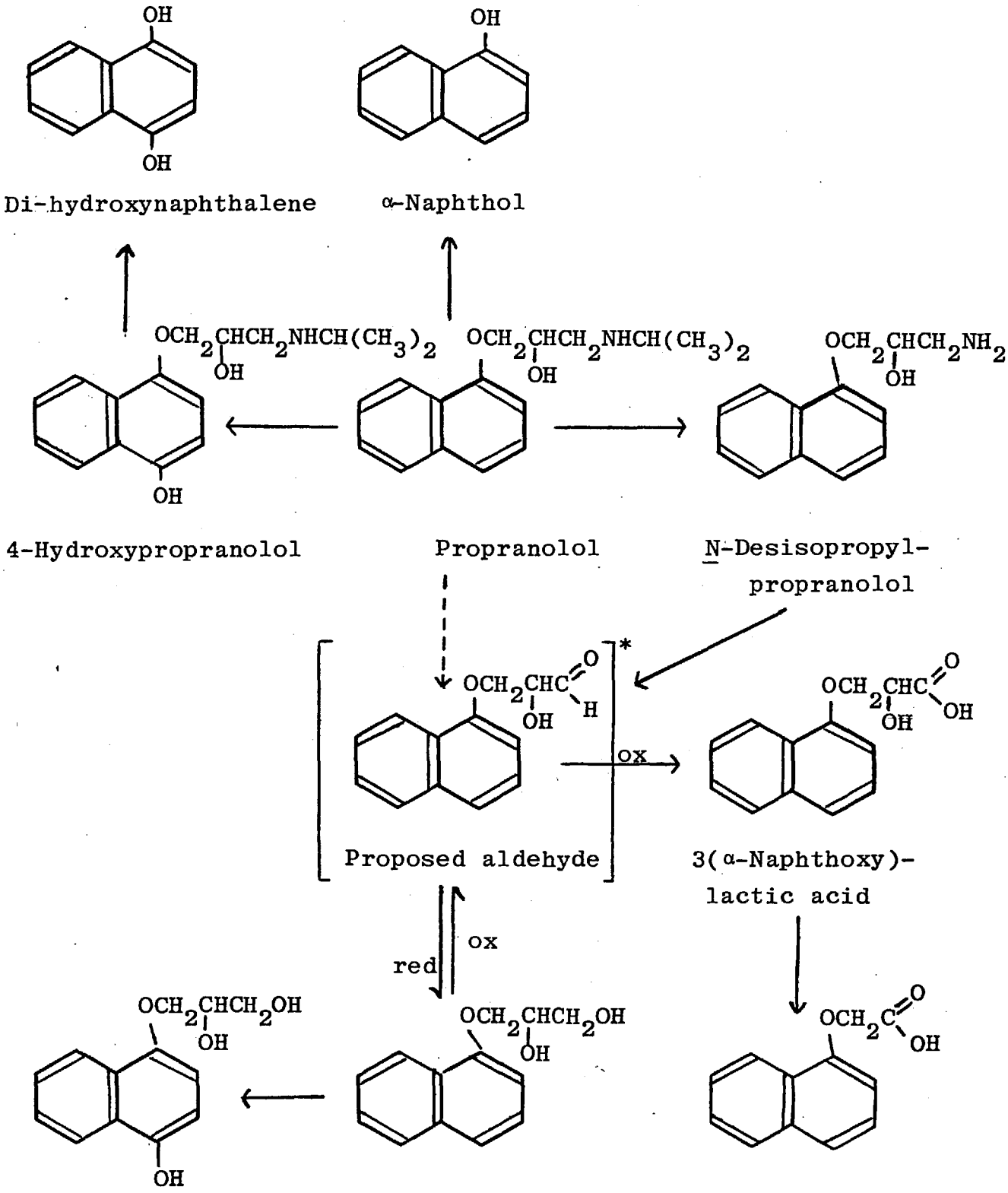
Adverse effects:- Heart failure may develop in patients with cardiac disease and increase in airway resistance can be life-threatening to asthmatics. Central effects include hallucinations, nightmares, insomnia and depression (Greenblatt and Shader, 1972; Greenblatt and Koch-Weser, 1973).

Metabolic fate:- Propranolol is well absorbed after oral administration, and is excreted in the urine after being almost completely metabolized (Hayes and Cooper, 1971). The metabolism of oral propranolol was studied in man and the dog by gas chromatography-mass spectrometry (Walle and Gaffney, 1972) and is outlined in Figure 1.9. 4-Hydroxy-propranolol, found only after oral and not intravenous administration, has β-blocking effects similar to propranolol (Fitzgerald and O'Donnell, 1971).

Differences in propranolol plasma protein binding have been used to explain inter-individual differences in plasma half-life of the drug (Evans et al., 1973a). Biotransformation of propranolol occurs mostly in the liver where the drug is thought to undergo a major first-pass effect (Evans et al., 1973b).

Figure 1.9. Metabolism of Propranolol

(after Walle and Gaffney, 1972)



* Postulated intermediary metabolite that has not been isolated

Adrenergic Neurone Blocking Drugs

The mechanism of action of these compounds is discussed on p.64 and their pharmacological, haemodynamic and adverse effects are similar to those of debrisoquine (see p. 68-70).

Metabolic fate of the unsubstituted guanidine adrenergic blocking drugs:-

Guanethidine is rapidly metabolized in the rat to three identified metabolites which together with the unchanged drug, are excreted in urine (McMartin, 1969; see Fig.1.10). Of an intraperitoneal injection of the [^{14}C]compound (10 mg/kg) after 8 h, the total metabolites were shown to account for 75% of the radioactivity, of which 55% was 2-(6-carboxyhexyl-amino)ethyl guanidine and its transformation product 6-carboxy-hexyl-2-iminoimidazolidine. However, excretion in man is prolonged; thus of an oral dose, of the ^{14}C-labelled compound after 48 h, about 40% of the ^{14}C was excreted in the urine and after 72 h, 23% was recovered in the faeces (Dollery et al., 1960).

The major route of metabolism of [^{14}C]guanoxan in the dog is reported to be by transamination to give arginine and guanidino-acetic acid. In man, however, the only metabolites detected were unconjugated 6- and 7-hydroxy-guanoxan in amounts ranging from 12 to over 50% of the dose (Jack et al., 1972).

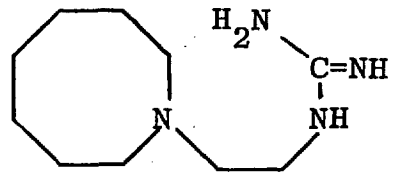
α-Methyldopa

α-Methyldopa (L-3-(3',4'-dihydroxyphenyl)-2-methylalanine; Aldomet), first shown to have hypotensive properties by Oates et al. (1960), is one of the major agents used in the treatment of hypertension today.

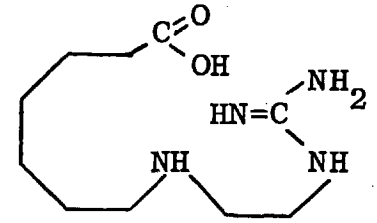
The exact mechanism by which this drug acts is, as yet,

Figure 1.10. Metabolic Fate of the Unsubstituted Guanidine Adrenergic Blocking Drugs

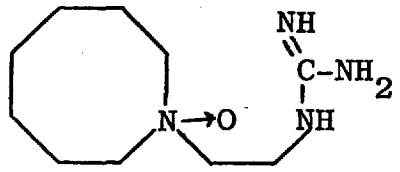
a) Guanethidine



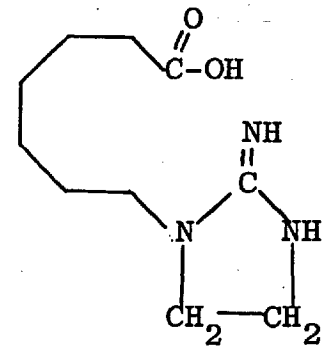
Guanethidine



2-(6-Carboxyhexylamino) ethyl guanidine

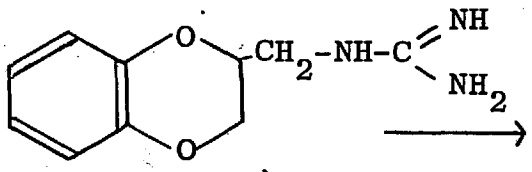


Guanethidine N-oxide

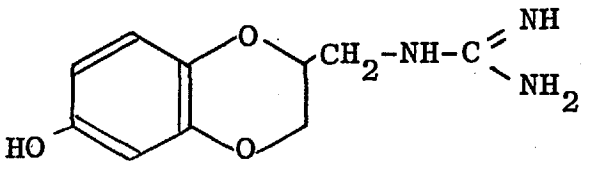


(6-Carboxyhexyl)-2-imino-imidazolidine

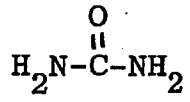
b) Guanoxan



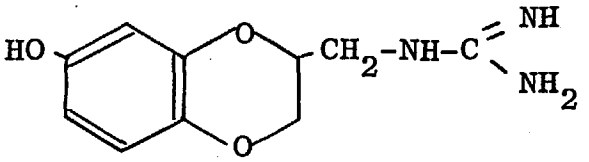
Guanoxan



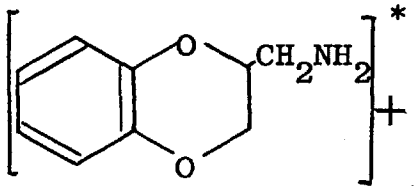
6-Hydroxy-guanoxan

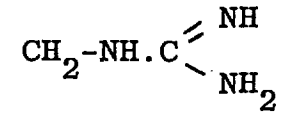


Urea

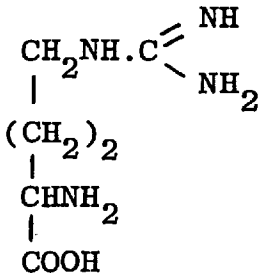


7-Hydroxy-guanoxan





Guanidino acetic acid



Arginine

$|^{14}C|$creatinine + $|^{14}C|$creatine

* Postulated intermediary metabolite that has not been isolated

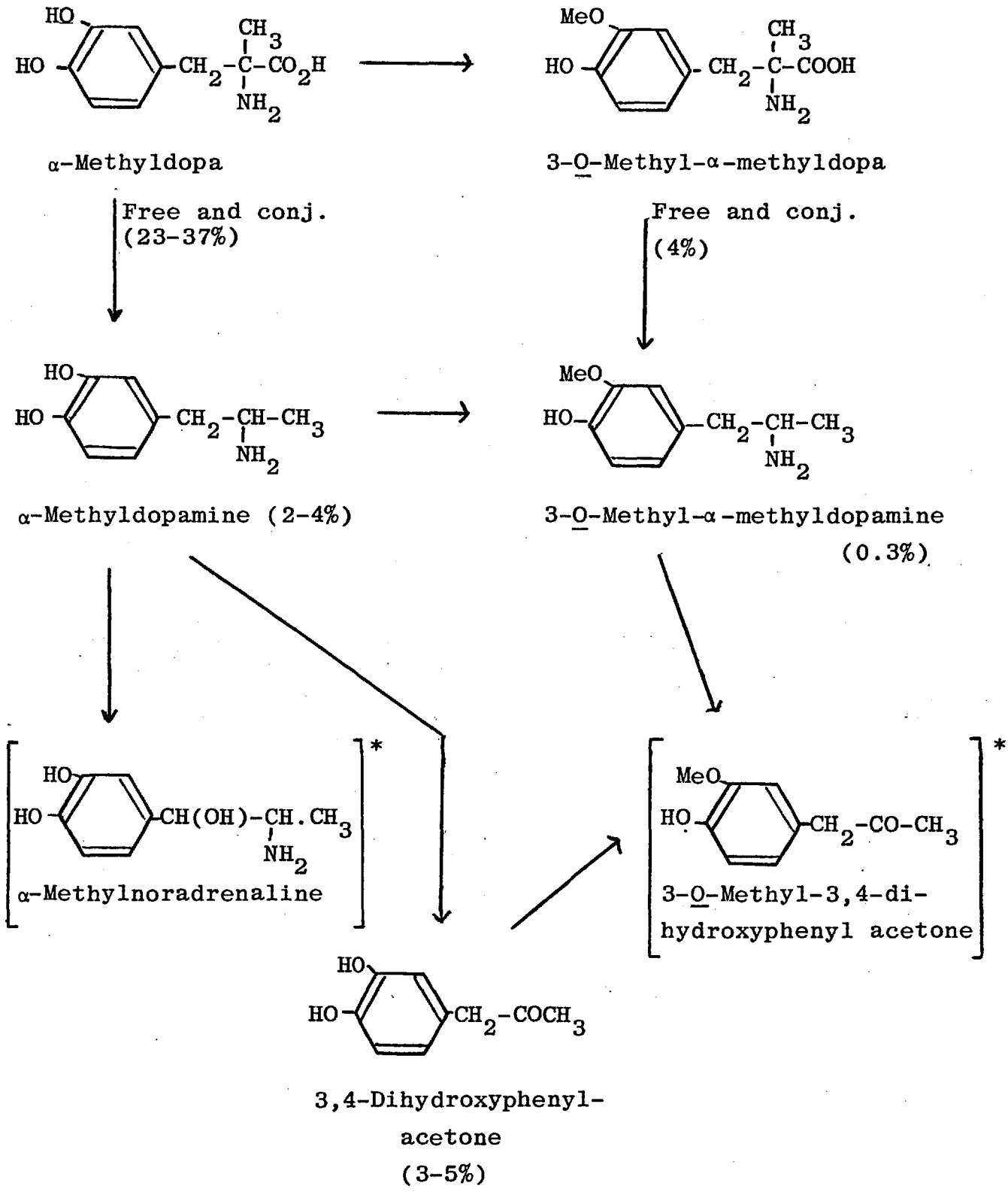
not clearly understood. The action of α-methyldopa was initially attributed to its dopa decarboxylase inhibitory effect, reducing the rate of synthesis of catecholamines and other phenylalkylamines. However, other dopa decarboxylase inhibitors were shown not to be hypotensive, so that the clinical usefulness of this drug did not appear to be related to enzyme inhibition.

Methyldopa is substituted for dopa as a substrate for transmitter synthesis resulting ultimately in the formation of methylnoradrenaline rather than adrenaline as the sympathetic transmitter (Carlsson and Lindqvist, 1962; Muscholl and Maître, 1963). Day and Rand (1963, 1964) postulated that this substitution accounted for impaired peripheral sympathetic neuronal function because methylnoradrenaline was less potent as a pressor substance than noradrenaline. However, in many animals in which α-methyldopa is hypotensive, α-methylnoradrenaline is approximately equipotent with noradrenaline (Trinker, 1971).

More recently, a central mode of action has been postulated for α-methyldopa. Certain doses of the drug which will reduce arterial pressure when injected intravertebrally, have no hypotensive effect on intravenous administration (Henning and Van Zwieten, 1968). Furthermore, the hypotensive effect produced by administration of methyldopa into the vertebral artery can be prevented only by pretreatment with specific dopa decarboxylase inhibitors that enter the central nervous system (Henning, 1969; Henning and Rubenson, 1971). The action of α-methyldopa is antagonised by dopamine-β-hydroxylase, suggesting that it is the metabolite, α-methylnoradrenaline, which is the active agent (Carlsson and Lindqvist, 1962).

Figure 1.11. Outline of the metabolic fate of α-methyldopa

(after Au et al., 1972)



* These postulated metabolites have not been isolated by the authors.

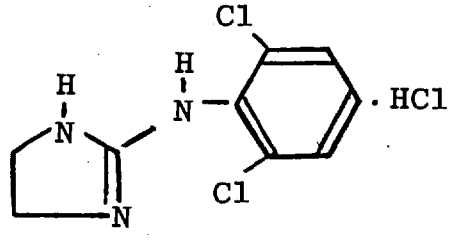
Methyldopa has been shown to decrease renin activity in man (Mohammed et al., 1969), which may contribute to its hypotensive action.

Adverse effects:- The most frequent untoward effect is mild sedation. Less frequent adverse effects include diarrhoea, blurred vision, nausea and a dry mouth.

Metabolic fate:- The active isomer, L-methyldopa, shows inter-individual differences in absorption between 26 and 74% of an orally administered dose (Sjoerdsma et al., 1963). The metabolism of α-methyldopa has been widely investigated. Studies using the ^{14}C-labelled drug (Au et al., 1972) showed that approximately 40% of an oral dose was excreted in the urine within 24 h and 60% in the faeces over 4 days. The faecal ^{14}C corresponded to unchanged α-methyldopa. The main urinary metabolite was free and conjugated α-methyldopa (23-37% of dose). Other metabolites were also identified and quantitated (see Fig.1.11).

Clonidine

Clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride; Catapres), lowers blood pressure when administered in microgram doses. Its mode of action is not fully understood.



There is a biphasic blood pressure response to intravenous clonidine in animals (Constantine and McShane, 1968), and man

(Barnett and Cantor, 1968). The initial brief hypertensive effect of the drug is thought to be caused by a direct peripheral α-adrenergic action (Barnett and Cantor, 1968). The second phase is characterized by acute hypotension (Merguet et al., 1968; Nayler et al., 1968). If the drug is given orally (Barnett and Cantor, 1968; Merguet et al., 1968), or in minute doses into the vertebral arteries (Bentley and Li, 1968) or cisterna magna (Kobinger and Walland, 1967), hypotension occurs without the initial rise in blood pressure. During the hypotensive phase, peripheral resistance is generally unaltered (Barnett and Cantor, 1968), which suggests a central mode of action. There is, in fact, growing evidence that these effects of clonidine are due to its stimulation of central noradrenaline reeeptors that are inhibitory to sympathetic nerve activity (Schmitt et al., 1971, 1973; Starke and Altmann, 1973). These receptors seem to be located in the lower brain stem (Schmitt et al., 1968; Shaw et al., 1971) and may be classified as α-adrenergic since clonidine stimulates peripheral α-receptors (Hoefke and Kobinger, 1966). Some investigators (Starke and Altmann, 1973) support the hypothesis of a presynaptic site of action, since the hypotensive responses to clonidine are prevented by pretreatment with desmethylimipramine (Reid et al., 1973).

Adverse effects:- The most frequent untoward effects are sedation and dry mouth (Addis and MacDougall, 1969). The observation of hypertensive crises following sudden withdrawal of clonidine, is the main argument against clinical use of the drug (Hansson et al., 1973).

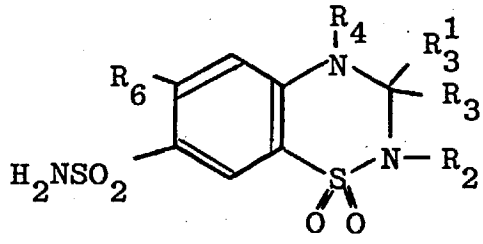
Metabolic fate:- Studies with ^{14}C-labelled clonidine in various species including man, show that the orally administered drug is well absorbed (Rehbinder, 1969). Of the dose, 65% or more is excreted in the urine, and of this approximately 40% is unchanged clonidine in man. Only partial characterization of the urinary metabolites has been achieved so far, indicating species differences in metabolism of the drug. The following number of metabolites have been detected: 4 in man, 6 in the monkey, 8 in the dog and 5 in the rat. p-Hydroxy-clonidine and its conjugates, the major metabolite in rat urine, has also been detected in human urine.

Diuretics

Diuretics are in common use in mild hypertension and are often prescribed in conjunction with other antihypertensive agents for the treatment of moderate to severe hypertension.

1. Thiazides

These are the most widely used diuretics. Most compounds of this group are 1,2,4-benzothiadiazine-1,1-dioxide analogues.



	R_2	R_3	R_3^1	R_4	R_6	Optimal dose in man m-
Chlorothiazide	H				Cl	500-2000
Hydrochlorothiazide	H	H	H	H	Cl	25-100
Benzothiazide	H	CH_2SCH_2			Cl	25-50
Polythiazide	CH_3	$CH_2SCH_2CF_3$	H	H	Cl	4-8

There are approximately 20 different compounds in this series. Saturation of the heterocyclic ring between the 3 and 4 position, and a variety of substituents on the 3 position, appear to increase potency. The addition of various functional groups at the 1,4 and 5 position decrease diuretic activity (Beyer and Baer, 1961).

Thiazides inhibit distal tubular mechanisms of electrolyte reabsorption and hence increase sodium and chloride ion excretion. Consequently they cause a reduction in extracellular fluid and plasma volume (Wilson and Freis, 1959; Gifford et al., 1961) with a concommitant decrease in cardiac

output (Aleksandrow et al., 1959). However, after several months of therapy, the diuretic effects of these compounds gradually diminish although their hypotensive action persists. At this time, peripheral resistance is clearly decreased, and this accounts for their antihypertensive effect (Tobian, 1967; Conway and Palmero, 1963). It is uncertain whether this reduction in resistance is mediated by a direct effect on arteriolar muscle or by changing the concentration of electrolytes within the arteriolar wall (Tobian, 1967).

Adverse effects:- Hypokalemia is the most common effect of chronic thiazide therapy, but this is not significant in patients on an adequate diet. Hyperglycaemia (Shapiro et al., 1961) and hyperuricaemia (Healey et al., 1959) also occur quite frequently.

Metabolic fate:- Orally administered [^{3}H]hydrochlorothiazide is rapidly absorbed in the rat. A high proportion, 43-69%, of the ^{3}H - label is excreted in the 0-48 h urine and the drug also undergoes biliary excretion (Sheppard et al., 1960). In dogs, the related compound, chlorothiazide is also partly excreted in the bile (Baer et al., 1959). Neither diuretic undergoes metabolic degradation. The urinary excretion of both compounds in man is rapid, almost all of an intravenous dose being excreted within 24 h.

Other less commonly used diuretics for hypertensive therapy are spironolactone and the high-ceiling diuretics.

2. Spironolactone

Spironolactone, (3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androstene-17α-yl)-propionic acid γ-lactone), a structural

analogue of the mineralocorticoid aldosterone, probably acts as a competitive inhibitor of the hormone (Kagawa et al., 1959; Liddle, 1961).

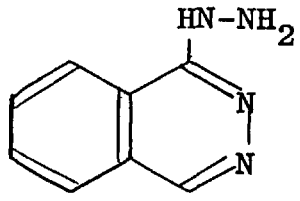
3. High ceiling diuretics

Furosemide (4-chloro-2-furfurylamino-5-sulphomoyl benzoic acid) and ethacrynic acid (2,3-dichloro-4-(α-ethyl acryloyl) phenoxyacetic acid) are termed "high ceiling" due to the fact that they have far greater peak diureses than any other agents (Mudge, 1966).

Vasodilators

Theoretically, one of the treatments of choice in hypertension, would appear to be the use of direct vasodilators to relax arterial smooth muscle. However, compensatory haemodynamic reflexes come into play, resulting in tachycardia and increased cardiac output, to counteract the reduced vascular resistance. Therefore, these drugs are combined with β-blockers or other antihypertensive agents for maximal clinical effectiveness.

Hydralazine: this is one of the several phthalazine-derived vasodilator compounds. These agents preferentially relax the smooth muscle on the precapillary arterioles, which constitute the greatest resistance to blood flow (Zacest, 1975).



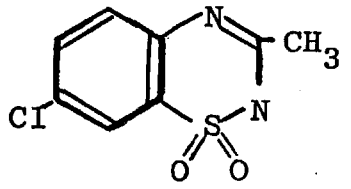
Most vasodilators cause an increase in plasma renin activity (Zacest, 1975).

Adverse effects:- Use of high doses may produce toxicity and symptoms resembling lupus erythematosus (Alarcon-Segovia et al., 1967).

Metabolic fate:- Hydralazine is well absorbed from the gastro-intestinal tract. Its action is principally terminated by metabolism, approximately 15% of an oral dose being excreted in the urine unchanged (McIsaac and Konda, 1964). The major metabolic transformations are N-acetylation, and ring hydroxylation with subsequent glucuronide formation.

The acetylation of hydralazine is a rare example of genetic variations in drug metabolism. The phenotypes for "slow" and "fast" acetylation separate into two populations which within themselves are continuously distributed. A recent pharmacokinetic study (Zacest and Koch-Weser, 1972), has shown that for a given dose of hydralazine, the slow acetylators have a higher plasma concentration of the drug than the fast acetylators, and a retrospective analysis (Perry et al., 1970) suggests that the incidence of hydralazine toxicity is largely confined to the slow acetylator group.

Diazoxide: although this compound is closely related to the thiazides, it has no diuretic properties.



Intravenous preparations of the drug cause a prompt fall in blood pressure below the normal range (Saker et al., 1968) and consequently it is very useful in hypertensive emergencies. Diazoxide directly dilates arterioles but has little effect on large veins (Thirwell and Zsoter, 1972).

Adverse effects: The drug causes marked salt and water retenion (Kincaid-Smith, 1975).

Less commonly used antihypertensive agents

Pargyline: N-Benzyl-N-methyl-2-propynylamine belongs to the "monoamine oxidase inhibitor" group of drugs. The mechanism by which these compounds elicit a hypotensive effect still remains obscure.

Veratrum alkaloids: These cause repetitive firing of afferent vagal fibres in the carotid sinus and left ventricle (Juhász-Nagy and Szentiránÿi, 1961), resulting in the Benzold-Jarisch reflex (a relatively brief response characterized by hypotension and bradycardia; Jarisch and Richter, 1939).

Nitrites: The haemodynamic effects of inorganic nitrite, various organic nitrites and nitrates are due to their vasodilatory properties.

Mechanism of Action of Debrisoquine and Other Adrenergic Neurone Blocking Agents

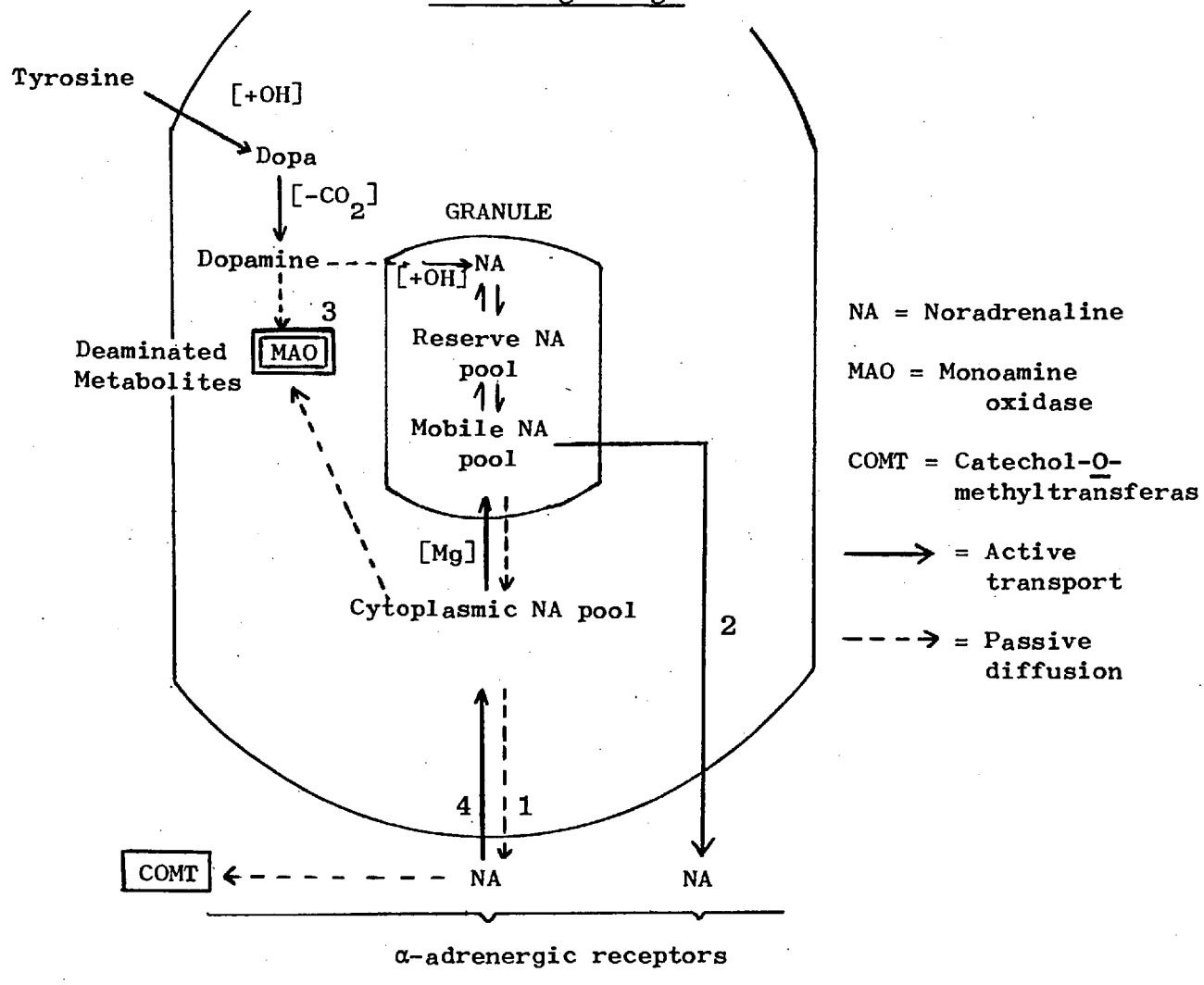
Debrisoquine is an adrenergic neurone blocking agent like bretylium, guanethidine and bethanidine. The exact mechanism by which these compounds exert their hypotensive effect is not understood, but seems to be related to their multiple metabolic actions on the sympathetic receptor-neurone complex summarized in Fig. 1.12.

Adrenergic neurone blocking drugs are taken up by, and concentrated within, the adrenergic nerve terminal (Boura and Green, 1965; Mitchell and Oates, 1970; Shah et al., 1974). This group of drugs have a common transport mechanism, since bethanidine uptake may be inhibited by guanethidine or debrisoquine (Mitchell and Oates, 1970). However, their accumulation is largely cocaine-sensitive (Shah et al., 1974) and may be inhibited by noradrenaline (Prasad et al., 1973), suggesting that the uptake process is the same as that for the neurotransmitter.

Once concentrated in the nerve terminal, adrenergic blocking drugs have a variety of actions. Bretylium, guanethidine and to a lesser extent debrisoquine, have a local anaesthetic effect on the neuronal membrane, which prevents action potentials from invading the nerve ending and releasing the neurotransmitter (Haeusler et al., 1968, 1969a,b, 1974).

Nevertheless, the hypotensive effect of these drugs is mainly attributed to their interference with the release of noradrenaline from sympathetic nerve terminals. Bretylium and guanethidine reduce the amount of noradrenaline released

Figure 1.12. Proposed Sites of Action of Adrenergic Neurone Blocking Drugs



Sites of action

1. Block of nerve-mediated release of noradrenaline from nerve endings.
2. Release of noradrenaline from granules. This occurs mainly with guanethidine but is the mechanism of blood pressure elevation caused by intravenous doses of guanidinium anti-hypertensives.
3. Inhibition of mitochondrial MAO - the site of intraneuronal noradrenaline metabolism.
4. Competitive inhibition of re-uptake of noradrenaline - a major route of inactivation of noradrenaline at the receptor site.

from the cat spleen following stimulation of the splenic nerve (Boura and Green, 1959; Abercrombie and Davies, 1963). Debrisoquine is thought to exert a similar pharmacological action (Moe et al., 1964).

The noradrenaline releasing action of this group of drugs is also well documented (Green, 1962; Abbs, 1966; Durant et al., 1970). Guanethidine, alone of the common neurone blocking agents, causes gross depletion of noradrenaline stores (Cass et al., 1960). Debrisoquine and bretylium on the other hand, cause initial localized depletion of noradrenaline stores in the intraneuronal vesicles (Malmfors and Abrams, 1970; Giachetti and Shore, 1967; Tomlinson and Mayor, 1972).

With the exception of guanethidine (Kuntzman and Jacobson, 1963), all the adrenergic neurone blocking drugs appear to inhibit intraneuronal monoamine oxidase reversibly, at concentrations comparable with those likely to occur within the nerve terminal (Giachetti and Shore, 1967; Pettinger and Horst, 1967; Medina et al., 1969). The anatomical sensitivity of enzyme inhibition by debrisoquine is suggested by increased sensitivity to intravenous tyramine but not to orally ingested tyramine (Pettinger et al., 1969). Hence with most neurone blocking agents, the released noradrenaline is protected by the inhibition of monoamine oxidase, but with guanethidine, the transmitter is metabolized and lost from the nerve ending. Nonetheless, the relationship between the adrenergic neurone blocking action and the intraneuronal monoamine oxidase inhibition of these drugs, is still in question. For example, doses of debrisoquine and bretylium

which produce monoamine oxidase inhibition do not block transmitter release (Malmfors and Abrams, 1970), whereas guanethidine which does not inhibit monoamine oxidase, inhibits the release of noradrenaline.

Thus adrenergic neurone blocking drugs have a variety of actions, some of which prevent the release of noradrenaline at nerve endings. However, the combination of two properties seem to be essential for the ability of a compound to cause adrenergic neurone blockade: uptake into and accumulation within adrenergic nerve endings and a weak membrane stabilizing property.

Clinical Pharmacology of Debrisoquine

1. Haemodynamic Effects

In pharmacological studies, debrisoquine is shown to have potent antihypertensive effects in normotensive and hypertensive human subjects and experimental animals (Abrams et al., 1964; Moe et al., 1964). A biphasic blood pressure response occurs after intravenous bolus doses of the drug to human subjects (Kakaviatos et al., 1964; Pocelinko and Abrams, 1964) and anaesthetized dogs (Moe et al., 1964). There is an initial pressor effect of one to four hours duration, followed by prolonged hypotension. In dogs, the blood pressure elevation is closely followed by a positive inotropic effect on the heart. In patients, 20-50 mg of intravenous debrisoquine results in an arterial pressure elevation of approximately 35 mg Hg and is associated with increased cardiac output and decreased peripheral vascular resistance. Transient hypertension does not occur when the drug is given orally unless very large doses (80 mg or greater) are used (Luria and Freis, 1965). After single oral therapeutic doses, onset of action of the drug varies between 4 to 10 h, causing a relatively small reduction of blood pressure as measured in the supine position but a substantial fall in the erect position. The duration of the hypotensive effect is between 9 and 24 h. The response of one patient to the same dose of debrisoquine given intravenously and orally is shown in Figure 1.13.

The pressor overshoot after the Valsalva manoeuvre is blocked by debrisoquine (Pocelinko and Abrams, 1964) indicating sympathetic blockade.

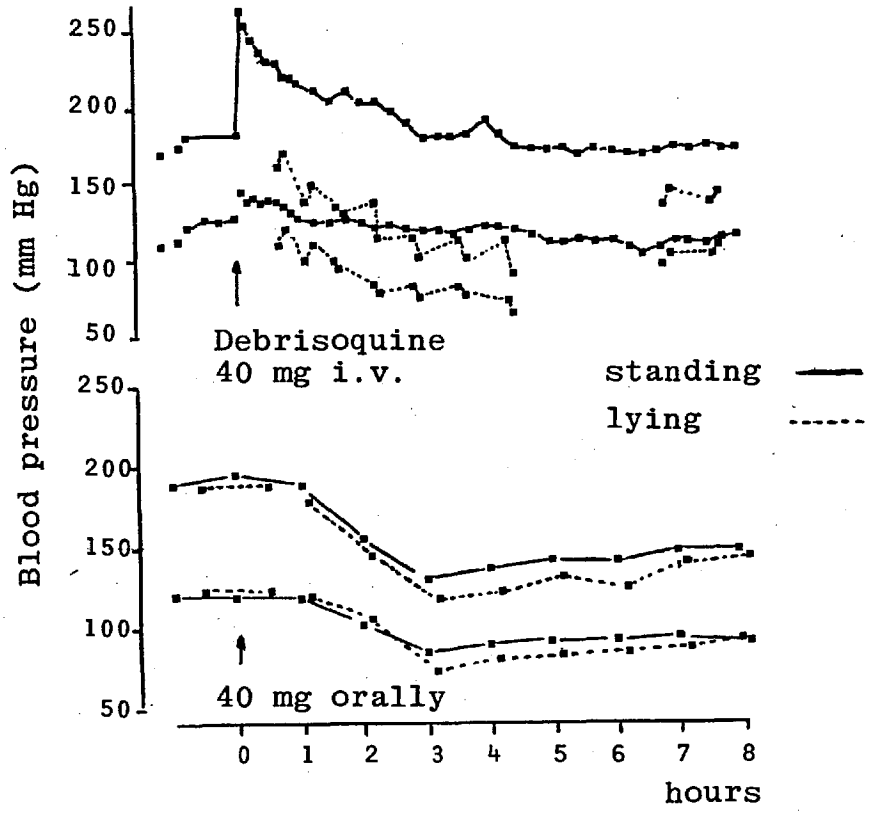


Figure 1.13. Effect of Intravenous and Oral Debrisoquine on Standing and Lying Arterial Blood Pressures

(after Athanassiadis et al., 1966)

Haemodynamic observations have been reported by two groups of workers and there is some discrepancy between their results. Onesti et al. (1966), have found that the drug lowers blood pressure by decreasing cardiac output. Whether this reduction is a direct action or a consequence of reduced venous pressure due to an effect on capacitance vessels was not established. They also observed no effect on arterial tone, but a significant reduction in renal blood flow. However, the other group of investigators (Abrams et al., 1964; Pocelinko and Abrams, 1964), found the hypotensive effect to be associated with diminished peripheral vascular

resistance, relatively good preservation of cardiac output and well-maintained renal blood flow, but diminished glomerular filtration in the erect position. These workers suggest the major functional effect of sympathetic blockade to be on arteriolar rather than venous tone.

2. Adverse Effects

The most frequent untoward reaction of debrisoquine which is often the dose-limiting factor, is postural hypotension. This is particularly evident after exercise and is, in fact, an exaggeration of the therapeutic response. Postural hypotension is manifested by dizziness, light-headedness and weakness on standing, especially in the morning, (Sommers et al., 1968; Belle et al., 1968). Less common side effects include nasal obstruction, nausea, mild diarrhoea and failure of ejaculation in older men (Heffernan and Carty, 1970; Rosendorff et al., 1968). Therapeutic doses of debrisoquine have been found to cause suppression of human paradoxical sleep and also increase intra-sleep restlessness (Dunleavy et al., 1971).

No toxic effects on the kidney, liver and bone marrow of patients have so far been observed (Orgain and Kern, 1970; Kitchen and Turner, 1966). Histological studies done at the neuronal level in the rat have shown that unlike guanethidine and bretylium, prolonged debrisoquine treatment does not cause permanent functional impairment or degeneration of adrenergic nerve terminals (Haeusler et al., 1974).

a) Acute toxicity

The acute LD_{50} values for debrisoquine sulphate have been determined by four different routes in mice (Abrams et al.,

1964)

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

The oral LD_{50} for the rat is 1580 mg/kg (Toxic Substances List, 1973).

b) Reaction to food and other drugs

Antagonism of the hypotensive action of debrisoquine by tricyclic antidepressant drugs has been shown in human subjects (Skinner et al., 1969). The precise mechanism involved is not clear, but may be due to inhibition of neuronal uptake of debrisoquine and hence prevention of its adrenergic-blocking action, by the antidepressant. Hypertensive responses due to the interactions of debrisoquine with phenylephrine and with tyramine have been observed (Amery and Deloof, 1970; Allum et al., 1974). The monoamine oxidase-inhibitory action of debrisoquine could account for this effect.

3. Therapeutic Efficacy

Several reports on the clinical use of debrisoquine have been published (Kitchen and Turner, 1966; Schultz, 1966; Gent and Bacon, 1967; Sommers et al., 1968; Cole and Adadevoh, 1970; Orgain and Kern, 1970). These differ in the assessment of effectiveness, duration of treatment and in the patient population selected. There is, however, general agreement that debrisoquine is an effective agent in the treatment of moderate or severe hypertension. An

outstanding feature of most clinical investigations on debrisoquine is the marked inter-individual differences in optimal dose requirement (General Practitioner Clinical Trials, 1969; Athanassiadis et al., 1966; Kitchen and Turner, 1966). The discovery of the underlying reason for this is one of the objectives of the present study. Doses used range from 20 to 400 mg daily, the average being about 70 mg (Athanassiadis et al., 1966; Kitchen and Turner, 1966).

Long-term therapy with the drug is reported to result in an increased daily dose requirement. Some workers have observed that an augmentation of up to 30% is necessary (Rosendorff et al., 1968; Kitchen and Turner, 1966) whereas others find that an increment in the dosage regimen is seldom needed.

Poor control of blood pressure in some patients treated with debrisoquine may usually be overcome by concommitant administration of a diuretic (Schultz, 1966; Orgain and Kern, 1970). This serves as a result of glomerular filtration rate reduction by the antihypertensive agent (Athanassiadis et al., 1966). One group of workers have claimed that debrisoquine potentiates the effect of other hypotensive drugs, long after debrisoquine treatment has been stopped (Bryant et al., 1964), but this effect has yet to be confirmed.

Although experience is limited, there seems at present, to be no contra-indication to the use of debrisoquine in pregnancy. Athanassiadis et al. (1966),treated a group of hypertensive women with the drug at some stage during their pregnancy and were unable to find any signs of adverse effects in the infants.

Comparison with other antihypertensives

Thus far, only two formal comparisons of debrisoquine with other antihypertensive drugs have been published, most of the information available being the result of uncontrolled observations. In the two double blind randomized clinical trials, using a cross-over design, no statistically significant difference was found between debrisoquine and guanethidine (Adi et al., 1975) and debrisoquine and α-methyldopa Heffernan et al., 1971). However, the nature and extent of the adverse effect differed. At equipotent doses, debrisoquine was better tolerated than guanethidine. Tiredness was caused by α-methyldopa treatment and postural hypotension was the most troublesome effect of debrisoquine.

Debrisoquine Metabolism and Disposition

1. Absorption, and pattern of elimination

Information concerning the fate of debrisoquine in man and laboratory animals is limited to data obtained by the Roche Clinical Research Department (see Kitchen and Turner, 1966). The drug appears to be well absorbed in man, 73% of an oral dose of [^{14}C]debrisoquine being excreted in the first day urine. These preliminary studies also reveal a varying pattern of elimination in the rat, depending on the route of administration. After intraperitoneal dosing, 52% of the dose was excreted in the urine and 42% in the faeces, whereas oral administration resulted in 37% of the dose appearing in the urine and 50% in the faeces.

2. Distribution

Distribution studies in the rat show that after intraperitoneal injection of [^{14}C]debrisoquine, the drug and its metabolic products can be found in the heart, liver and intestine 6 h after injection. However, at 16 h, metabolic products are only found in the intestine, urine and faeces. The drug cannot be detected in the brain at any time, and is bound to plasma protein only to a small extent (15-30%). There is a linear relationship between dosage of debrisoquine and accumulation *in vivo*, which suggests that the drug is not bound to specific saturable cellular binding sites (Medina *et al.*, 1969).

3. Metabolism

Williams (1959) has stated that "the biochemical reactions of the guanidino group in foreign compounds are not known, but

the evidence available suggests that it is relatively stable *in vivo*". The only information available regarding the nature of the debrisoquine metabolites is in agreement with this statement (Kitchen and Turner, 1966; Medina *et al.*, 1969).

Objectives of the Present Study

A project on debrisoquine was undertaken with a view to achieving the following objectives:

a) To establish the metabolic fate and pattern of elimination of the drug in a laboratory species and man.

b) To establish the pharmacokinetics of the drug and its metabolites in plasma.

c) To investigate the basis for inter-individual differences in the therapeutic dose requirement of the drug.

d) To determine the effect of other drugs (i.e. diuretics) that are commonly used in conjunction with debrisoquine.

CHAPTER TWO

MATERIALS AND METHODS

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Materials

Radiochemical Synthesis of [^{14}C]Debrisoquine Hydrochloride

[^{14}C]Cyanamide

This compound was prepared essentially as described by Graff *et al.* (1951).

A solution of potassium [^{14}C]cyanide (1.13 mg, 1 mCi) (The Radiochemical Centre, Amersham, Bucks., U.K.) and non-radioactive potassium cyanide (649 mg) in water (6.5 ml) was added over a fifteen minute period to a stirred and cooled (0-5°C) mixture of bromine (0.5 ml) and water (7.0 ml). After a further fifteen minutes, the pale yellow solution was extracted with diethyl ether (3 x 10 ml) and the combined ethereal extracts were dried over anhydrous sodium sulphate for 3 h. The ethereal solution was then treated without removal of the sodium sulphate, with 5 ml of a 2.9-M solution of ammonia in methanol. Ammonium bromide soon precipitated from the solution and the mixture was left at 0°C overnight to complete the precipitation. The solids were filtered off, and the solvent removed in a rotary evaporator. The oily product was dried *in vacuo* over phosphorus pentoxide for 20 h and treated with dry ether to precipitate further ammonium bromide which was then filtered off. On evaporation, the filtrate yielded 0.368 g of crystalline [^{14}C]cyanamide.

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

This was prepared by a modification of the method of Wenner (1965) as follows:-

The above |^{14}C|cyanamide and 1,2,3,4-tetrahydroiso-

quinoline hydrochloride (1.56 g) were suspended in toluene (8.0 ml). The mixture was stirred and heated to reflux for 6 h. This soon separated into two phases and after 2-3 h the lower phase became extremely viscous. After cooling and allowing to stand for 15 h, the upper toluene layer was decanted off and the lower glass-like layer taken up in boiling ethanol (2.0 ml) and the solution cooled in an ice-bath. Acetone (5.0 ml) was added to give a crystalline solid. This was recrystallized twice from ethanol to give 576 mg of 3,4-dihydro-2(1H)-isoquinoline [^{14}C] carboxamidine hydrochloride (specific activity 0.5 µCi/mg), m.p. 176-179°C (Wenner quotes 179-181°C). The radiochemical yield was 28.8%, calculated on the potassium [^{14}C] cyanide. The radiochemical purity was determined by reverse isotope dilution and paper chromatography in systems C & D and thin layer chromatography in systems A & B and was found to be >97%.

Reference Compounds

3,4-Dihydro-2(1H)-isoquinoline carboxamidine (debrisoquine) sulphate (Ro 5-3307/1; Declinax) pka approximately 12.0, m.p. 274-276°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 (dec.)

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

All the above listed compounds were gifts from Hoffman La Roche.

3,4-Dihydro-2(1H)-isoquinoline carboxamidinoxime-p-toluene sulphonate (Sa-S-17), m.p. 112-114°C (gift from Sterling-Winthrop Research Institute, Rensselaer, N.Y., U.S.A.).

Synthesis of 4-Hydroxy-Debrisoquine Sulphate

2-(1-Hydroxy-2-nitroethyl)-benzaldehyde lactol

This was obtained by the method of Baer and Achmatowicz (1964).

A mixture of o-phthalaldehyde (7.73 g) and nitromethane (137.0 ml) was stirred overnight at 25-30°C in the presence of anhydrous sodium carbonate (15.5 g). The sodium carbonate was then filtered and the solution evaporated to dryness in a rotary evaporator. The residue was washed several times with benzene, and filtered. The resulting white crystalline lactol (2.93 g) was recrystallized from chloroform and had m.p. 120-122°C (dec.). (Literature value 121-123°C (dec.)).

4-Hydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride

This was prepared by the method of Nakao et al. (1966). The 2-(1-hydroxy-2-nitroethyl)-benzaldehyde lactol was reduced in two batches, by the following procedure.

A solution of the lactol (1.0 g) in 50% acetic acid (10 ml) was hydrogenated in the presence of platinum dioxide (40 mg) for 2-3 h under a pressure of 40 p.s.i. The platinum dioxide was then removed by filtration and the remaining solution was evaporated to dryness in vacuo at 35°C. The pale yellow oil thus formed was washed with water (2 x 10 ml) to remove the acetic acid. The resulting dark yellow oil was dissolved

in the minimum volume of ethanol and treated with a slight excess of dry HCl in ethanol. The solution was evaporated to dryness and the residue taken up in ethanol (2 x 10 ml) and further evaporated to remove the remaining acetic acid. The 4-hydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (458 mg) was recrystallized from ethanol (m.p. 196°C).

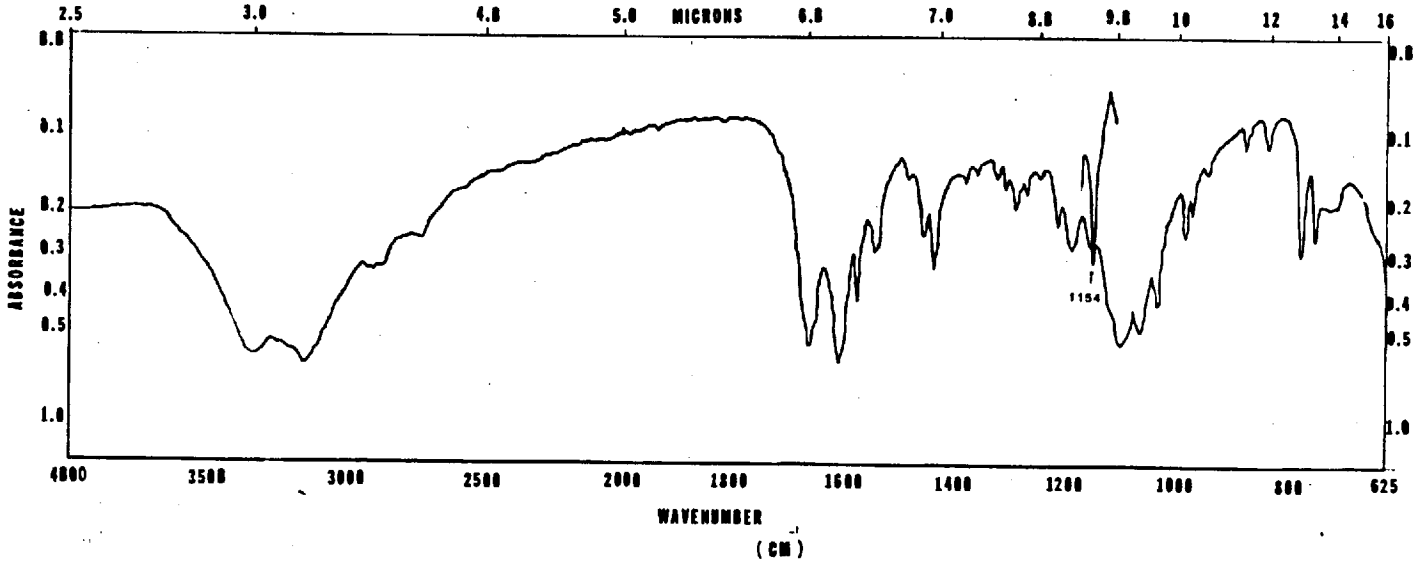
4-Hydroxy-3,4-dihydro-2(1H)-isoquinoline carboxamidine sulphate (4-Hydroxy-debrisoquine)

The final condensation step was performed by a modification of the method by Wenner (1965):

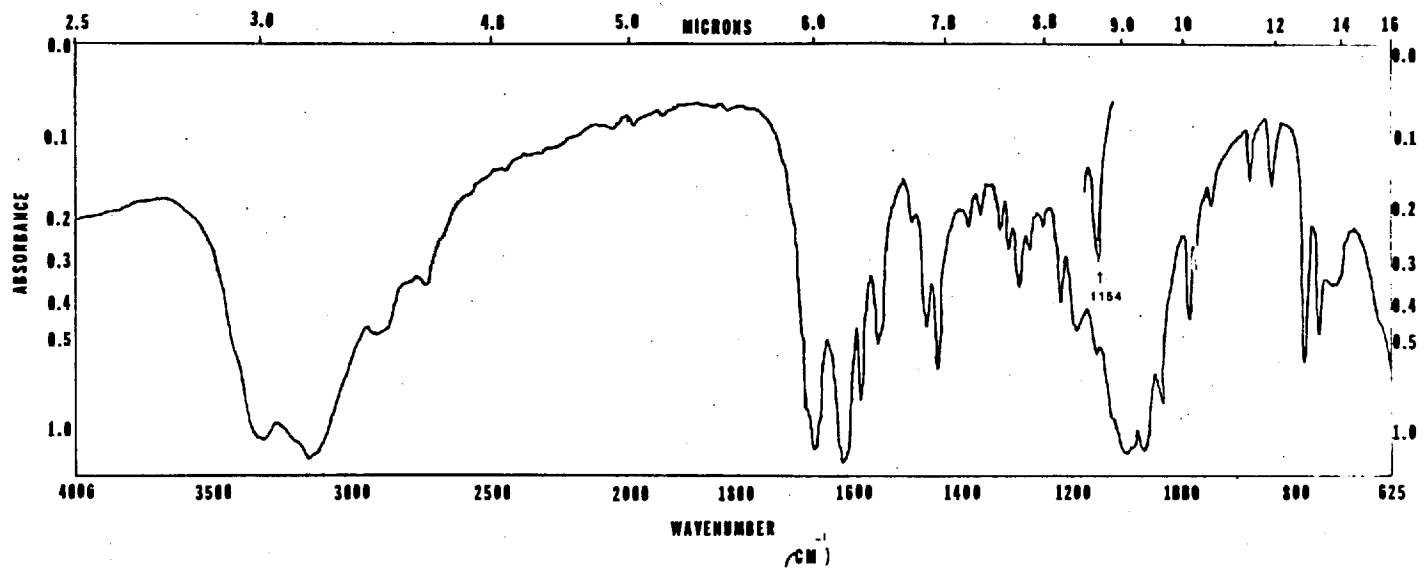
4-Hydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (458 mg) was dissolved in water, and the aqueous solution filtered and extracted with dichloromethane to remove any impurities. The solution was then made strongly basic with 40% sodium hydroxide, and the milky base liberated was extracted with dichloromethane (3 x 10 ml). The bulked solvent extracts were dried by filtering through phase separating paper (Whatman P.S.) and evaporated under reduced pressure. The pale yellow base (416 mg) thus liberated, was suspended in water (2 ml) and this solution was mixed with S-methylpseudothiourea sulphate (425 mg) in water (3 ml). The unstoppered reaction mixture was allowed to stand at room temperature in a fume-cupboard overnight. After 16 h, crystals of 4-hydroxy-debrisoquine began to separate. These were filtered to give 68 mg of product (m.p. 253-254°C).

The infra-red spectrum (see Fig. 2.1.) was identical to a sample of 4-hydroxy-debrisoquine provided by Roche Products (Welwyn Garden City, Herts., U.K.; m.p. 253-254°C). In each case the position of hydroxylation was determined by n.m.r. (see Fig. 2.2.).

Figure 2.1. Infra-red Spectra of 4-Hydroxy-debrisoquine

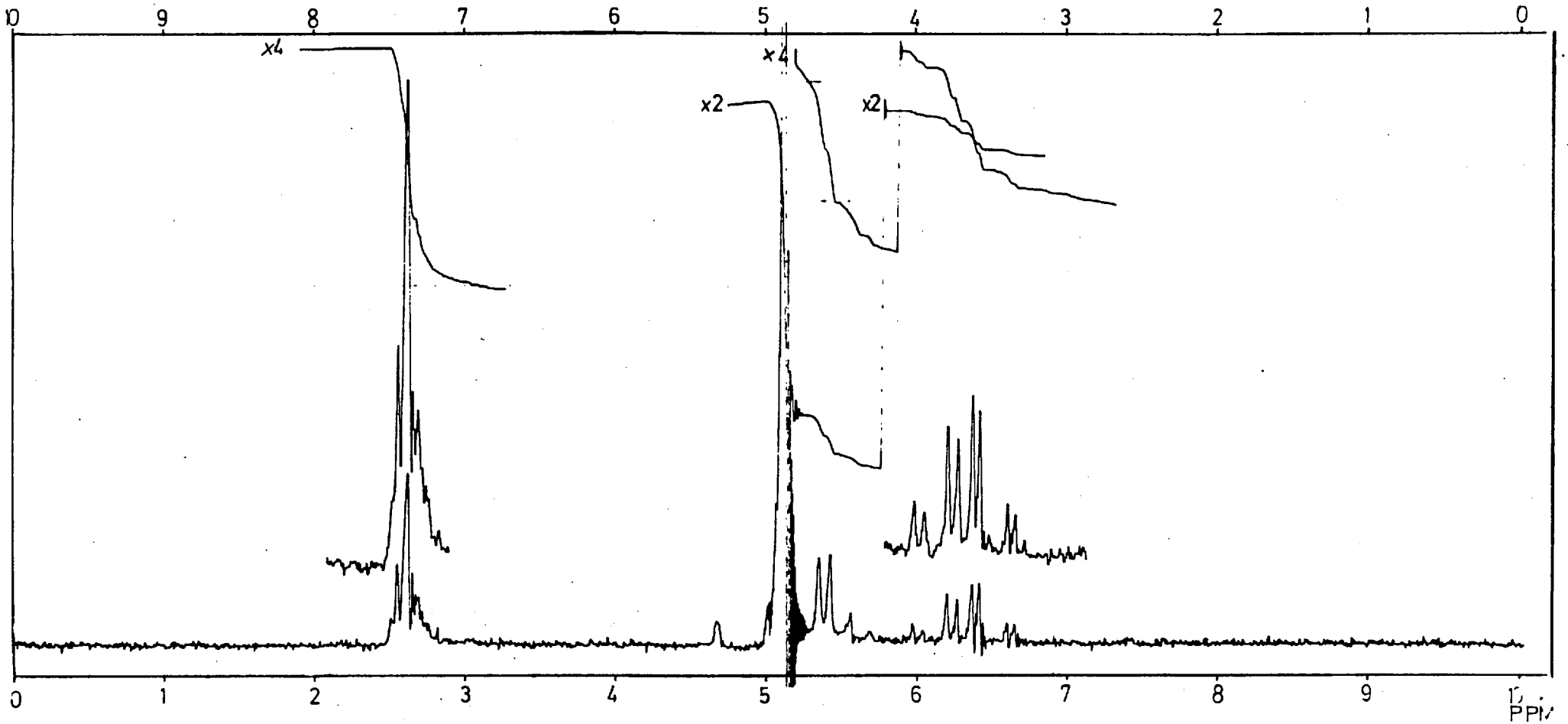


a) synthetic sample



b) standard compound

Figure 2.2. N.m.r. Spectrum of Synthetic 4-Hydroxy-debrisoquine



Synthesis of N-Carbamoyltetrahydroisoquinoline

The method used was essentially that of Bamber and Dieckmann (1893). Tetrahydroisoquinoline hydrochloride (1.68 g; 10 mmol) and potassium cyanate (0.81 g; 10 mmol) were dissolved separately in the minimum of water. The two solutions were then mixed and allowed to stand at room temperature. Shiny leaflets separated which were filtered and dried *in vacuo*. N-Carbamoyltetrahydroisoquinoline (yield = 1.4 g; 81%) had m.p. 169°C (identical to literature value).

Methods

Animals

Female Wistar albino rats (0.2 kg body weight) were obtained from Allington Farm, Porton Down, Wilts. U.K.) and maintained on a standard diet of Oxoid 41B (Oxo, Poole, Dorset, U.K.). Human subjects were male volunteers from this department and patients at St. Mary's Hospital, Harrow Road, London, W.9.

Administration of ^{14}C-labelled debrisoquine

Rats were given an aqueous solution of [^{14}C]debrisoquine hydrochloride (10 mg; 5 μCi) orally, by gastric intubation. Human subjects were given orally a mixture of debrisoquine sulphate (30 mg) and [^{14}C]debrisoquine hydrochloride (10 mg; 5 μCi), dissolved in water or contained in a gelatine capsule.

Collection of excreta

Rats were housed individually in glass metabolism cages ("Metabowl"; Jencons Scientific Apparatus Ltd., Hemel Hempstead,Herts., U.K.) designed for separate urine and faeces collection. They were allowed free access to water, but food was withheld for the first 24 h to avoid contamination of the urine.

Human excreta were collected in plastic bottles and plastic-lined tins as appropriate.

All excreta were stored at -15°C.

Collection of expired air

The Metabowls allowed for complete trapping of expired carbon dioxide. A vacuum pump was used to draw air from each Metabowl through a drying trap of anhydrous calcium chloride, then through two traps containing 400 ml and 150 ml

respectively of a solution of redistilled ethanolamine in 2-methoxyethanol (1:2 v/v) which absorbed the carbon dioxide present (Jeffay and Alvarez, 1961).

Collection of blood samples

Blood samples (20 ml) were taken from human subjects at one to two hourly intervals after dosing, by multiple venous puncture. The blood was immediately transferred into tubes containing lithium heparin-coated granules (Searle; High Wycome, Bucks., U.K.) and centrifuged to separate the plasma from the red blood cell fraction.

Radiochemical techniques

^{14}C was determined with a scintillation spectrometer (Packard models 3214 and 3320).

Urine: Samples (0.1-1.0 ml) were counted for radioactivity in duplicate in vials containing a dioxan-based scintillator fluid (Bray, 1960; 15 ml). Quench correction was performed by the "channels ratio" method (Bridges et al., 1967).

Faeces: All faecal samples were homogenized for counting. Human faecal homogenate, being strongly coloured unlike the rat excreta, was bleached by adjusting to pH 10 with 40% NaOH and treating with an equal volume of hydrogen peroxide (100 vol.). The mixture was left to stand at room temperature for 48 h, then neutralized. Aliquots of bleached human faecal homogenate (0.5 ml) and untreated rat faecal homogenate (0.5 ml) were counted for radioactivity after suspension in Bray's scintillator with thixotropic gel (Cab-O-Sil; Koch-Light Laboratories Ltd., Bucks., U.K.).

Expired air: Aliquots (2 ml) of the trapping solution containing dissolved carbon dioxide, were counted in a toluene:2-methoxyethanol (2:1 v/v) scintillation medium containing 5.5 g/l of 2,5-diphenyloxazole (PPO) as described by Jeffay and Alvarez (1961). The efficiency of counting was determined by the use of [^{14}C]toluene internal standard (Packard Instrument Co. or Radiochemical Centre, Amersham, Bucks., U.K.).

Plasma samples: Untreated plasma samples (1-2 ml) were counted in the same way as the faecal homogenates. Aliquots of plasma were also extracted and counted directly by the following procedure:

Extraction of plasma samples

Plasma samples (2-6 ml) were adjusted to pH 13.5 with 40% NaOH and extracted with chloroform (4 x 6 vol.) by agitating on a mechanical shaker for 15 min. The plasma was saturated with NaCl prior to the final extraction. The combined extracts were evaporated to dryness *in vacuo*, the residue taken up in methanol (2 ml) and counted directly for radioactivity.

The validity of this method was determined by spiking plasma samples (2 ml) with known amounts of [^{14}C]debrisoquine (50-200 d.p.m.) and estimating the efficiency of extraction, which was found to be 86.3 ± 1.9% (S.D.)

Radiochromatogram scanning

Paper chromatograms and thin-layer plates were scanned on a Packard model 7200 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 these in the scintillation spectrometer. The paper chromatography system D was used and is discussed on p. 100.

Reverse isotope dilution technique for debrisoquine estimations

Debrisoquine sulphate (approx. 1 g accurately weighed), was added to the urine sample to be analysed (containing approx. 150,000 d.p.m.). The compound was thoroughly dissolved by the addition of water and the solution made strongly basic (pH >13) with 40% NaOH. The solution was then saturated with NaCl, and extracted with dichloromethane (3 x 2 vol.). The pooled extract was 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 off and recrystallized from ethanol to constant specific radioactivity.

Concentration of metabolites

Urine samples (0-24 h) were initially concentrated by freeze drying (Freeze Drier Model B67, New Brunswick Co. Inc., New Brunswick, N.Y., U.S.A.) and leaching the solid residue with methanol. The resulting extracts were reduced to a small volume, by evaporation under reduced pressure, and stored overnight at -15^{o}C to precipitate urea. After filtering under suction, the solution was evaporated to dryness *in vacuo* and the residue re-dissolved in water.

Further concentration and purification of this aqueous metabolite solution was achieved by extraction onto Amberlite XAD-2 resin (B.D.H. Chemicals Ltd., Poole, Dorset, U.K.), which is a non-ionic styrene-divinyl benzene copolymer. The resin was first prepared by washing successively with acetone, methanol and distilled water as described by Mulé *et al.* (1971) and then packed in a glass column, the size of which varied depending on the volume of solution to be treated (urine:resin, 2:1 v/v). The aqueous metabolite fraction was then passed through the column at a rate of 5 ml/min and aliquots of the resulting eluate (1 ml) were counted for radioactivity as an index of adsorption of the metabolites onto the resin. The column was then washed with an excess of distilled water and allowed to run dry after which any residual water was expelled by means of a stream of N_2 gas. The adsorbed radioactivity was eluted with methanol until over 90% was recovered in the eluate. The methanolic solution was evaporated to dryness and the residue dissolved in the minimum of methanol.

Separation of metabolites

The concentrated metabolites were chromatographed on Whatmann 3 mm paper and developed in solvent system D. A loading of 50-100 µl of solution per 5 cm of paper was used and the compounds were located by radiochromatogram scanning. The chromatographic band corresponding to each metabolite fraction was cut out and eluted separately by submerging in a tank containing methanol for periods up to 2 h. The methanol extracts were then reduced *in vacuo* to a small volume, in preparation for the final purification stage.

Purification of metabolites.

The isolated metabolite fractions were chromatographed on plastic-backed silica gel 60 F_{254} plates (E. Merck A.G., Darmstadt, Germany; 0.25 mm thick), pre-eluted with methanol and developed in solvent system A or B. After location of the metabolite bands by radiochromatogram scanning, these were cut out and eluted in the same manner as the paper chromatograms.

Gas-Liquid Chromatography

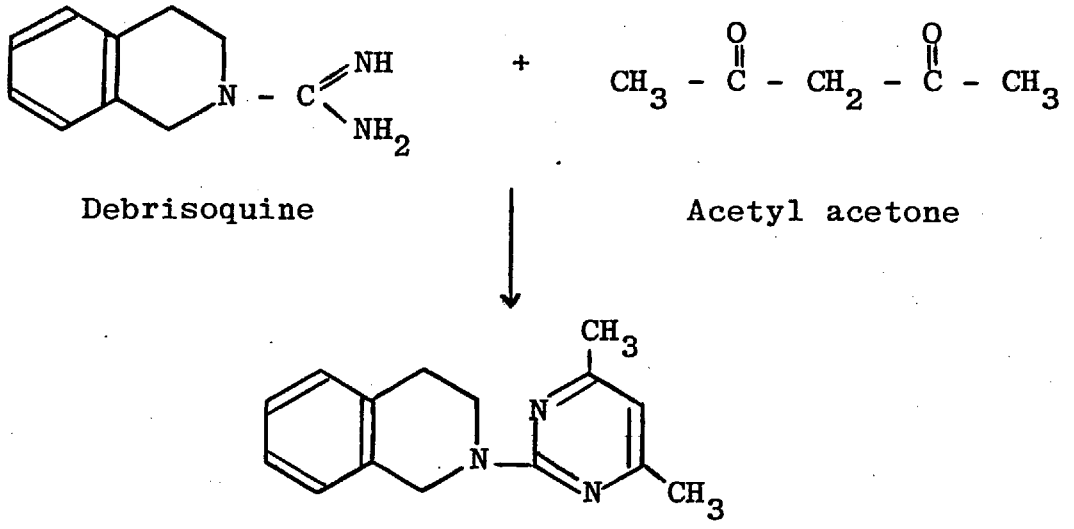
Different procedures were employed for qualitative and quantitative gas-liquid chromatographic (g.l.c.) analysis. The derivatization and chromatographic conditions for each will be discussed separately.

Qualitative Procedure

The reference compounds, freeze-dried urinary extracts, and isolated metabolite fractions were initially derivatized with acetyl acetone essentially by the methods of Shenyakin et al. (1967) and Vetter-Diechtl et al. (1968):

To a solution of the sample (100 µg) in 50% saturated aq. sodium bicarbonate (0.2 ml) and methanol (0.8 ml), was added freshly distilled acetyl acetone (0.2 ml). After incubation at 50°C for 48 h, water (4.8 ml) was added. Basic compounds were extracted with chloroform (3 x 12.0 ml) after adjusting the derivatization mixture to pH 8.5, and acidic compounds were extracted in the same way at pH 4.0.

Although underivatized debrisoquine can be extracted into organic solvents at a pH greater than 12.0, as described previously, it is not possible to extract any of the underivatized metabolites under similar conditions (i.e. at various pH values ranging from 3-12). However, the reaction with acetyl acetone, incorporates the highly basic amidine moeity of these compounds into a relatively non-polar 4,6-dimethyl pyrimidine which may be readily extracted.



Debrisoquine + Acetyl acetone

2-(4,6-Dimethyl-2-pyrimidyl)-
1,2,3,4-tetrahydro isoquinoline.

Further derivatization of the pyrimidine derivatives involved either silylation or methylation.

Silylation. The samples, dissolved in benzene or pyridine, were treated with N,O-bis-(trimethylsilyl)-acetamide (BSA; Phase Separations Ltd., Queensferry, Flintshire, U.K.) or N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) + 1% trimethyl chlorosilane (TMCS; Pierce Chemicals Co., Rockford, Ill., U.S.A.). The mixture was incubated at 60°C for 5-10 min prior to injection on the column.

Methylation. Solutions of the samples in methanol were methylated with ethereal diazomethane. This was prepared from the reaction of N-methyl-N-nitroso-p-toluene sulphonamide (Diazald R; Aldrich Chemical Co. Ltd., Gillingham, Dorset, U.K.) in ether with saturated alcoholic potassium hydroxide, and the resulting ethereal diazomethane was distilled at room temperature. A portion of this was added to the sample and

diazomethane was removed under a stream of N_2 gas (Vogel, 1956).

An alternative on-column methylation procedure was also employed, which involved co-injection of the sample with 0.2M-trimethylanilinium hydroxide in methanol (MethElute™; Pierce and Warriner (U.K.) Ltd., Chester, Cheshire, U.K.).

G.l.c. conditions. The chromatograph used was a Hewlett Packard F & M Scientific 402 fitted wtih a flame ionization detector.

Glass column: 3% OV-225 on Chromosorb W.AW-DMCS (100-120 mesh)
Length of column = 1.22 m (4 ft)
3 mm internal diameter

H_2 pressure: 20 p.s.i.
Air pressure: 25 p.s.i.
N_2 pressure: 40 p.s.i.
N_2 flow rate: 28 ml/min
Oven temp.: 200°C
Injection temp: 240°C
Detector Temp.: 250°C

Quantitative Procedures

1. Debrisoquine estimation by hydrolysis and trichloroacetyl (TCA) derivative formation

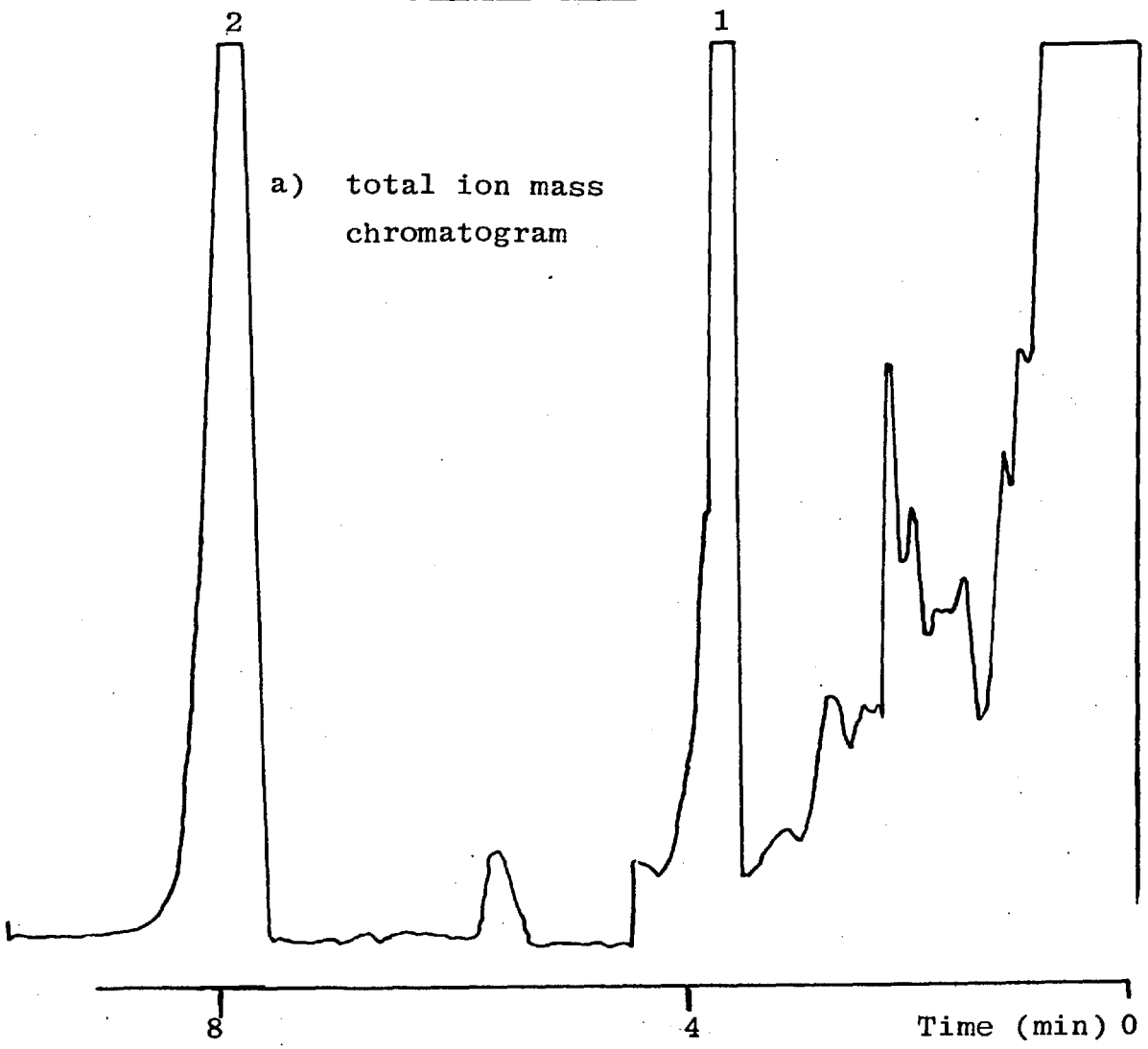
The method used was an adaptation of the hydrolysis and derivatization procedure of Hengestmann et al. (1974)

Urine (5 ml) from debrisoquine-treated subjects, with phenformin (10 µg) added as an internal standard, was adjusted

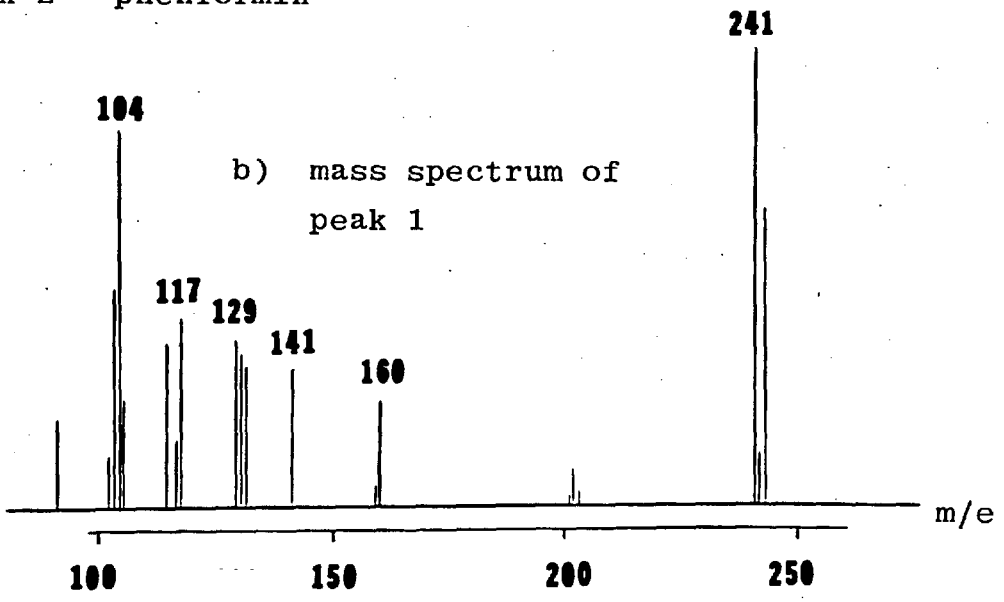
to pH >12 with 50% NaOH (0.5 ml). This allowed extraction of debrisoquine into dichloroethane (2 x 20 ml), followed by its back-extraction into 0.5M-HCl (2 x 4 ml). The guanidino group was then removed by hydrolysis with 40% KOH (1.5 ml) at 110°C for 4 h in screw-cap septum vials (Pierce, Rockford, Ill., U.S.A.). After cooling, the mixture was extracted with hexane (2 x 4 ml). The resulting tetrahydroisoquinoline moeity contained in the pooled aspirated hexane fraction (6 ml), was derivatized by the addition of trichloroacetyl chloride (0.2 ml) and pyridine (0.1 ml). The reaction was left at room temperature for 20 min after which it was washed successively with 2M-HCl (2 x 1 ml), 0.1M-NaOH (2 x 1 ml) and water (2 x 1 ml). The mixture now containing TCA-tetrahydroisoquinoline, was dried over sodium sulphate for 30 min, filtered and the hexane removed with a stream of nitrogen. The residue thus formed was re-dissolved in hexane (200 µl) and a portion of this solution (2 µl) was injected on the column. Evidence for the formation of the halogen acyl-tetrahydroisoquinoline was obtained by gas-liquid chromatography - mass spectrometry (g.l.c.-m.s.; see Fig. 2.3). Although trichloroacetyl chloride was used only the dichlorotetrahydroisoquinoline was found to be present (from mass spectrum). Possibly one of the chlorine ions was lost initially in the ion source of the mass spectrometer.

Quantitation of urinary debrisoquine levels was achieved by the construction of a standard curve of the ratio of debrisoquine/phenformin peak height against concentration of debrisoquine. This was done on each occasion that the samples were analysed. The linear range of the standard curve was

Figure 2.3. G.l.c.-m.s. of Dichloroacetyltetrahydroisoquinoline



Peak 1 = dichloroacetyltetrahydroisoquinoline
Peak 2 = phenformin



G.l.c.-m.s. System III

1-16 µg/ml and the minimum amount detectable was 1 µg/ml of urine.

G.l.c. conditions. The chromatograph was a Hewlett Packard F & M Scientific 402 equipped with an electron capture detector (tritium adsorbed onto titanium foil).

Glass column: = 3% OV-1 on Chromosorb G.
Length of column = 1.22 m (4 ft)
3 mm internal diameter

Carrier gas (5% argon in methane) flow rate = 35 ml/min

Injection port temperature = 210°C

Column temperature = 190°C

Detector temperature = 210°C

2. Debrisoquine and 4-hydroxy-debrisoquine estimations by derivatization with hexafluoroacetyl acetone

Development of this method by Erdmansky and Goehl (1975) made possible for the first time, the analysis of subnanogram quantities of mono-substituted guanidino-containing drugs, without a g.c.-m.s. unit. Furthermore, this technique does not require as much time or sample manipulation as the previously discussed quantitative procedure.

To urine (0.1 ml) in a screw-cap septum vial was added 0.01M-HCl (5 µl) containing phenformin (30 µg) as an internal standard. The mixture was buffered with 1M-$NaHCO_3$ (50 µl) and benzene (0.5 ml) and hexafluoroacetyl acetone (50 µl) were added. The reaction vial was then heated in an aluminium heating block at 100°C for 2 h. After removal from the heating block, 3M-NaOH (5 ml) was added to hydrolyse the excess hexafluoroacetyl acetone. The sample was vortexed, centrifuged and a portion of the benzene phase (2 µl) was

injected on the g.l.c. column.

Debrisoquine and 4-hydroxy-debrisoquine in urine were quantitated by the construction of a standard logarithmic plot of the ratios of phenformin/debrisoquine peak height. against concentration of debrisoquine and the ratio of 4-hydroxy-debrisoquine/phenformin peak height versus concentration of 4-hydroxy-debrisoquine. This was done on every occasion that samples were analysed. The standard curve was linear in the range 20-100 ng/ml and the minimum amount detectable was approximately 1-2 ng/ml of urine.

Phenolic metabolites were detected by the addition of BSA (10% in benzene; 50 µl) to a portion of the benzene layer (50 µl). The solution was mixed and 2 µl injected immediately.

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

Glass column: = 3% OV-1 on Chromosorb W HP 80-100 mesh
Length of column = 1.83 m (6 ft)
3 mm internal diameter

Carrier gas (5% argon in methane) flow rate = 40 ml/min

Injection port temperature = 230°C

Column temperature = 190°C

Detector temperature = 230°C

Paper and Thin-Layer Chromatography

The chromatographic separation of this group of guanidine compounds proved to be unusually difficult. The large number of thin-layer chromatographic (t.l.c.) systems employed for untreated urine gave only two composite radioactive bands. Hence t.l.c. was only used as a final purification step in the isolation of biosynthetic metabolites.

Paper chromatography proved to be more useful providing all samples were adjusted to pH 2 prior to spotting. At values above pH 2, the phenomenon of "double spotting" of standard compounds was observed, which could be due to the occurrence of different ionic forms.

T.l.c. systems

A. Chloroform:methanol:water (5:5:1 v/v)

B Benzene:methanol (1:1 v/v)

Plastic-backed Silica Gel 60 F_{254} plates were used.

Paper systems

C. Propan-2-ol:ammonia (sp. gr. 0.88):water (20:1:2 v/v)

D. Butan-2-ol:formic acid:water (100:12:10 v/v)

Whatman No. 1 or 3 mm paper was used.

The age of solvent system D was found to have a marked effect on the development of the chromatograms. Aged solvent, over a week old, which gave relatively well resolved bands was invariably used. Furthermore, it was observed that standard debrisoquine streaked extensively on paper and this effect could be prevented by the addition of control urine to the standard solution before spotting.

Table 2.1. shows paper chromatographic characteristics of debrisoquine and related compounds.

Table 2.1. R_F Values and Colour Reactions of Debrisoquine and Related Compounds

Compounds	R_F values in solvents C	R_F values in solvents D	Colour with nitroprusside reagent
Debrisoquine*	0.47	0.73	Pink
4-Hydroxy-debrisoquine*	0.33	0.28	Pink
5-Hydroxy-debrisoquine†¶	0.25	0.58	Purple
6-Hydroxy-debrisoquine†	0.22	0.57	Purple
7-Hydroxy-debrisoquine†	0.22	0.57	Purple
8-Hydroxy-debrisoquine†¶	0.22	0.58	Purple
3,4-Dihydro-2(1H)-isoquinoline carboxamidinoxime	0.70 & 0.63	0.75	Orange
N-Carbamoyltetrahydroisoquinoline‡	0.78	0.81	Brown

* detected as a reddish spot with diacetyl reagent

† gave a reddish spot with diazotized 4-nitroaniline

¶ detected as a purple spot with Gibb's reagent

‡ gave a yellow spot with DMAB (see text)

Spray Reagents

Sodium nitroprusside - hydrogen peroxide (Hofmann and Wünsch, 1958). This spray reagent had the following composition: 5% aqueous sodium nitroprusside (2 ml), 3% aqueous sodium hydroxide (1 ml), 3% aqueous hydrogen peroxide (5 ml) and water (15 ml). Guanides appear as coloured spots (see Table 2.1).

Diacetyl reagent. The reagent was made up of equal volumes of 0.1% aqueous diacetyl, 20% aqueous potassium hydroxide and 2.5% ethanolic 1-naphthol. Guanides appear as reddish spots.

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 re-sprayed with 0.5M-NaOH in ethanol (Wickström and Salvesen, 1952). The phenols were detected as red spots.

4-Dimethylaminobenzaldehyde (DMAB). DMAB (0.5% w/v) was dissolved in a mixture of 2M-HCl and ethanol (1:1 v/v). N-Carbamoyltetrahydroisoquinoline gives a canary yellow colour on standing for 12 h.

Gibb's reagent. The paper chromatograms were dipped in a solution of 2,6-dichlorobenzoquinone-4-N-chloroimine in ethanol (0.05% w/v), allowed to dry in air, then sprayed with saturated aqueous $NaHCO_3$ solution. This reaction is specific for phenolic compounds with an unsubstituted para-position. and gives spots of differing colours depending on the position and nature of the substituents. 5-Hydroxy- and 8-hydroxy-debrisoquine gave purple spots whereas the 6-hydroxy- compound showed a very pale blue colouration and the 7-hydroxy-debrisoquine did not react at all.

Spectroscopy

Infra red spectra of samples as KBr discs (0.75% w/w) were recorded on a Perkin-Elmer spectrophotometer Model 137B

Ultra-violet spectra were recorded on a Unicam Recording UV Spectrometer (SP 1800). Table 2.2 gives the extinction coefficients (ε) of aqueous solutions, solutions of reference compounds at acidic and basic pH values.

Proton magnetic resonance spectrum. A Perkin-Elmer R-12A was used.

Mass spectra were obtained by two methods:

Direct insertion.

A Varian MAT CH5 mass spectrometer was used. Samples were introduced into the source using a direct insertion probe fitted with a gold cup.

G.l.c.-m.s.

Three difference g.l.c.-m.s. systems were used, denoted I, II and III as appropriate in the text.

I Finnigan 1015D g.l.c.-m.s. using a 1.52 m x 2 mm column of 3% OV-225 on Chromosorb W, programmed from 180°C to 240°C at 2°C/min using helium as a carrier gas.

II Varian MAT 311A mass spectrometer coupled to a Varian Aerograph 1400 using a column of 3% OV-225 on Chromosorb W programmed from 200°C to 270°C at 6°C/min using helium as a carrier gas.

III Varian MAT CH5 mass spectrometer coupled to a Varian Aerograph 1700 using a column of 3% OV-1 on Chromosorb G; isothermal at a column oven temperature of 190°C, using helium as a carrier gas.

Table 2.2. U.V. Extinction Coefficients (ε) of Debrisoquine and Related Compounds at Acidic and Basic pH Values

Compound	λ_{max} (nm)	ε l/Mol /cm* pH 2	pH 13
Debrisoquine	262	249	319
	270(s)	175	245
4-Hydroxy-debrisoquine	260	210	275
	270(s)	141	195
8-Hydroxy-debrisoquine	271	3078	-
	276(s)	2810	-
	290	-	2734
3,4-Dihydro-2(1H)-isoquinoline carboxamidinoxime.	250-252	917	-

(s) = shoulder

* Molecular weights of the free bases of these compounds were used in calculating ε.

\- = not detected

Enzymatic Hydrolyses

Suitable volumes of urine and isolated metabolite solution, were adjusted to pH 5 with 2M-acetic acid and incubated with either an equal volume of β-glucuronidase solution (Ketodase; Warner-Chilcott, Eastleigh, Hants., U.K.) or 0.2 vol. of an aryl-sulphatase preparation (Type H-2; Sigma Chemical Co., St. Louis, Miss., U.S.A.; which contains some β-glucuronidase) and 0.5 vol. 0.2M-sodium acetate/acetic acid buffer (pH 5) for 24 h at 37°C. Saccharic acid-1,4-lactone (2 mg; Sigma Chemical Co., London, U.K.) was added to the sulphatase incubations to inhibit the β-glucuronidase activity. Several control samples were prepared containing boiled enzyme, saccharic acid-1,4-lactone or phenolphthalein diglucuronide. The formation of phenolphthalein from its glucuronide, indicated by the pink colouration on addition of alkali, demonstrated β-glucuronidase activity. The hydrolysis was terminated in each case by the addition of an excess of methanol and the precipitated enzyme was removed by filtration through sintered glass. The filtrate was decreased in volume under reduced pressure and used for chromatography.

Alkaline Hydrolysis

Urine (1 ml) was made alkaline by the addition of 10% NaOH, using phenolphthalein as an indicator. The solution was boiled for 1 h and then neutralized by initially adding acetic acid and then $NaHCO_3$ until effervescence stopped. A portion of the hydrolysed urine was then used for chromatography.

CHAPTER THREE

METABOLISM OF DEBRISOQUINE IN RAT AND MAN

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Metabolism of Debrisoquine in Rat and Man

Rat Studies

The excretion pattern of [^{14}C] debrisoquine in the rat is represented in Table 3.1 and Figure 3.1. The mean values and standard errors about the mean (S.E.M.) were calculated from the individual excretion figures on each collection day. Of an oral dose of debrisoquine hydrochloride (50 mg/kg), 67.0 ± 4.0% was recovered in the 24 h urine. A significant proportion (10.5 ± 1.0%) was detected in the 0-48 h faeces and no radioactivity was found in the expired air.

Paper chromatography in systems C and D and radiochromatogram scanning, revealed the presence of four radioactive bands. Figure 3.2 represents a typical radiohistogram (developed in system D), where the four fractions have been designated m-1, m-2, m-3 and m-4. Incubation of rat urine with β-glucuronidase resulted in partial disappearance of m-1 to give a peak of higher R_F value, hence suggesting that m-1 is a glucuronide. The conjugate was stable under mild alkaline hydrolysis indicating that it is probably not an ester glucuronide, since the latter would be susceptible to hydrolysis under these conditions (Williams, 1947). Incubation with sulphatase containing saccharic acid-1,4-lactone, suggested that there were no sulphate conjugates present since no shift of the peak m-1 was observed.

Separation and isolation of the four metabolite fractions (m-1, m-2, m-3 and m-4) was achieved as described previously in Chapter Two. The characterization of each metabolite band will be discussed separately.

Table 3.1 Elimination of ^{14}C in the Rat after an Oral Dose of [^{14}C] Debrisoquine

Recovery of ^{14}C Expressed as a % of the Dose

In Urine

Day	Rat 1	Rat 2	Rat 3	Rat 4	Mean ± S.E.M.	
1	64.2	78.8	64.2	60.8	67.0	4.0
2.	70.8	84.7	67.2	67.3	72.5	4.2
3	72.5	86.0	67.8	68.6	73.7	4.2
4	72.8	86.6	68.9	69.2	74.4	4.2
5	73.1	87.1	69.4	69.6	74.8	4.2

In Faeces

Day	Rat 1	Rat 2	Rat 3	Rat 4	Mean ± S.E.M.	
1 + 2	8.2	11.3	9.5	12.8	10.5	1.0
3	8.9	13.2	9.8	14.2	11.5	1.3
4	9.1	13.4	10.1	14.6	11.8	1.3
5	9.3	13.5	10.2	14.8	12.0	1.3

Total

Day	Rat 1	Rat 2	Rat 3	Rat 4	Mean ± S.E.M.	
1 + 2	79.0	96.0	76.7	80.1	83.0	4.4
3	81.4	99.2	77.6	82.8	85.3	4.8
4	81.9	100.0	79.0	83.8	86.5	4.7
5	82.4	100.6	79.6	84.4	86.8	4.7

Figure 3.1. Cumulative Excretion of ^{14}C in the Rat after an Oral Dose of [^{14}C]Debrisoquine

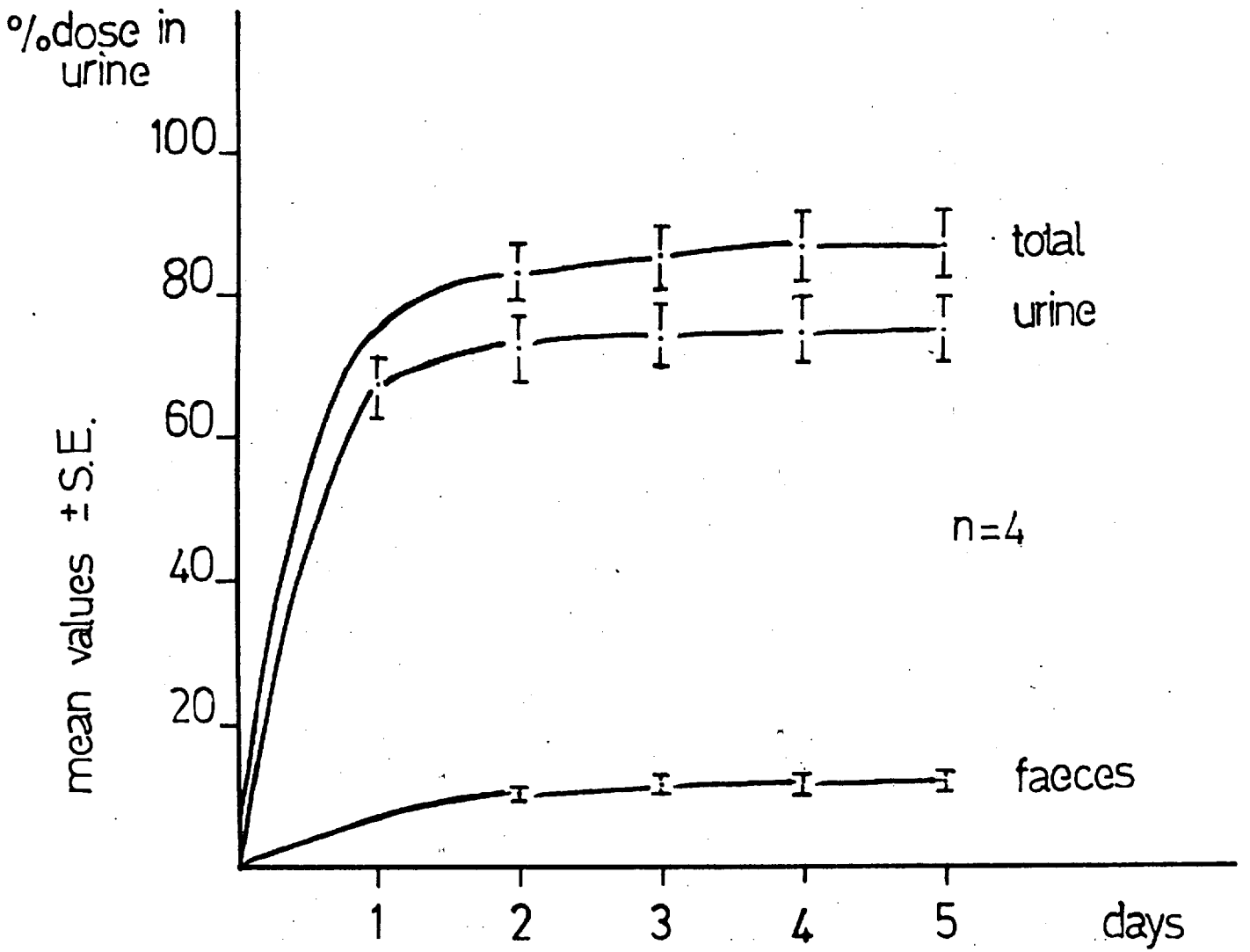
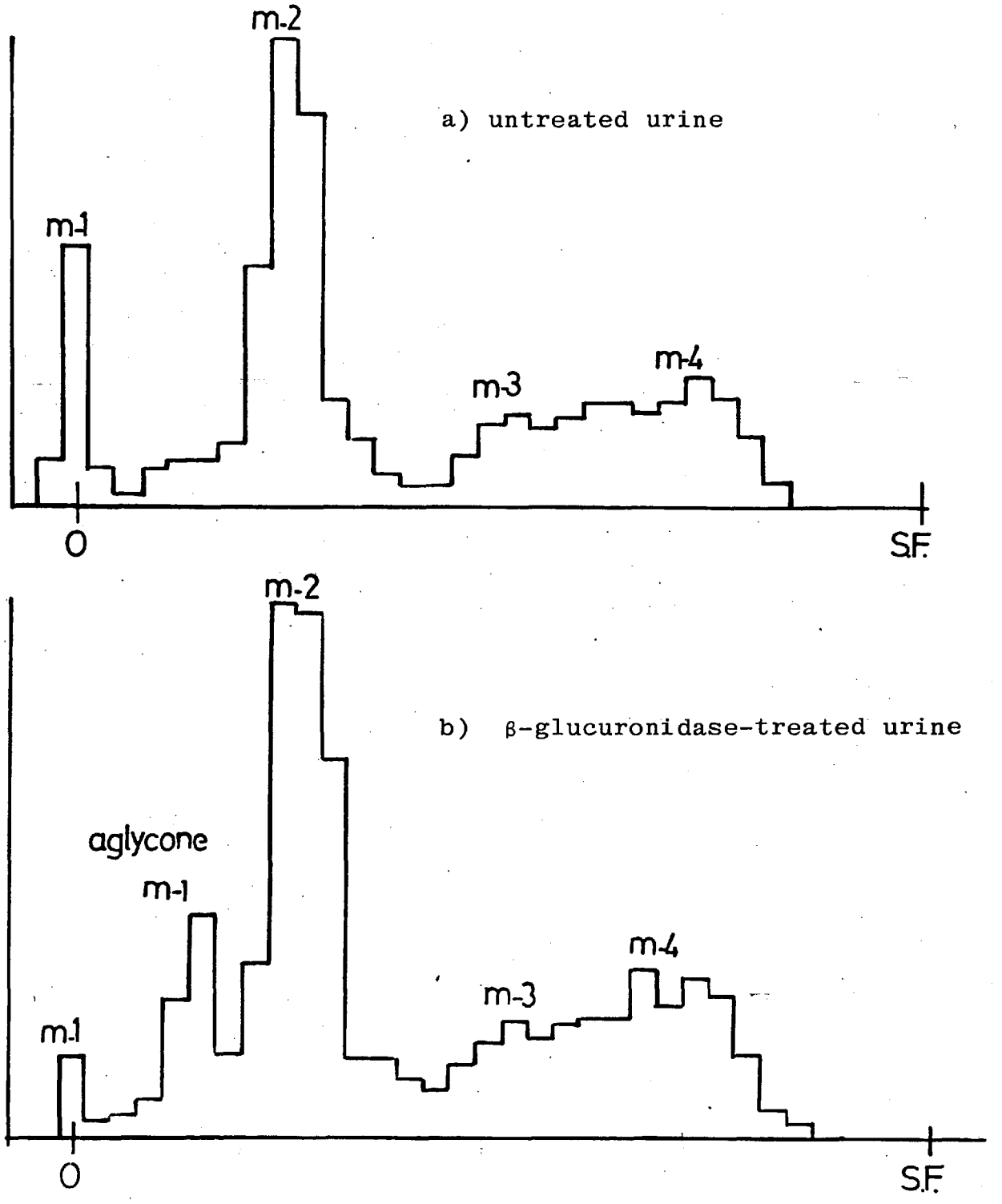


Figure 3.2. Radiohistogram of 24 h Urine from Rats Administered an oral Dose of [^{14}C]Debrisoquine



m-2: The properties of m-2 were found to be the same as those of standard 4-hydroxy debrisoquine on paper chromatography (systems C and D; see Table 2.1), and g.l.c. using the acetyl-acetone derivatization method (see Fig. 3.3).

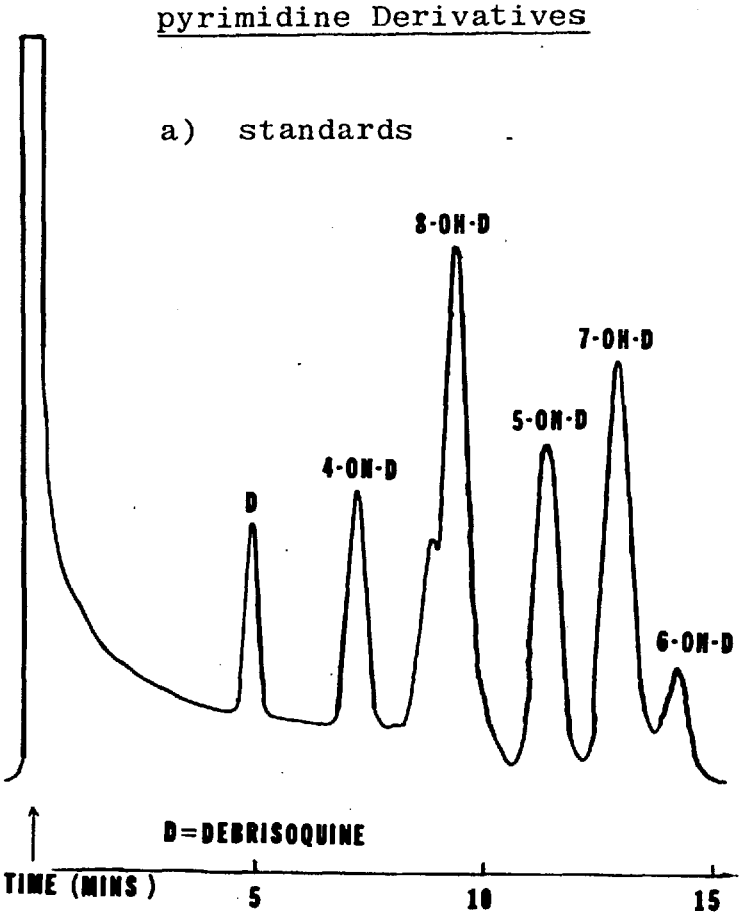
A mass spectrum of m-2 obtained by direct insertion, revealed the presence of a parent ion 16 mass units higher than debrisoquine (see Fig. 3.4) suggesting mono-oxygenation of the parent compound. The mass spectrum obtained for m-2 was compared to the spectra of the standard hydroxylated derivatives, 4-hydroxy-, 5-hydroxy-, 6-hydroxy-, 7-hydroxy- and 8-hydroxy-debrisoquine (Fig. 3.5). Again, this also indicated that m-2 was 4-hydroxy-debrisoquine. Unequivocal evidence for the identity of this metabolite fraction was obtained by g.l.c.-m.s. of the silylated 4,6-dimethylpyrimidine derivatives of m-2, synthetic 4-hydroxy-debrisoquine and one of the standard phenols, 8-hydroxy-debrisoquine (see Fig. 3.6). The mass spectra of derivatized m-2 and standard 4-hydroxy-debrisoquine were identical, each having a small molecular ion of m/e 327 and a base peak of m/e 237, as compared with the phenol where the parent ion (m/e 327) is in fact the base peak.

m-4: The mobility of m-4 on paper chromatography (systems C and D; see Table 2.1) and g.l.c. (Fig. 3.3) suggested that this fraction corresponded to unchanged debrisoquine.

A mass spectrum of m-4 obtained by direct insertion was equivalent to that of debrisoquine, having a parent ion of m/e 175 and major peaks of m/e 132, 104, 91 and 77 (postulated fragmentation pattern given in Appendix). Mass spectra of the 4,6-dimethylpyrimidine derivatives of m-4 and standard

Figure 3.3. G.l.c. Profile of Trimethylsilyl-4,6-dimethyl-pyrimidine Derivatives

a) standards



b) fractions m-2 and m-4 isolated from rat urine

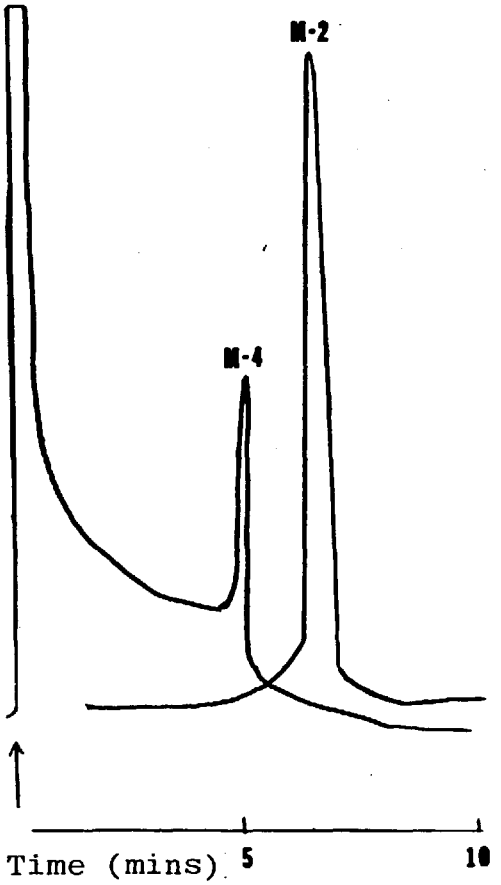
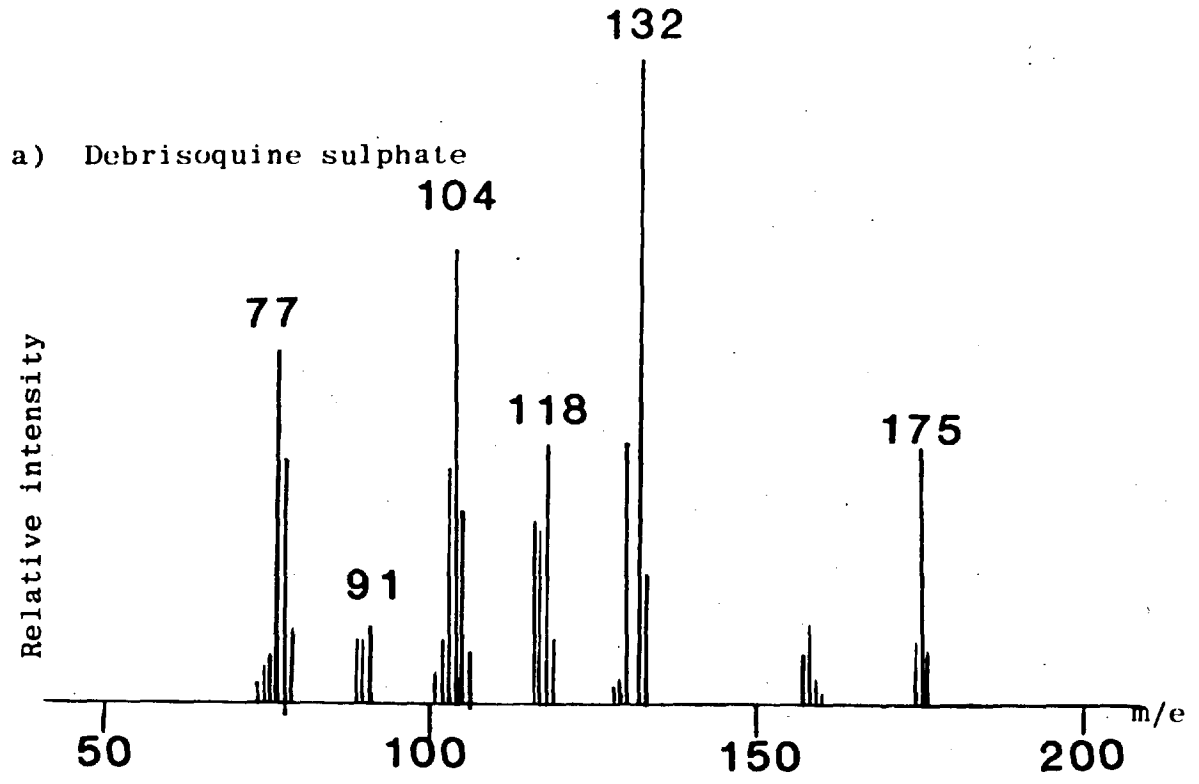
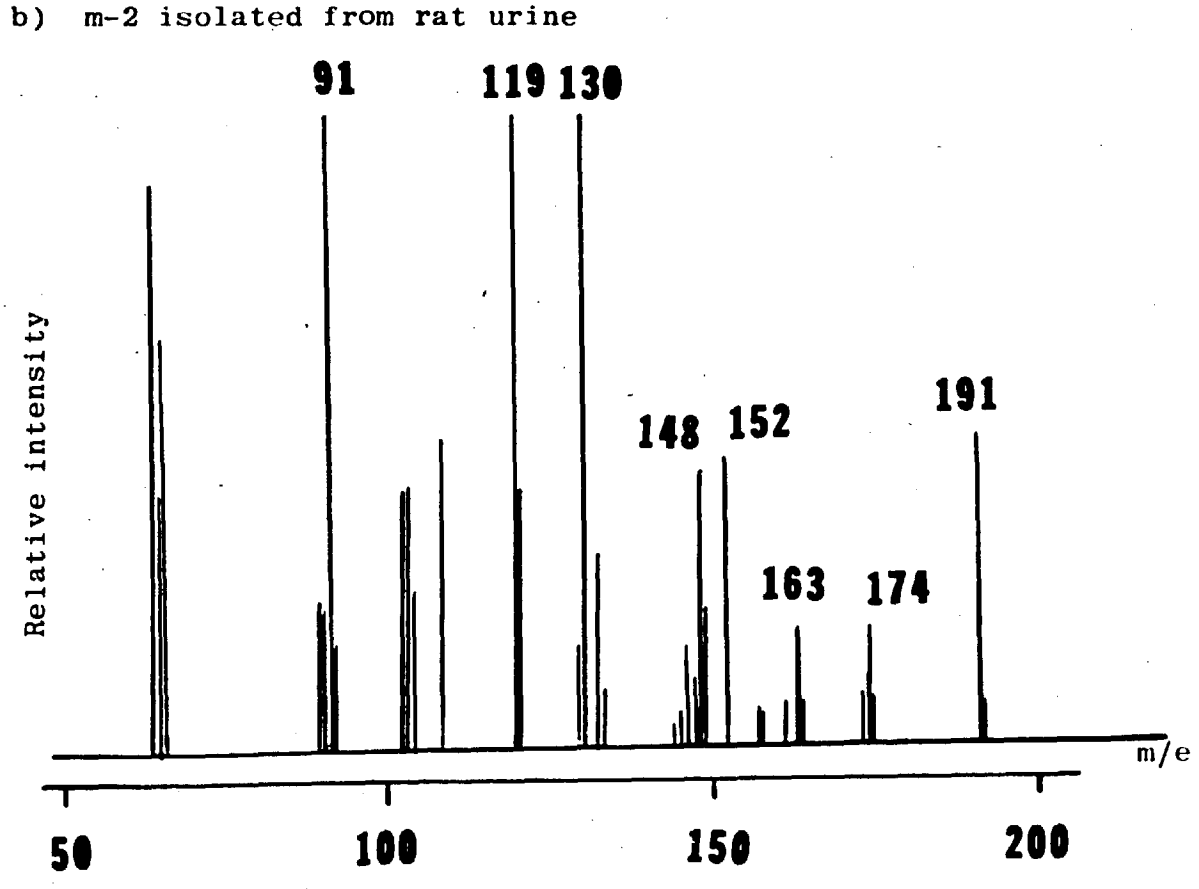


Figure 3.4. Mass Spectra by Direct Insertion

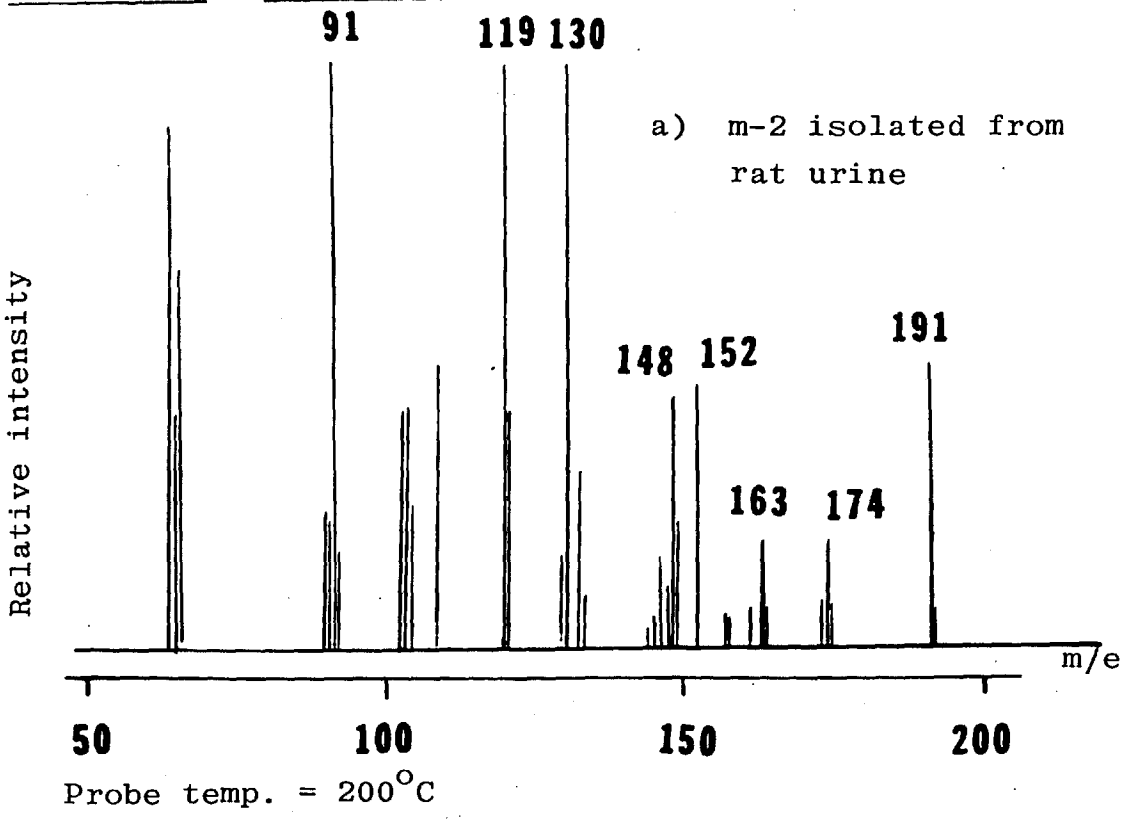


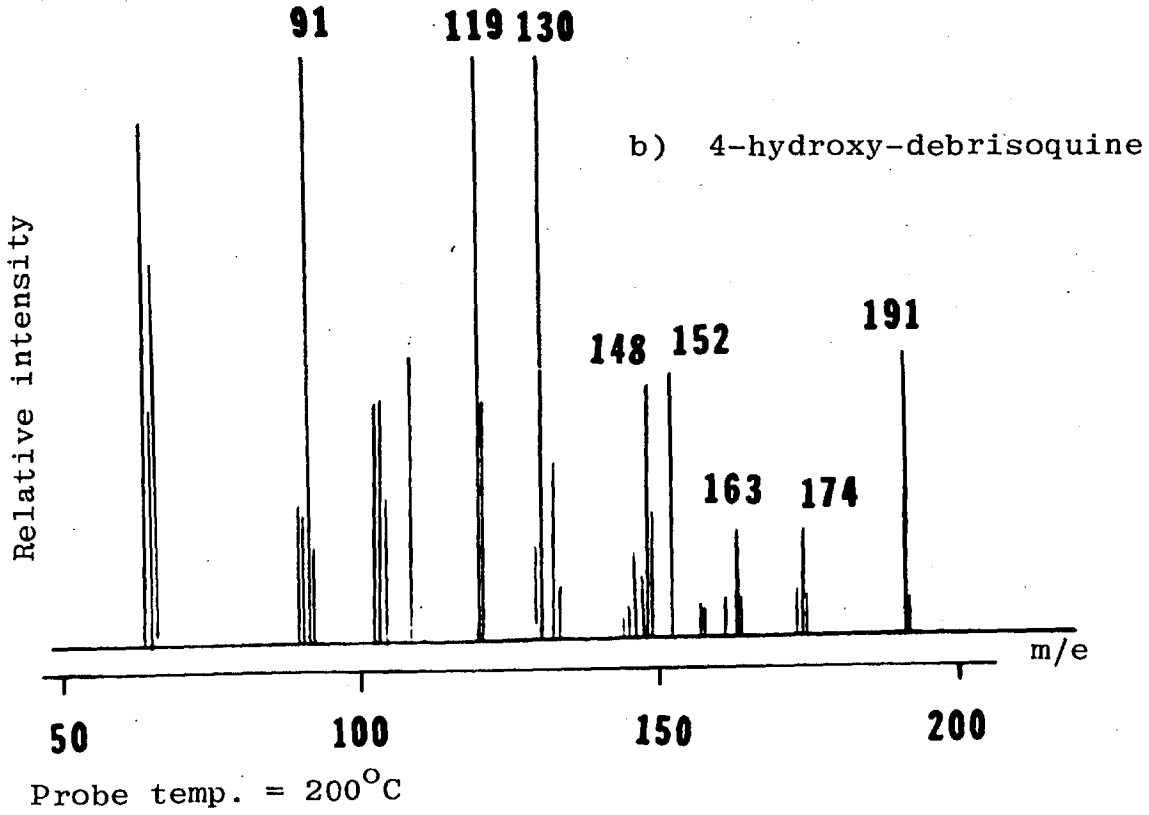
Probe temp. = 200°C



Probe temp. = 200°C

Figure 3.5. Mass Spectra by Direct Insertion

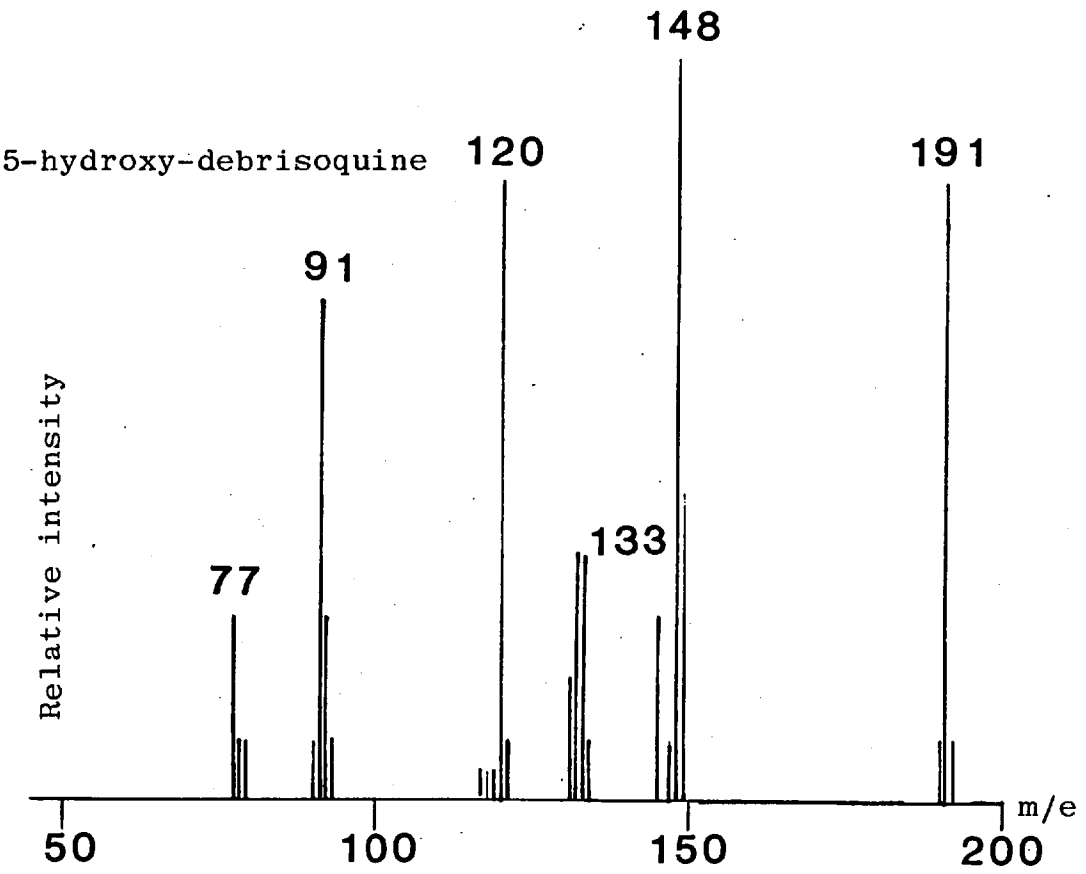




Cont/...

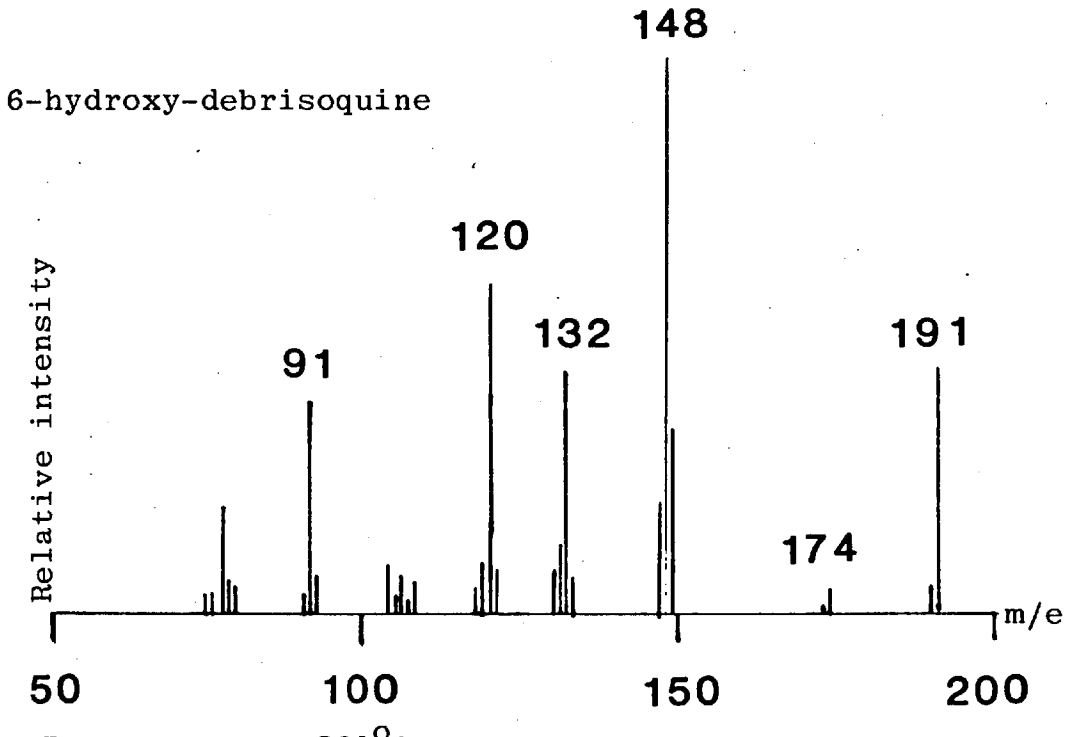
Figure 3.5. Continued

c) 5-hydroxy-debrisoquine



Probe temp. = 225°C

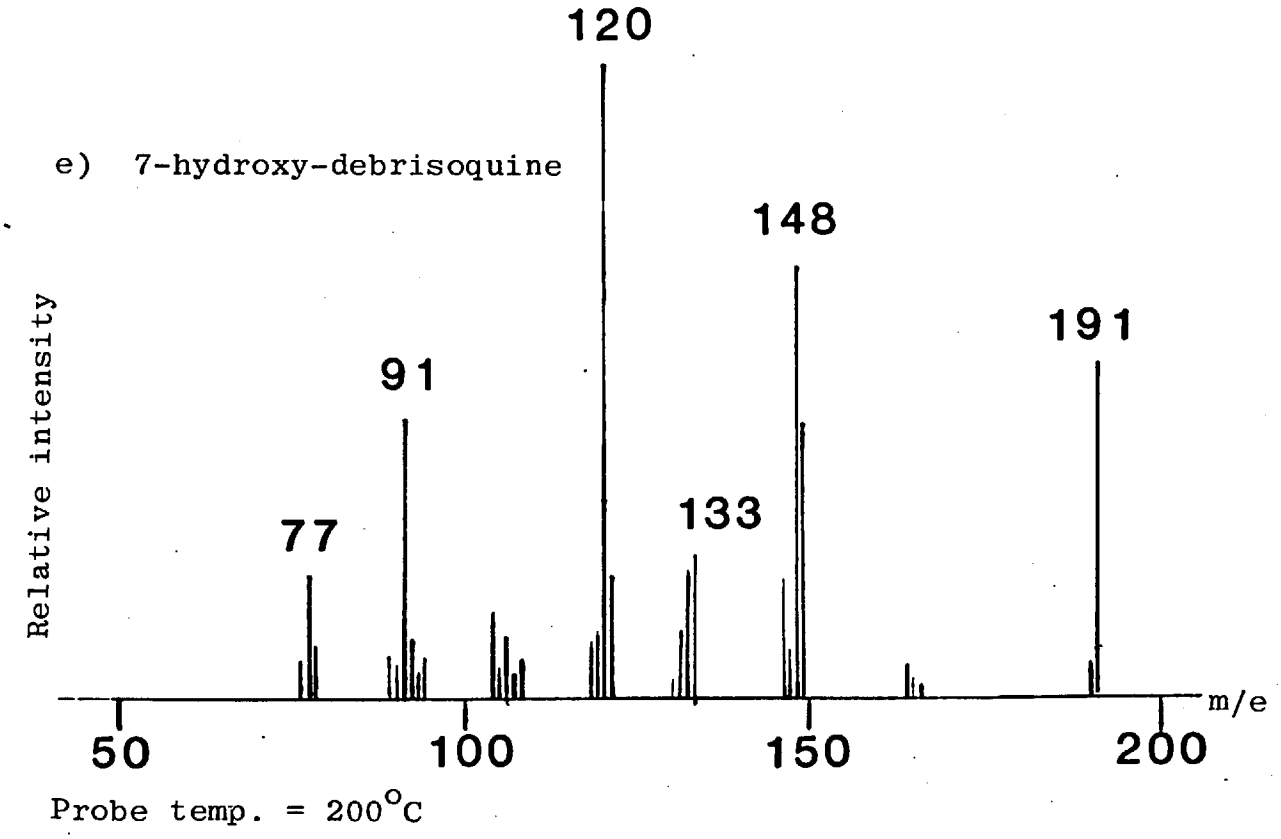
d) 6-hydroxy-debrisoquine



Probe temp. = 200°C

Cont/...

Figure 3.5. Continued



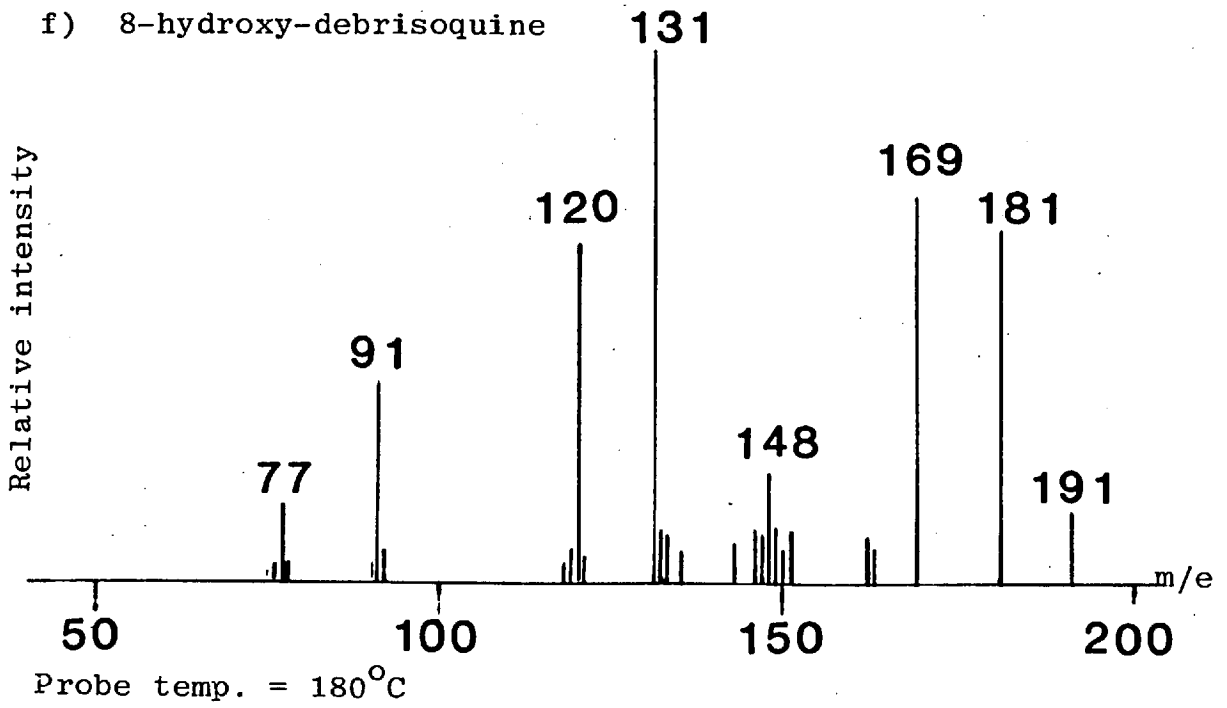
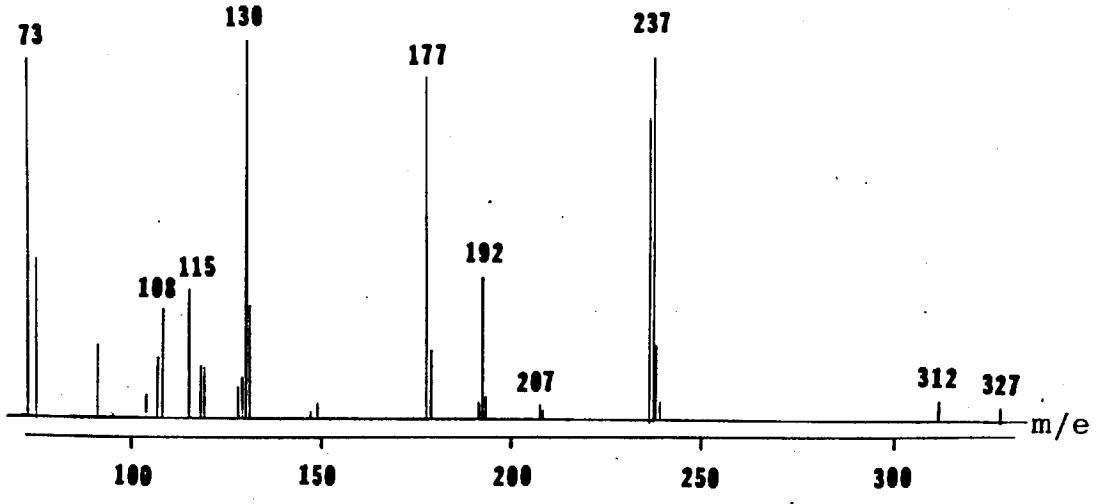
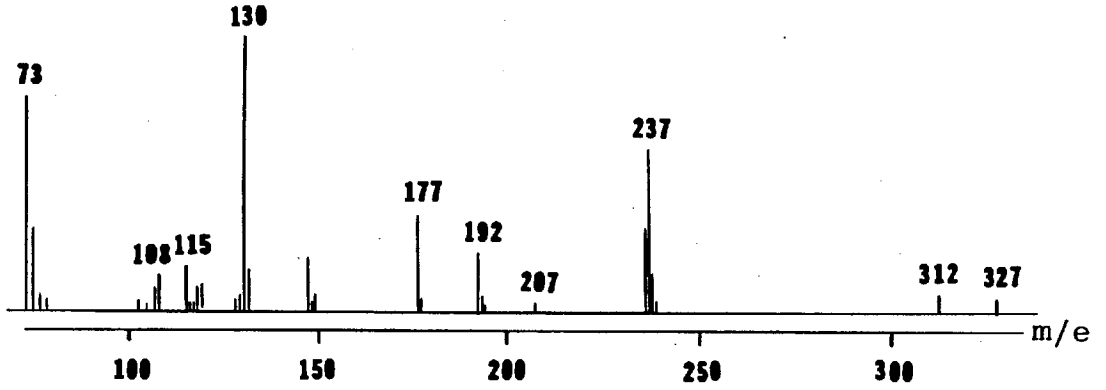


Figure 3.6. G.l.c.-m.s. of Trimethylsilyl-4,6-dimethyl-pyrimidine Derivatives

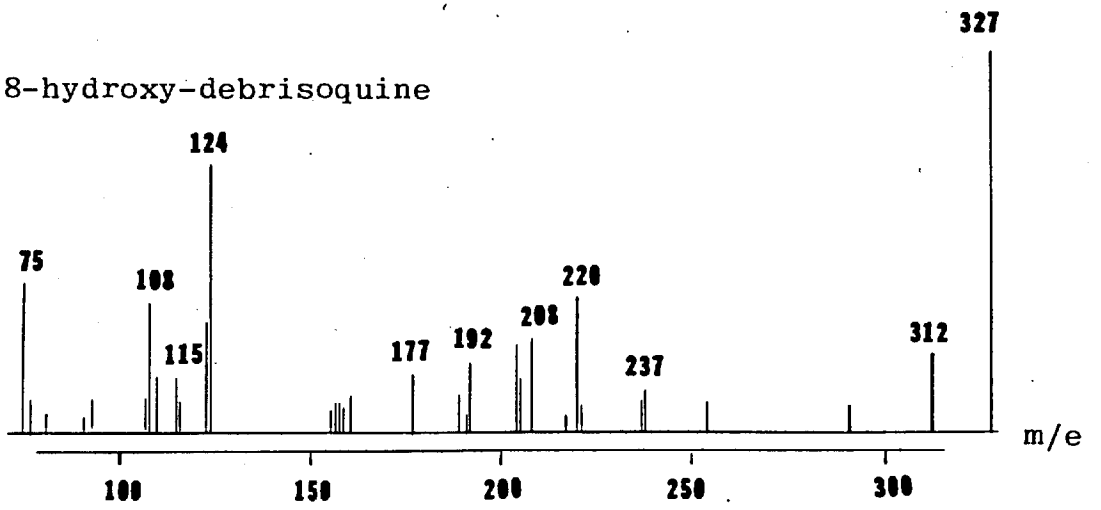
a) m-2 isolated from rat urine



b) 4-hydroxy-debrisoquine



c) 8-hydroxy-debrisoquine



G.l.c.-m.s. System III

debrisoquine, obtained by g.l.c.-m.s. were also identical (Fig. 3.7). The identity of m-4 was thus established as debrisoquine.

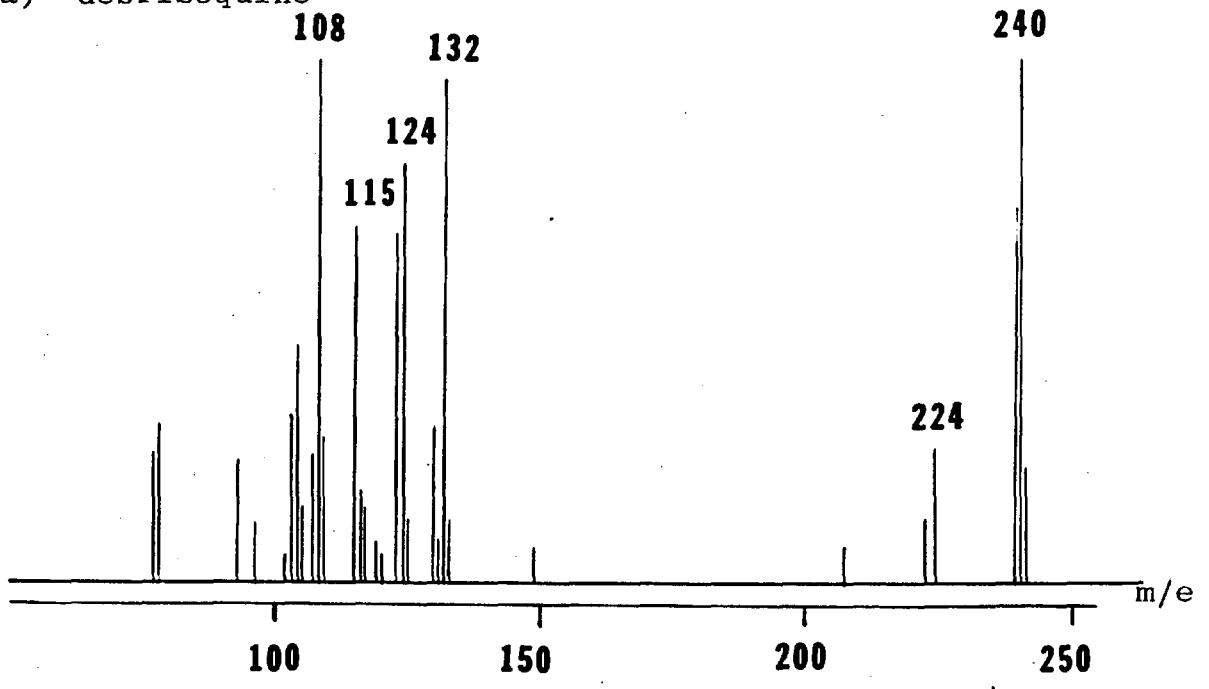
m-1: The aglycone of m-1, obtained by incubation of the sample with β-glucuronidase, was derivatized with acetylacetone. Of the total radioactivity in the derivatization mixture, 39% was extracted at pH 9, 31% at pH 4 and the remainder was unextractable even after a further derivatization.

The acidic and basic fractions were examined by g.l.c.-m.s. The acidic fraction was treated with ethereal diazomethane and found to contain two esterified carboxylic acid derivatives of debrisoquine which could be separated on the g.l.c. column (see Fig. 3.8). These compounds gave similar mass spectra with a molecular ion of m/e value 285 (Fig. 3.8). The acidic metabolites are probably formed by hydroxylation at either the 1 or 3 position, followed by ring cleavage, with subsequent oxidation.

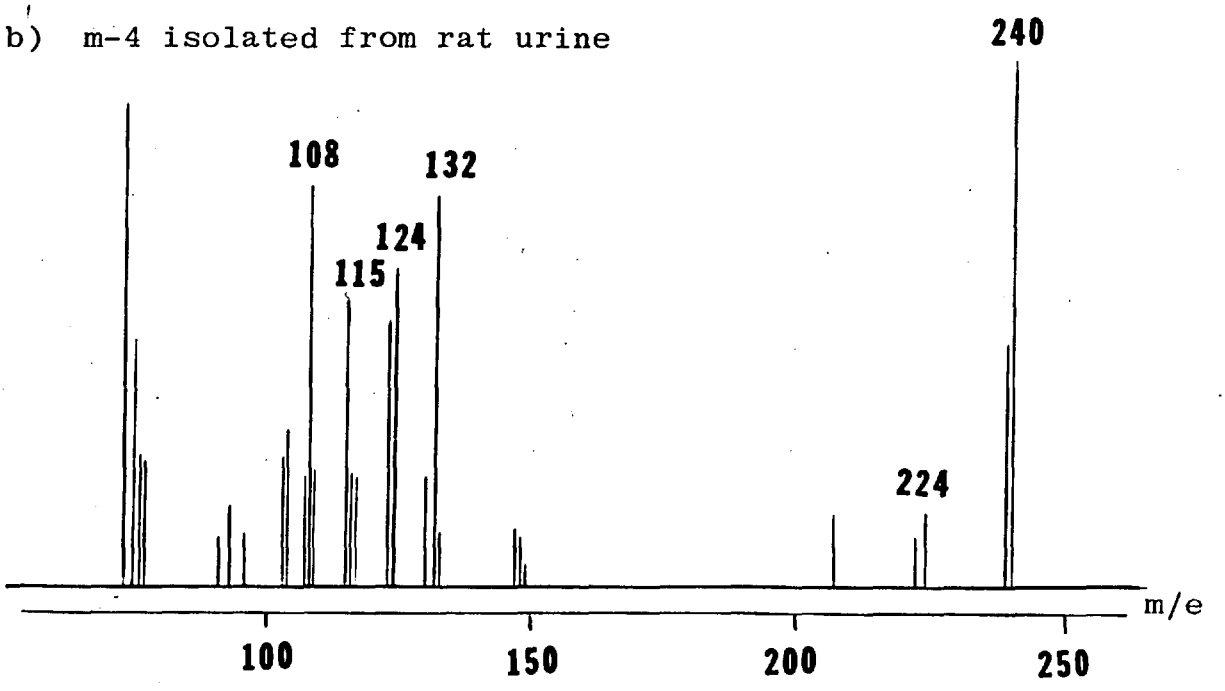
The basic fraction was analysed after silylation with BSTFA + 1% TMCS and Figure 3.9 shows the traces obtained when the sample was run using both wide scan and mass fragmentographic facilities. Mass spectra were taken on several peaks and debrisoquine, 4-hydroxy-debrisoquine, 6-hydroxy-debrisoqine and a di-hydroxylated debrisoquine derivative were shown to be present (see Fig. 3.9). However, judging from the mass fragmentographic trace, by far the greatest proportion of this fraction was accounted for by the di-hydroxylated debrisoquine derivative. Comparison of the postulated fragmentation pattern of this compound with that of 4-hydroxy-debrisoquine and

Figure 3.7. G.l.c.-m.s. of Trimethylsilyl-4,6-dimethyl-pyrimidine Derivatives.

a) debrisoquine

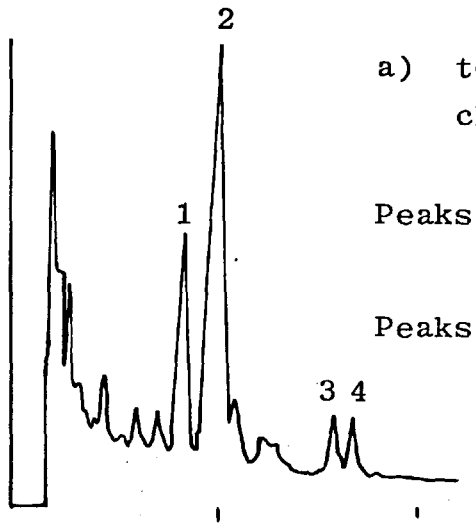


b) m-4 isolated from rat urine



G.l.c.-m.s. System III

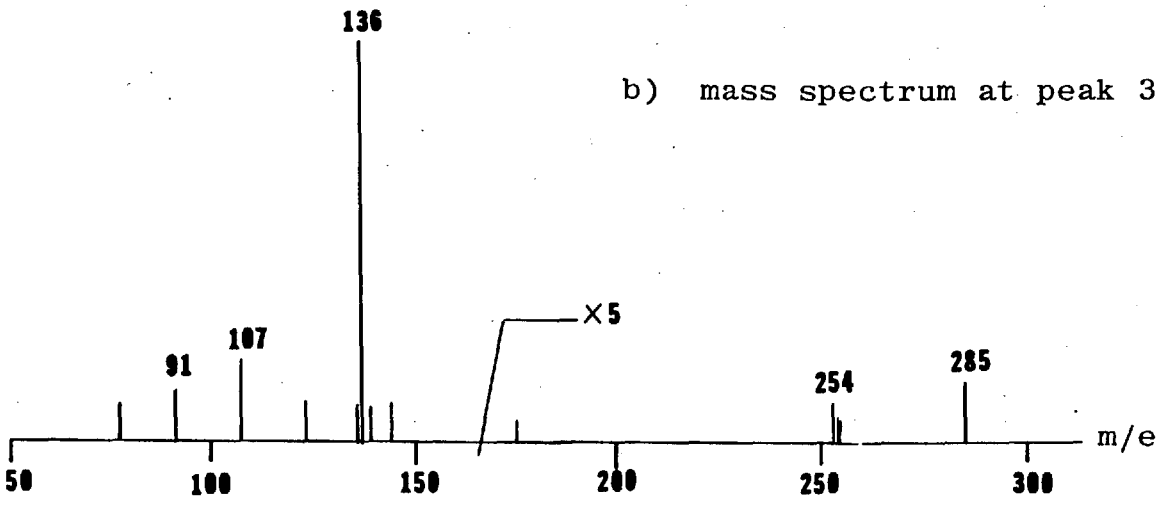
Figure 3.8. G.l.c.-m.s. of the Acidic Metabolite Fraction from Rat Urine



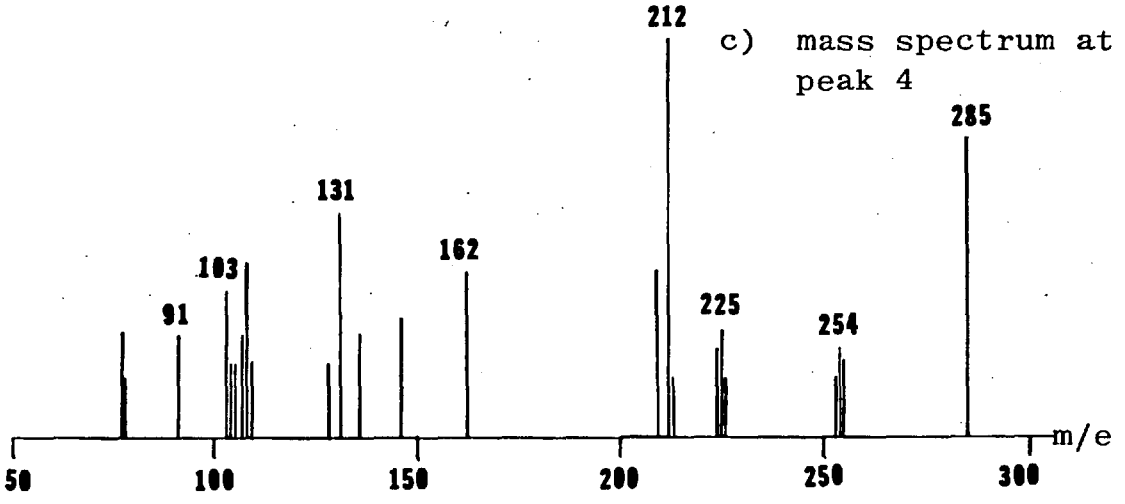
a) total ion mass chromatogram

Peaks 1 & 2 not debrisoquine related

Peaks 3 & 4 acidic metabolit



b) mass spectrum at peak 3



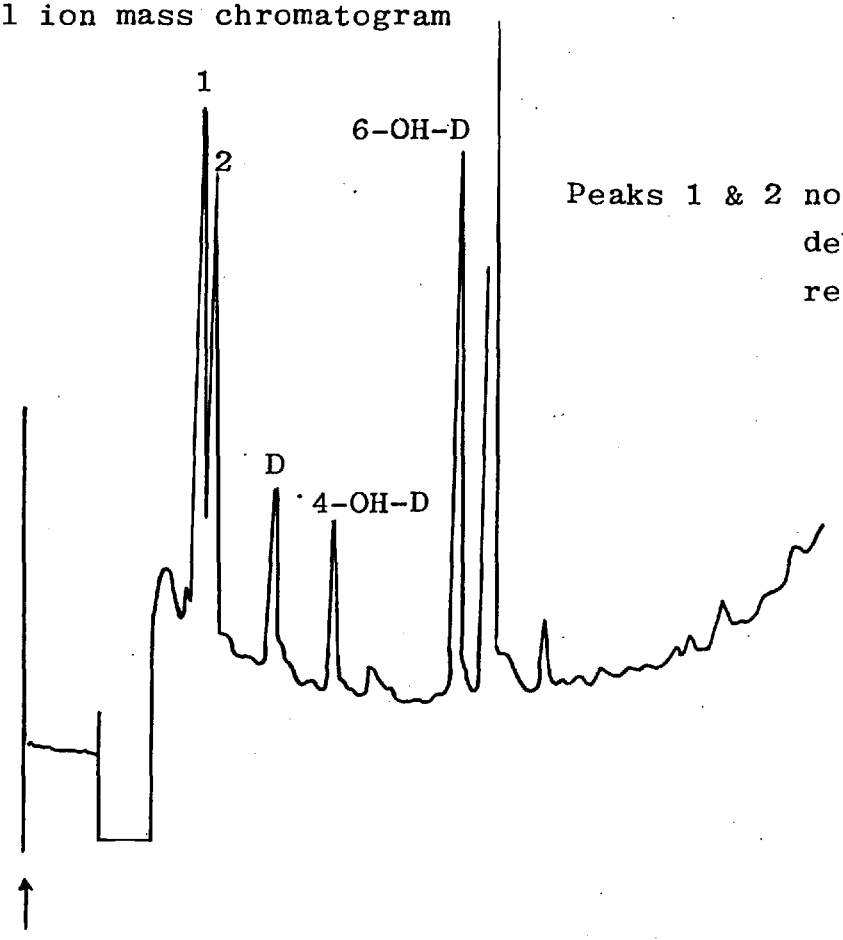
c) mass spectrum at peak 4

G.l.c.-m.s. System I

Figure 3.9. G.l.c.-m.s. of the Basi m-1 Fraction from Rat Urin

di-OH-D

a) total ion mass chromatogram



b) single ion mass chromatogram

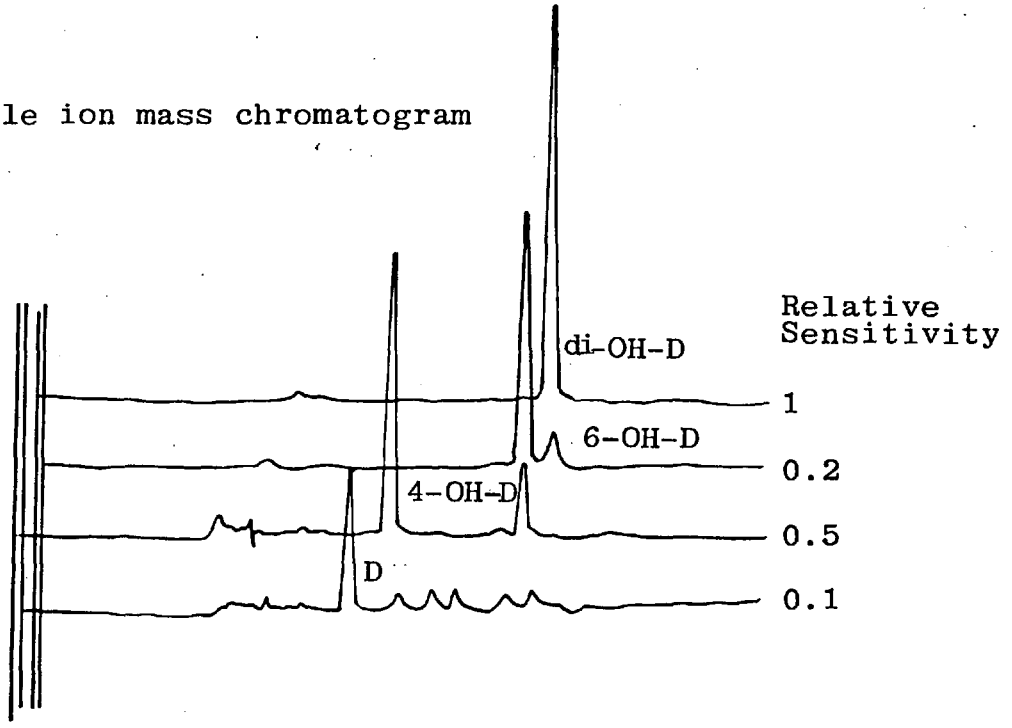


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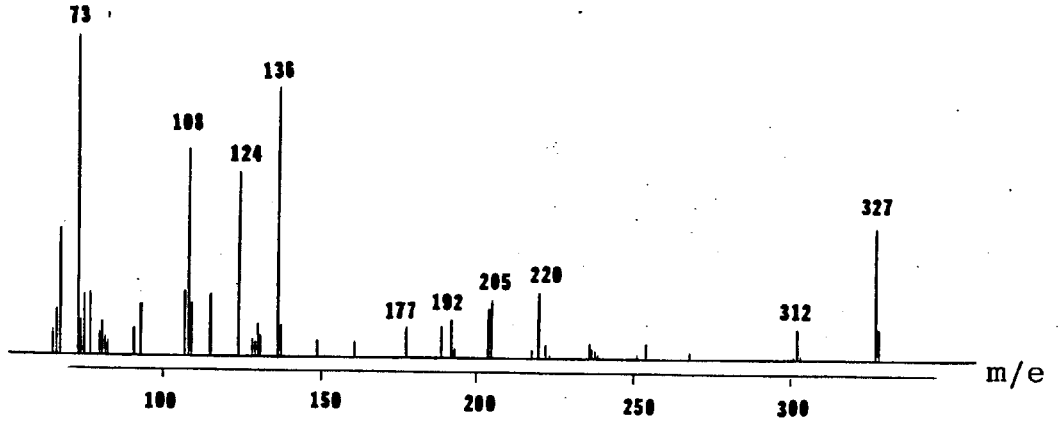
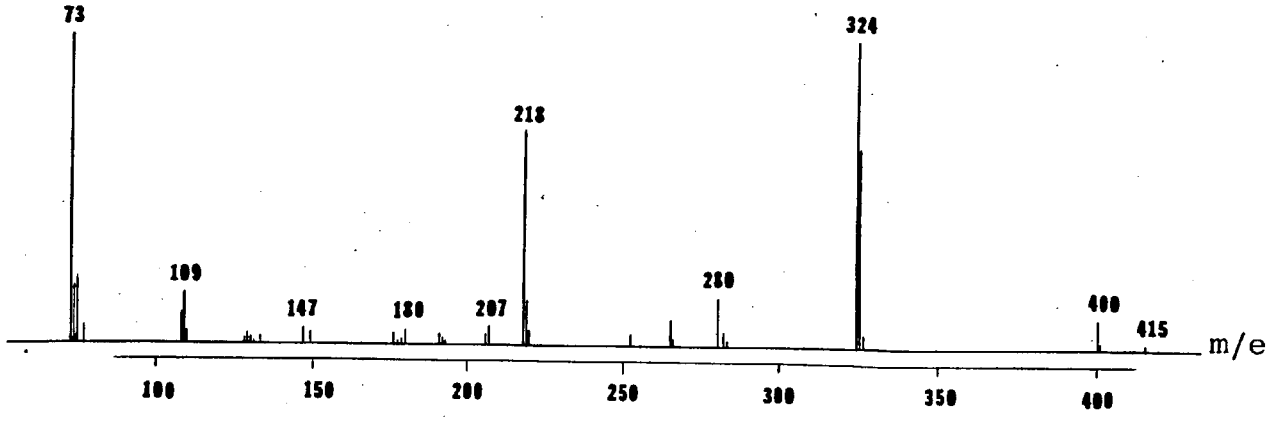
Figure 3.9. Continued

c) mass spectrum of 6-hydroxy-debrisoquine



d) mass spectrum of di-hydroxy-debrisoquine



G.l.c.-m.s. System I

the standard phenol 6-hydroxy-debrisoquine (see Appendix), suggests that the compound is hydroxylated on both the aromatic and heterocyclic rings.

A possible explanation for the presence of trace amounts of debrisoquine, 4-hydroxy-debrisoquine and 6-hydroxy-debrisoquine, is that they are retained at the origin of the paper chromatogram during the m-1 isolation procedure.

m-3: The radioactive band, m-3, was found to have the same R_F on paper chromatography in systems C and D as the standard phenols (see Table 2.1) and also gave a positive colour reaction with diazotized 4-nitroaniline and Gibb's reagent, which are phenolic spray reagents. On mass spectrometry, by direct insertion, a parent ion of m/e 191 was seen (see Fig. 3.10), which also suggests mono-oxygenation of the parent drug. however, further investigation by ultra-violet scanning spectrometry revealed that, unlike 8-hydroxy-debrisoquine, no obvious bathochromic shift could be obtained on changing the pH of the solution from acid to base (see Fig. 3.11).

G.l.c. analysis of this metabolite fraction was not possible using the acetylacetone derivatization procedure. However, whole rat urine was later analysed using the more sensitive hexafluoroacetylacetone method (see Fig. 3.12). This revealed the presence of all four phenols with a predominance of 5- and 8- hydroxy-debrisoquine.

Quantitation of metabolites:- Quantitation of the different metabolite fractions was achieved mainly by paper chromatography and reverse isotope dilution (as described in Chapter

Figure 3.10. Mass Spectrum by Direct Insertion of m-3 Isolated from Rat Urine

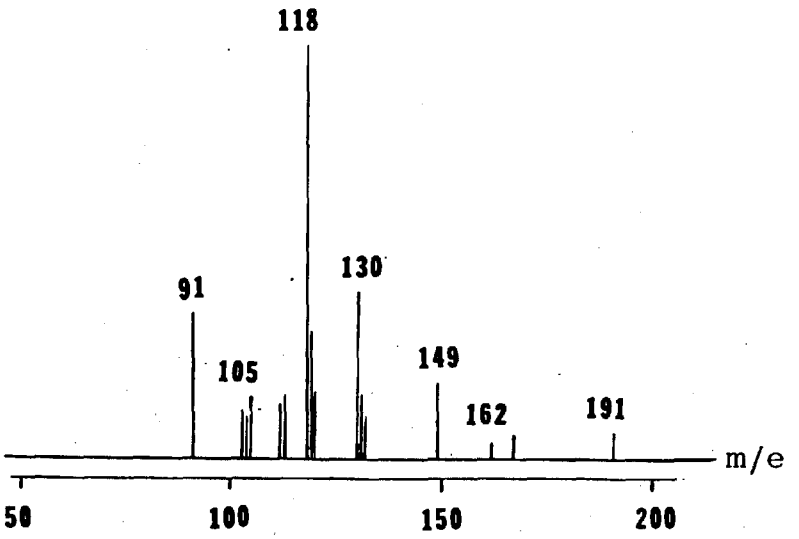


Figure 3.11. Ultra-violet Spectra

a) 8-hydroxy-debrisoquine b) m-3 isolated from rat urine

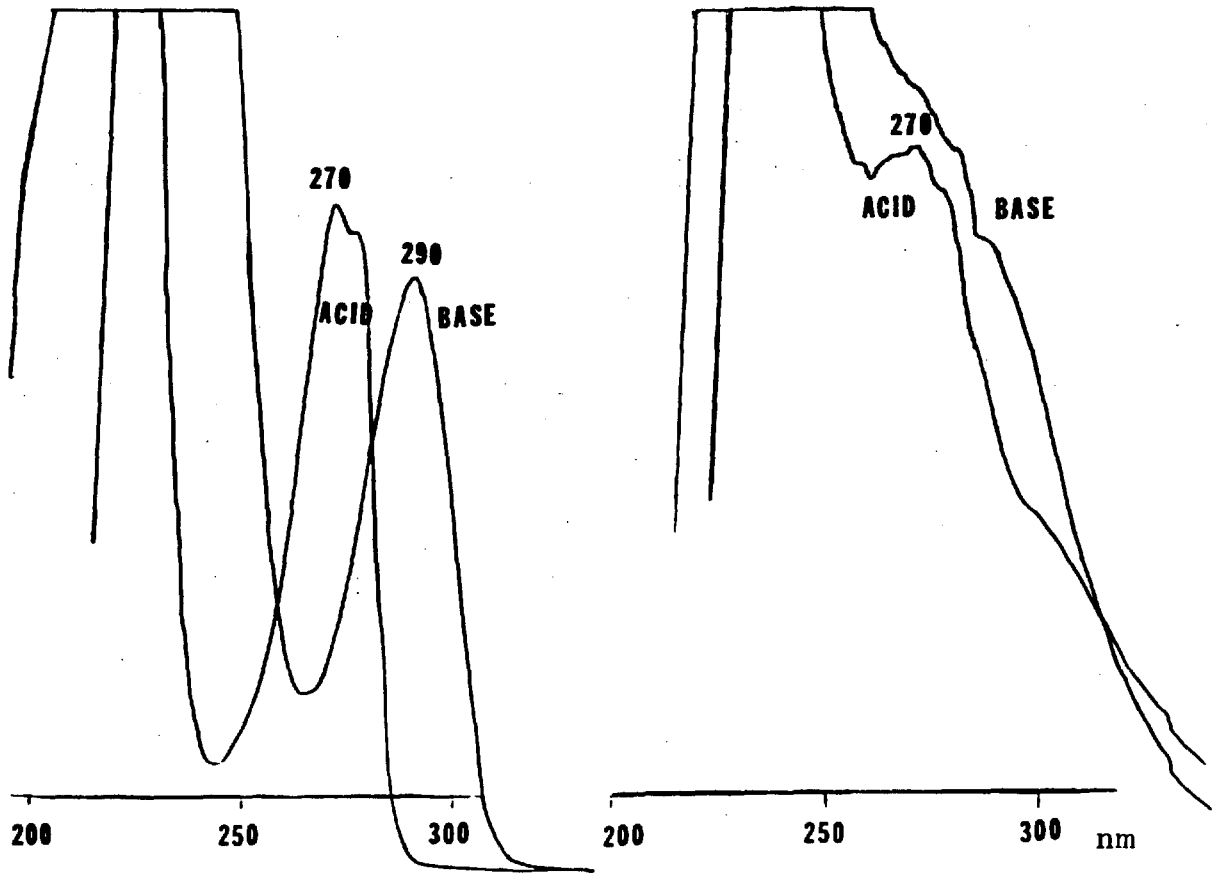
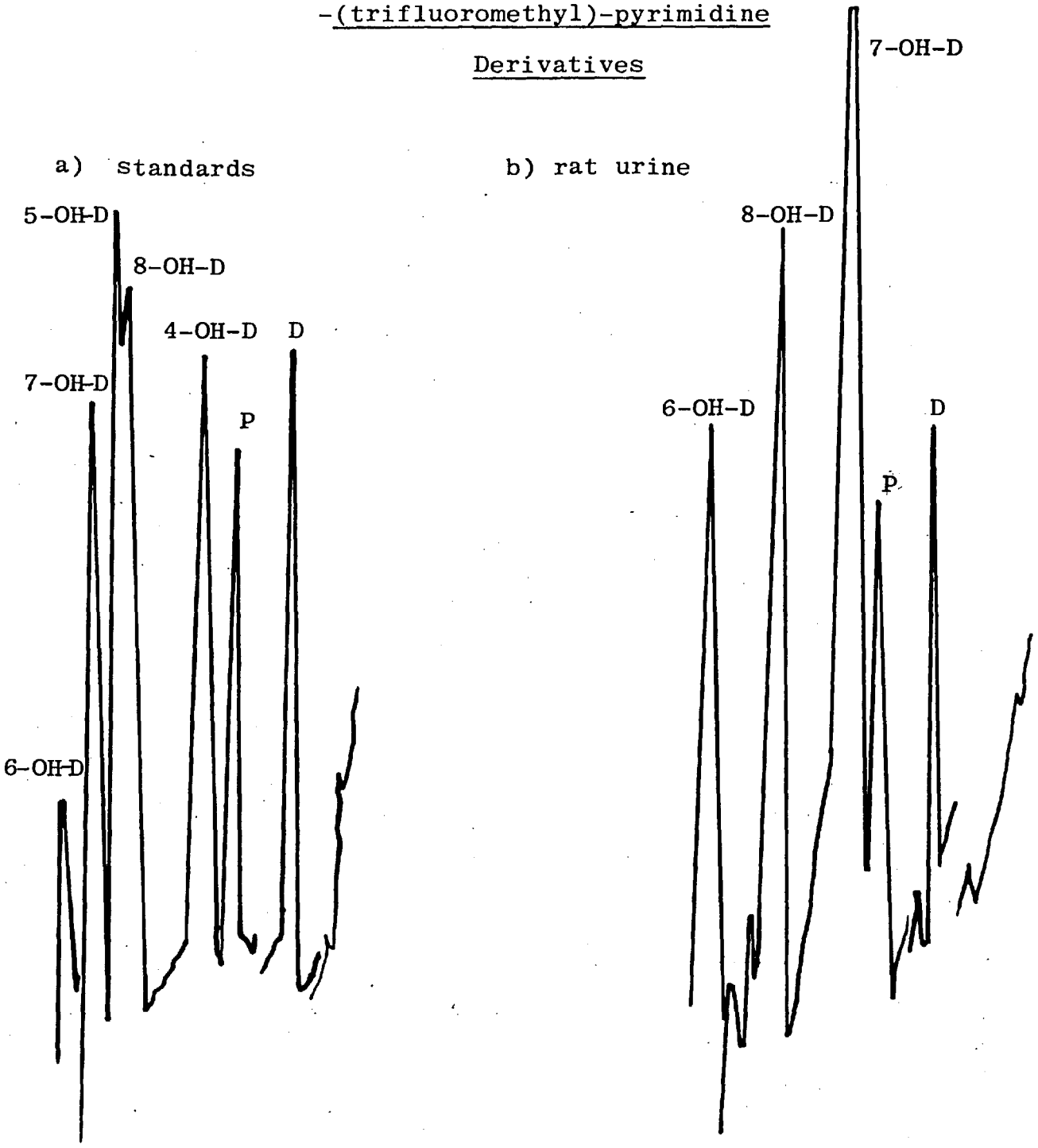


Figure 3.12. G.l.c. Profile of Trimethylsilyl-4,6-di-(trifluoromethyl)-pyrimidine Derivatives



D = Debrisoquine

P = Phenformin

Two). Although a value was obtained for the composite phenolic fraction, m-3, figures for the individual phenols by g.l.c. using the hexafluoroacetylacetone method have not as yet been obtained.

The rat was shown to metabolize debrisoquine to a great extent, only 10% of the dose being excreted as the unchanged drug (see Table 3.2). The major metabolite, 4-hydroxy-debrisoquine, constitutes 35% of the dose. The peak at the origin, m-1 and the phenolic band each account for a further 10%.

Human Studies

The excretion pattern after a single oral dose of [^{14}C]debrisoquine (40 mg; 5 μCi) was studied in four normotensive subjects. Urine samples were collected at various time intervals during the first day, concommitantly with blood pressure measurements. The drug is well absorbed in man, 74.7 ± 1.2% of the dose being excreted in the urine within 24 h (see Table 3.3). An average of 8.3 ± 3.1% of the radioactivity was recovered in the faeces over a three-day period.

The metabolic fate of debrisoquine in man was found to be similar to that in the rat as judged by the chromatographic pattern in system D (see Fig. 3.13). However, man does not appear to form the glucuronide conjugate of m-1. G.l.c. analysis of human urine using the acetylacetone derivatization method with comparison to the standard compounds and rat urine, revealed the presence of debrisoquine and 4-hydroxy-debrisoquine. This was also confirmed by g.l.c.-m.s. in system II (see Fig. 3.14). However, g.l.c. analysis using the hexa-

Table 3.2. Metabolism of [^{14}C]Debrisoquine in the Rat Expressed as % of the Dose in 0-24 h Urine

Metabolite R_F	Probable identity	% of Dose in 0-24 h urine*
0.00)	m-1	9.1 (7.2-11.6)
0.14)	(see text)	1.6 (1.6-2.0)
0.28	4-Hydroxy-debrisoquine	34.9 (29.9-40.1)
0.60	Phenolic band†	11.5 (7.8-13.4)
0.77	Debrisoquine	8.8 (6.9-12.2)

Descending paper chromatography on Whatman No. 1 paper in solvent system D.

* Mean value for 3 rats with range in parentheses

† Presence of the phenols in the m-3 radioactive band has only been shown qualitatively. No quantitative figures are available for the various phenols.

Table 3.3 Elimination of ^{14}C in Four Normotensive Subjects After an Oral Dose of [^{14}C]Debrisoquine

Recovery of ^{14}C Expressed as a % of the Dose

In Urine

Day	R.L.	L.G.D.	R.L.S.	A.L.	Mean ± S.E.M.
1	75.2	75.7	71.5	76.7	74.8 1.2
2	81.8	87.5	82.8	82.3	83.6 1.3
3	84.6	88.4	86.7	83.7	85.8 1.1

In Faeces

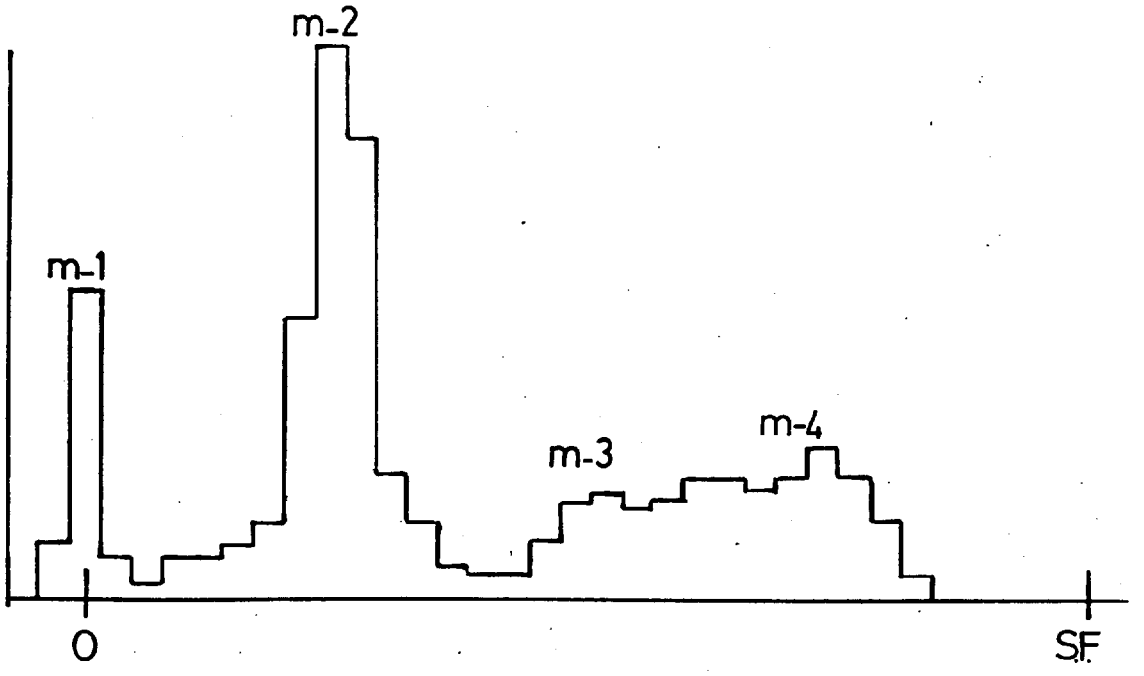
Day	R.L.	L.G.D.	R.L.S.	A.L.	Mean ± S.E.M.
1	15.1	0.0	3.1	0.0	4.5 3.1
2	16.6	2.1	6.8	3.9	7.4 3.2
3	16.8	2.1	7.5	6.7	8.3 3.1

Total

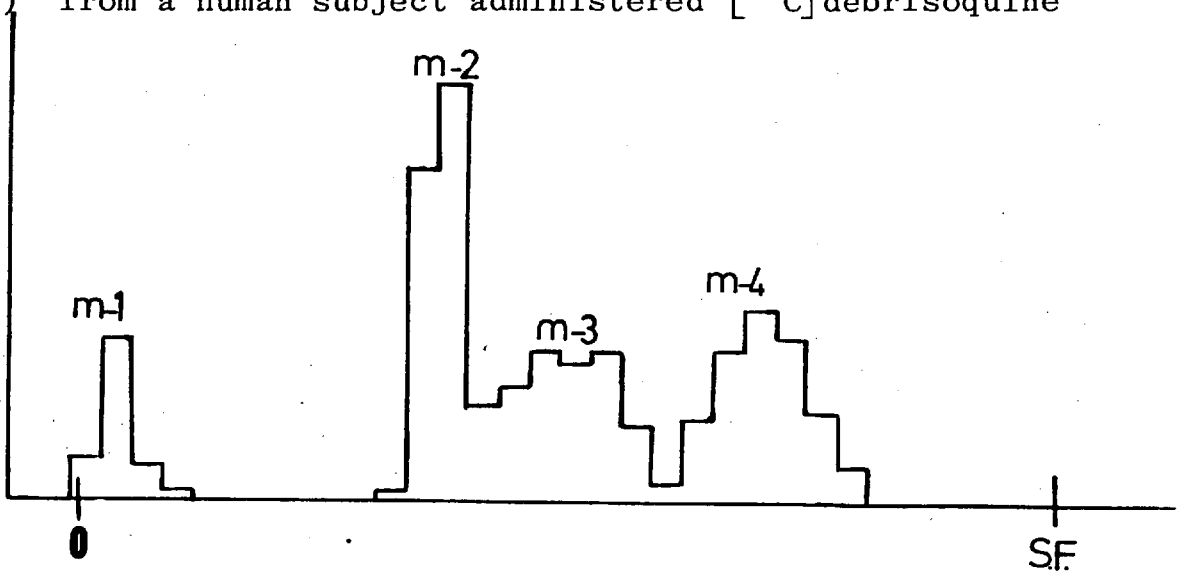
Day	R.L.	L.G.D.	R.L.S.	A.L.	Mean ± S.E.M.
1	90.3	75.7	74.4	76.7	79.3 3.7
2	98.4	89.6	89.6	86.2	88.9 2.9
3	101.4	90.4	94.2	90.4	94.1 2.6

Figure 3.13. Radiohistograms of 24 h Urines

a) from rat administered [^{14}C]debrisoquine

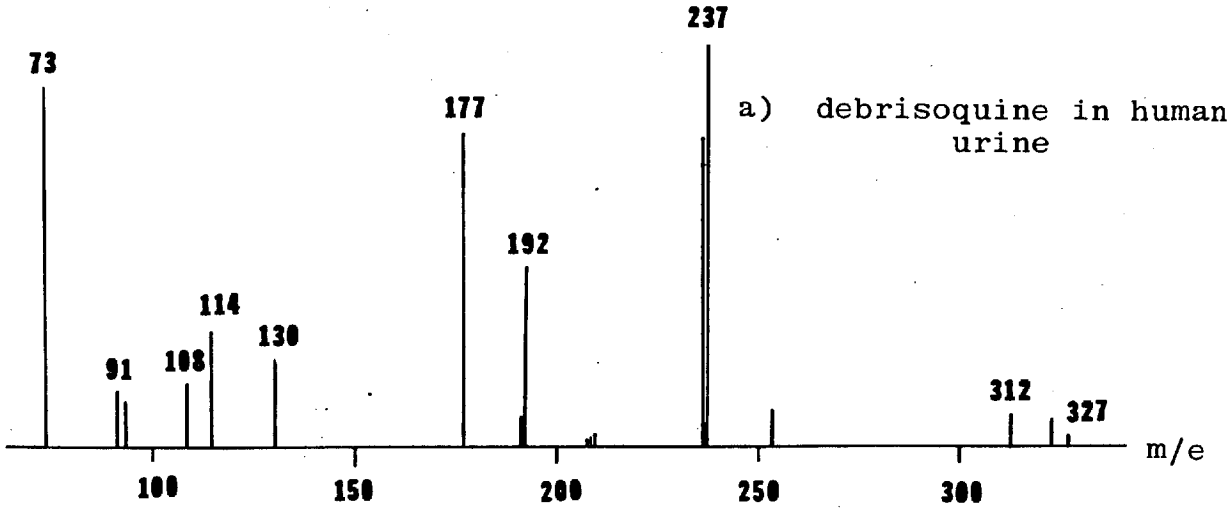


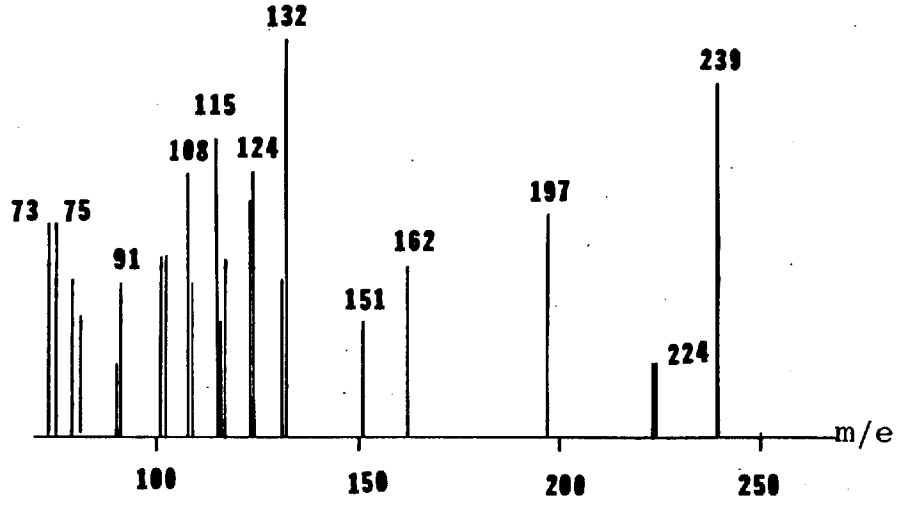
b) from a human subject administered [^{14}C]debrisoquine



Paper chromatography Solvent System D

Figure 3.14. G.l.c.-m.s. of Trimethylsilyl-4,6-dimethyl -pyrimidine Derivatives



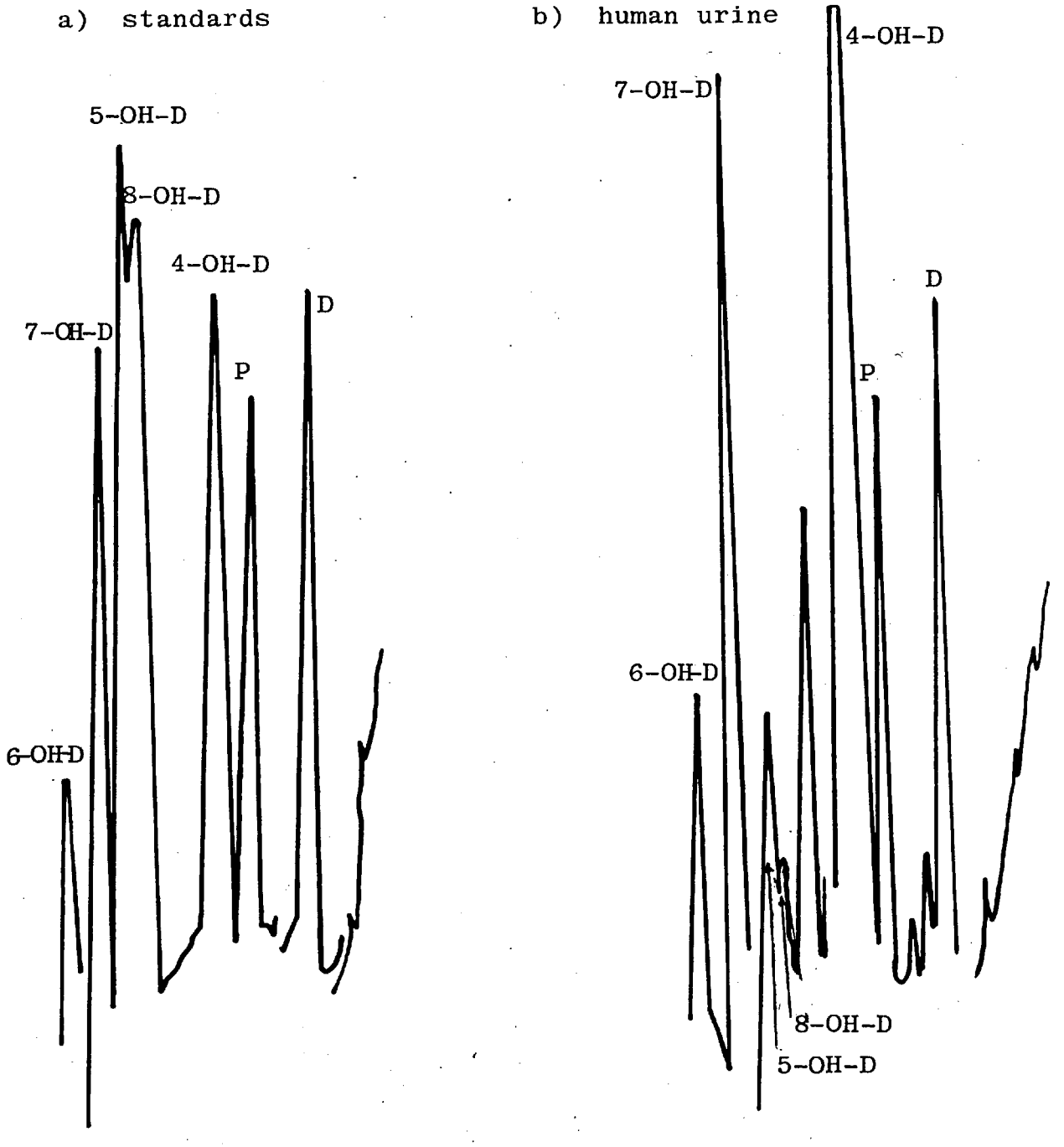


G.l.c.-m.s. System II

fluoroacetylacetone procedure showed the presence of phenols. A species difference in the position of hydroxylation was apparent; man appears to form predominantly 7-hydroxy-debrisoquine (see Fig. 3.15).

Quantitation of metabolites: Unlike the rat, which consistently excretes similar proportions of the various metabolite bands, a marked quantitative difference was found between human subjects. The urinary metabolite levels were estimated at different time intervals, for each subject, as shown in Table 3.4. Of the four normotensives, only R.L.S. showed a statistically significant lowering of blood pressure ($P < 0.05$). From the total 0-24 h excretion figures (Table 3.4) it can be seen that urinary debrisoquine levels estimated by reverse isotope dilution and g.l.c. show a good correlation. The amount of unchanged drug excreted varies between 16 and 60%; 4-hydroxy-debrisoquine varies between 5 and 24%; m-1, the composite peak at the origin shows an inter-individual range of 2-11%; and the phenolic band varies between 7 and 33%.

Figure 3.15. G.l.c. Profile of Trimethylsilyl-4,6-di-(trifluoromethyl)-pyrimidine Derivatives



D = Debrisoquine

P = Phenformin

Table 3.4. Metabolites of [^{14}C]Debrisoquine in the Urine of Four Normotensive Subjects, expressed as % of the Dose at Various Time Intervals from 0-24 h

Subject R.L.

Time (h)	Probable identity of metabolites: m-1 (see text) R_F 0.00 & 0.14*	4-Hydroxy-debrisoquine R_F 0.33*	Phenolic band† R_F 0.58*	Debrisoquine¶ R_F 0.73*	
1	0.1	0.6	0.5	0.8	
2	0.6	3.3	2.3	3.5	
3	0.6	4.3	3.3	4.2	
4	0.7	3.3	2.2	2.7	
6	0.7	4.3	5.1	1.7	
8	1.0	2.8	2.8	1.5	
10	0.7	2.3	3.1	1.8	
15	0.5	0.1	3.9	1.0	
24	0.9	3.2	3.6	1.4	
Total 0-24 h	5.8	24.2	26.8	18.8	(18.0)‡

Subject G.D.

1	0.3	1.0	0.3	1.8	
2	2.3	6.9	3.3	7.5	
3	1.4	3.7	3.8	3.4	
4	1.1	2.4	2.8	2.6	
6	1.7	1.6	5.7	3.3	
10	0.7	0.1	3.0	1.7	
15	0.9	0.2	3.5	1.6	
24	0.7	0.8	2.8	1.3	
Total 0-24 h	9.1	16.7	25.2	23.2	(23.0)‡

Cont/...

Table 3.4. Continued

Subject R.S.

Time (h)	Probable identity of metabolites: m-1 (see text) R_F 0.00 & 0.14*	4-Hydroxy-debrisoquine R_F 0.33*	Phenolic band† R_F 0.58*	Debrisoquine¶ R_F 0.73*	
1	-	-	0.1	1.7	
2	-	-	4.2	11.4	
3	-	-	1.0	6.9	
4	-	-	-	5.5	
6	-	-	1.0	8.9	
10	-	-	1.0	9.8	
24	1.5	5.4	-	10.6	
Total 0-24 h	1.5	5.4	7.3	54.8	(54.0)‡

Subject A.L.

1	0.6	0.4	4.1	0.9	
2	2.2	0.4	11.6	2.2	
3	1.4	1.6	4.3	1.8	
4	1.4	2.6	0.2	5.4	
6	1.5	2.9	-	5.9	
8	1.7	0.8	7.8	0.5	
24	2.4	6.5	4.7	1.9	
Total 0-24 h	11.2	15.1	32.7	18.6	(18.5)‡

* Descending paper chromatography in solvent system D. - = not detected

† Presence of the phenols has only been shown qualitatively. Quantitation of the individual phenol has not been achieved.

¶ Debrisoquine levels at the various times were estimated by reverse isotope dilution

‡ Total 0-24 h debrisoquine levels obtained by g.l.c.

CHAPTER FOUR

CLINICAL PHARMACOLOGY OF DEBRISOQUINE

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Investigation of the Basis for Inter-Individual Differences in Response to Debrisoquine

A review of the literature reveals that clinical management of hypertension with debrisoquine is complicated by marked individual variations in dose requirement of the drug (see Chapter One). Metabolism studies in our Department have shown that single oral doses (40 mg) of [^{14}C] debrisoquine were well absorbed (approx. 75% of the drug excreted in the urine in the first day; Table 3.3), and that the drug was metabolized by hydroxylation to an extent which varied between subjects (see Table 3.4).

An open study involving ten normotensive subjects and a number of hypertensive patients with normal renal function (plasma creatinine clearance <124 μm/l), was undertaken to investigate a possible correlation between responsiveness and extent of metabolism.

The normotensive subjects each received 40 mg debrisoquine orally on a treatment day and their lying, standing and post-exercise (after a brisk 50 m walk) blood pressures were measured using a random zero sphygmomanometer.(Gelman Hawkley, Lancing, Sussex). Urine samples were collected at similar time intervals (0, 1, 2, 3, 4, 6, 8, 24 and 48 h) and their debrisoquine content determined by g.l.c. The blood pressure of these subjects was measured at identical times on a control day in the absence of the drug, by the same observer using a random zero sphygmomanometer.

The cumulative urinary excretion of debrisoquine in the ten subjects showed a seven-fold range in the percentage

of the dose as unchanged drug in 48 h (see Fig. 4.1). The average of the "mean standing blood pressure" (i.e. the diastolic blood pressure + one third of the pulse pressure) obtained 0, 1, 2, 3, 4, 6, 8 and 24 h after debrisoquine was compared with the value obtained on the control day (using an unpaired t-test; see Table 4.1). The three subjects that showed a statistically significant hypotensive effect, excreted more unchanged debrisoquine (34, 51 and 58% of the dose) than the seven non-responders. Subject R.S., who excreted most unchanged drug had a standing mean blood pressure more than 2 S.D. below his control value 32 h after receiving the drug.

Four of these subjects (R.S., A.L., R.L. and G.D.) were dosed with [^{14}C]debrisoquine (40 mg; 5 μCi) and plasma levels of the drug were assayed radiochemically. A substantial difference was also found in the plasma drug concentrations of these subjects (see Fig. 4.2). The only responder, R.S., had a consistently high plasma debrisoquine concentration (15-58 ng/ml) over 24 h, whereas in the typical non-responder, A.L., by far the major proportion of the plasma radioactivity was found to correspond to the metabolites (average debrisoquine concentration 4-7 ng/ml). In the other two subjects, R.L. and G.D., peak debrisoquine levels appeared 1-2 h after administration, and were replaced by the appearance of its metabolites.

The mean rates of excretion of debrisoquine in responders and non-responders were compared (see Fig. 4.3). Peak excretion rates for both groups occurred 3-4 h after ingestion of the drug. However, the excretion rates were shown to be higher for responders than non-responders. The fall in the

Figure 4.1. Cumulative Excretion of Unchanged Debrisoquine in the Urines of Ten Normotensive Subjects Dosed Orally with 40 mg Debrisoquine

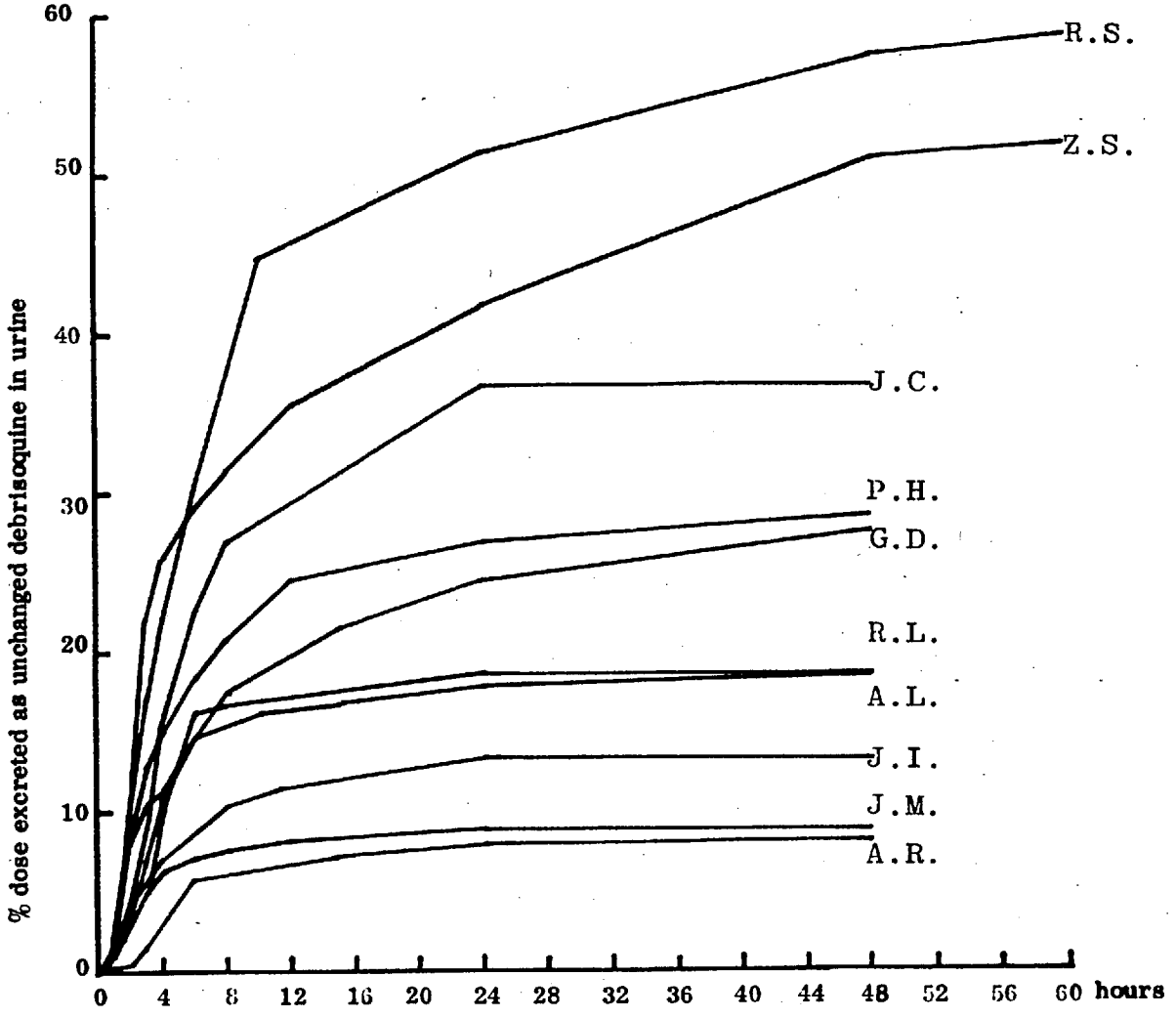


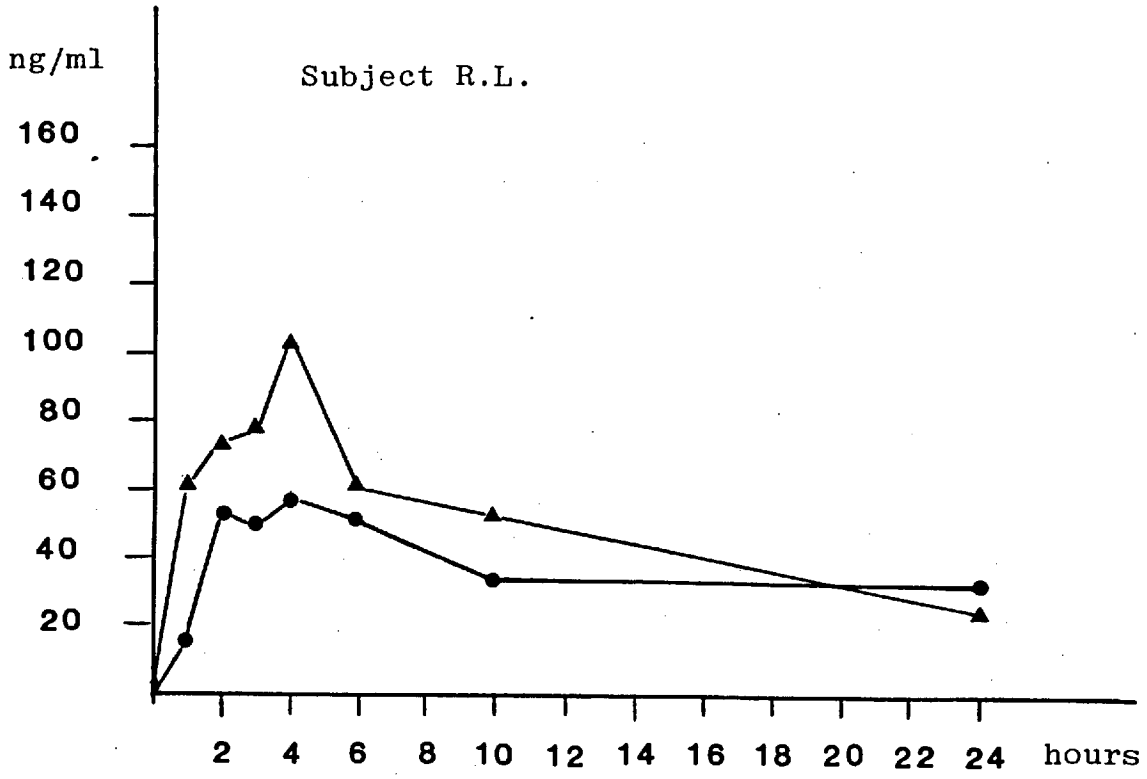
Table 4.1. Hypotensive Effect of Debrisoquine (40 mg) on Ten Normotensive Subjects

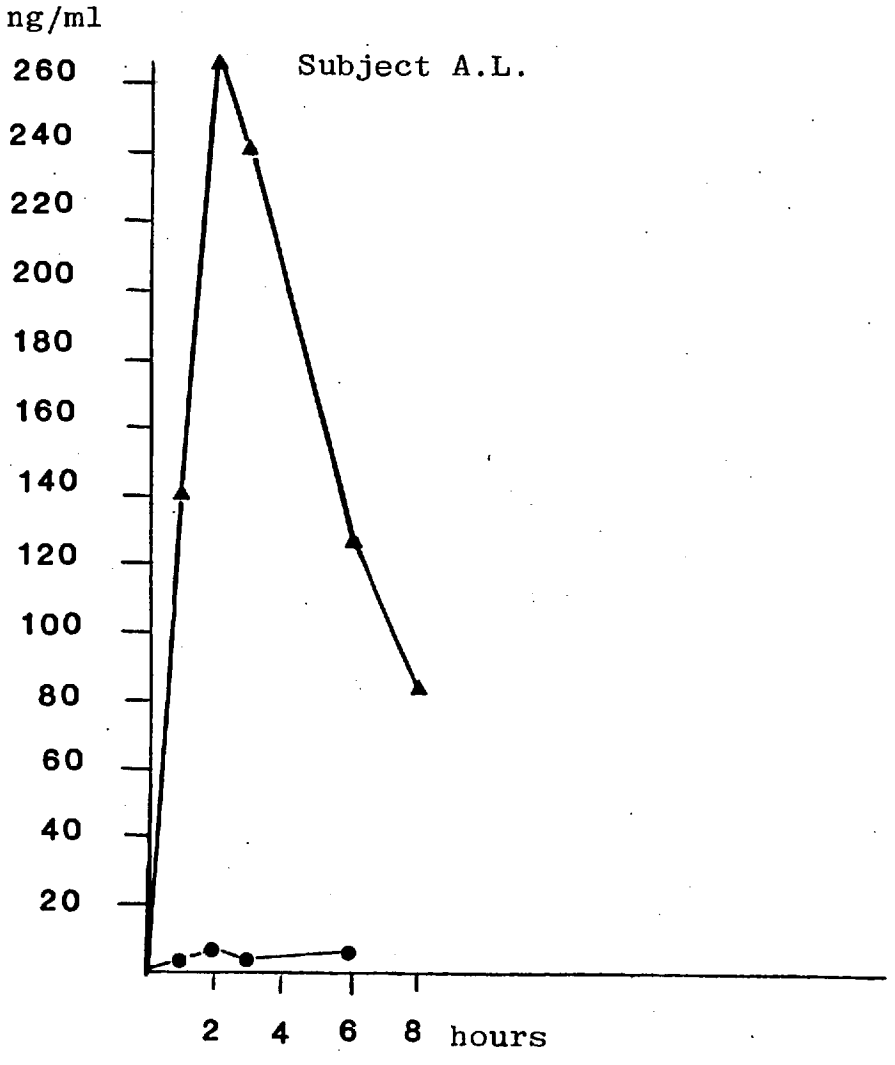
Subject	Mean Standing BP (mm Hg)		% of dose in urine by 48 h as unchanged debrisoquine
	Control day	Treatment day	
Z.S.	105.8 ± 4.8 (SD)	92.2 ± 8.8**	51
J.C.	90.9 ± 3.5	82.1 ± 9.9*	34
R.S.	99.3 ± 6.4	74.9 ± 13.5**	58
G.D.	104.3 ± 7.4	102.5 ± 9.0	23
R.L.	99.1 ± 5.6	95.0 ± 6.7	18
P.H.	85.0 ± 5.7	84.8 ± 9.5	28
J.I.	84.4 ± 7.0	89.0 ± 12.0	13
A.R.	93.1 ± 6.6	91.0 ± 6.3	8
J.M.	90.4 ± 5.5	85.0 ± 4.4	9
A.L.	92.5 ± 9.6	89.7 ± 6.7	18

* $P < 0.05$

** $P < 0.01$

Figure 4.2. Plasma Profiles of Four Normotensive Subjects Dosed Orally with [^{14}C]Debrisoquine (40 mg; 5 µCi)





▲= total plasma metabolites ●= plasma debrisoquine

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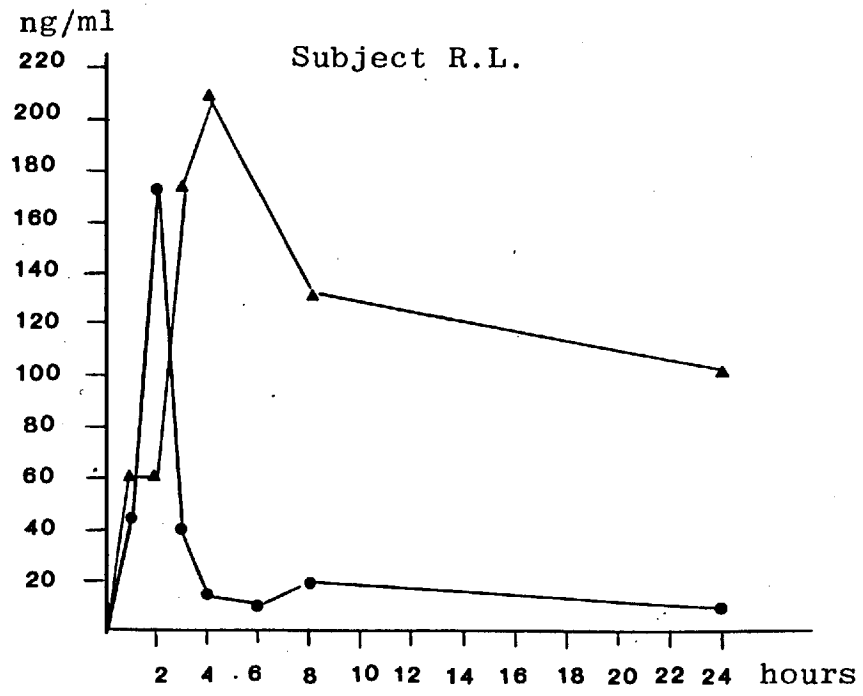
Figure 4.2. Continued



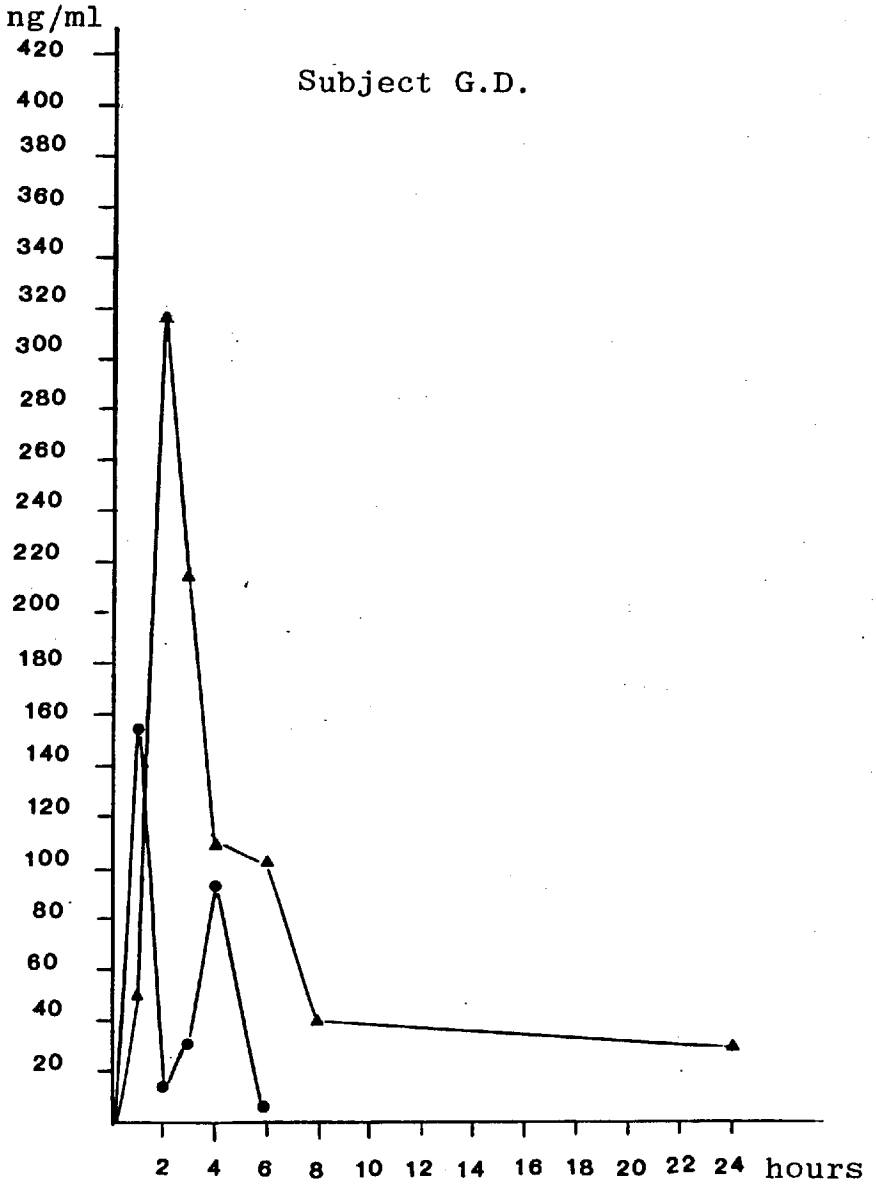
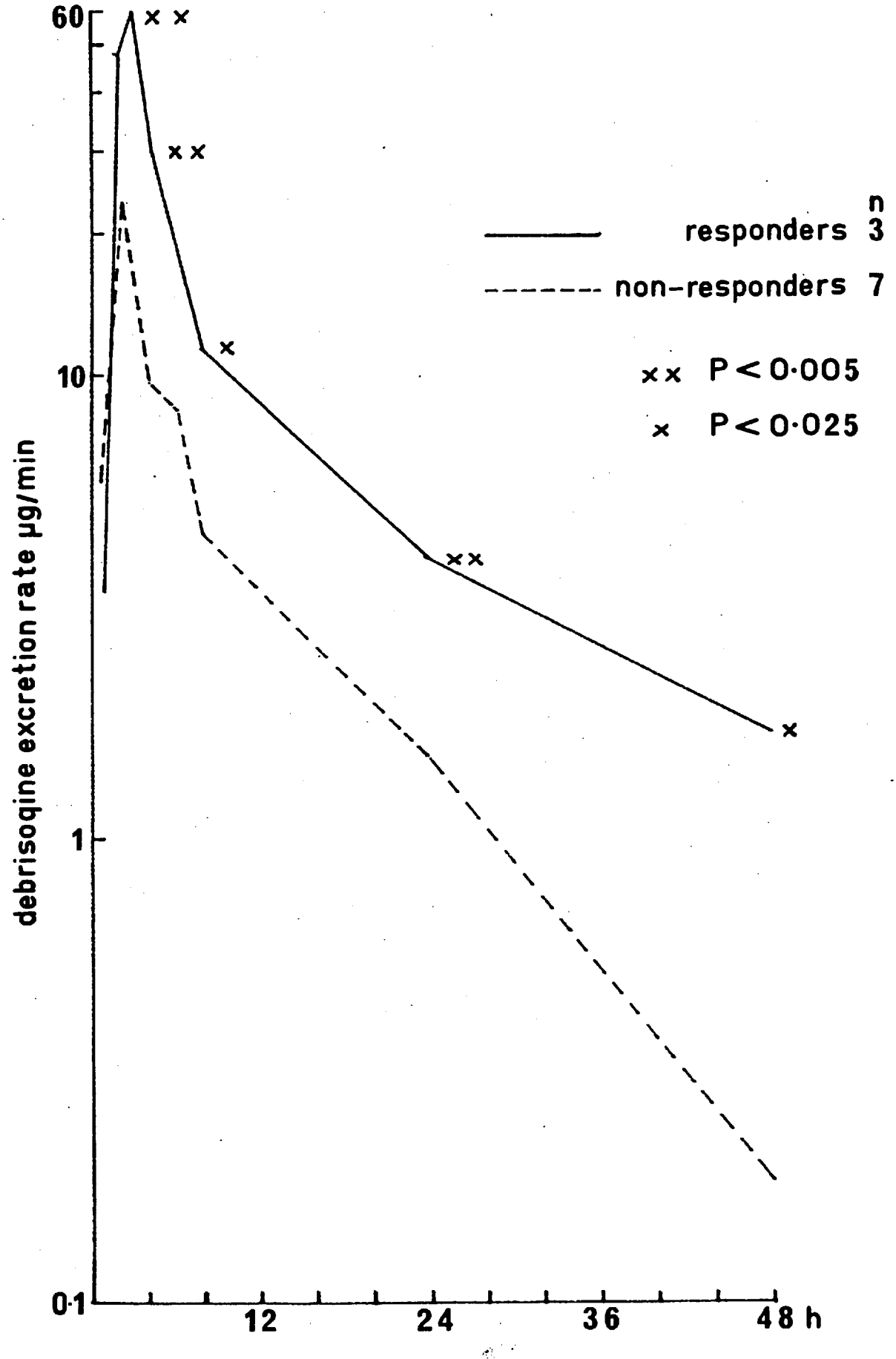


Figure 4.3. Urinary Excretion Rates of Debrisoquine by Ten Normotensive Subjects after an Oral Dose of 40 mg Debrisoquine



excretion rates with time for both groups is compatible with a two or more compartment model but there is insufficient data for a detailed pharmacokinetic analysis.

The seven hypertensive subjects involved in this investigation all attended a general outpatient clinic. To the drug regimen of these patients, who were being maintained on a thiazide diuretic alone or a combination of thiazide and amiloride, was added a maintenance dose of debrisoquine. Each patient collected urine for a period of 24 h before attending the clinic, and the debrisoquine content was determined. Lying and standing blood pressures were measured using a random zero sphygmomanometer, by a nurse with no involvement in the investigation, and compared with readings obtained prior to debrisoquine therapy. Six of the patients underwent another such test day, the second occasion being after an increase in the debrisoquine dosage had been prescribed.

The range of the percentage dose excreted as unchanged debrisoquine was similar to that found in normotensive subjects. The response by each subject, measured as the percentage fall in mean standing blood pressure, was compared both with the daily dose and the 24 h urinary debrisoquine content. A significant correlation ($r = 0.87$) was obtained between response and the amount of unchanged drug excreted (see Fig. 4.4). However, the correlation between response and the daily dose was found to be poor ($r = 0.48$; Fig. 4.5).

Figure 4.4. Relationship between Response to Debrisoquine and 24 h Urinary Excretion of Unchanged Debrisoquine in 13 Hypertensive Subjects

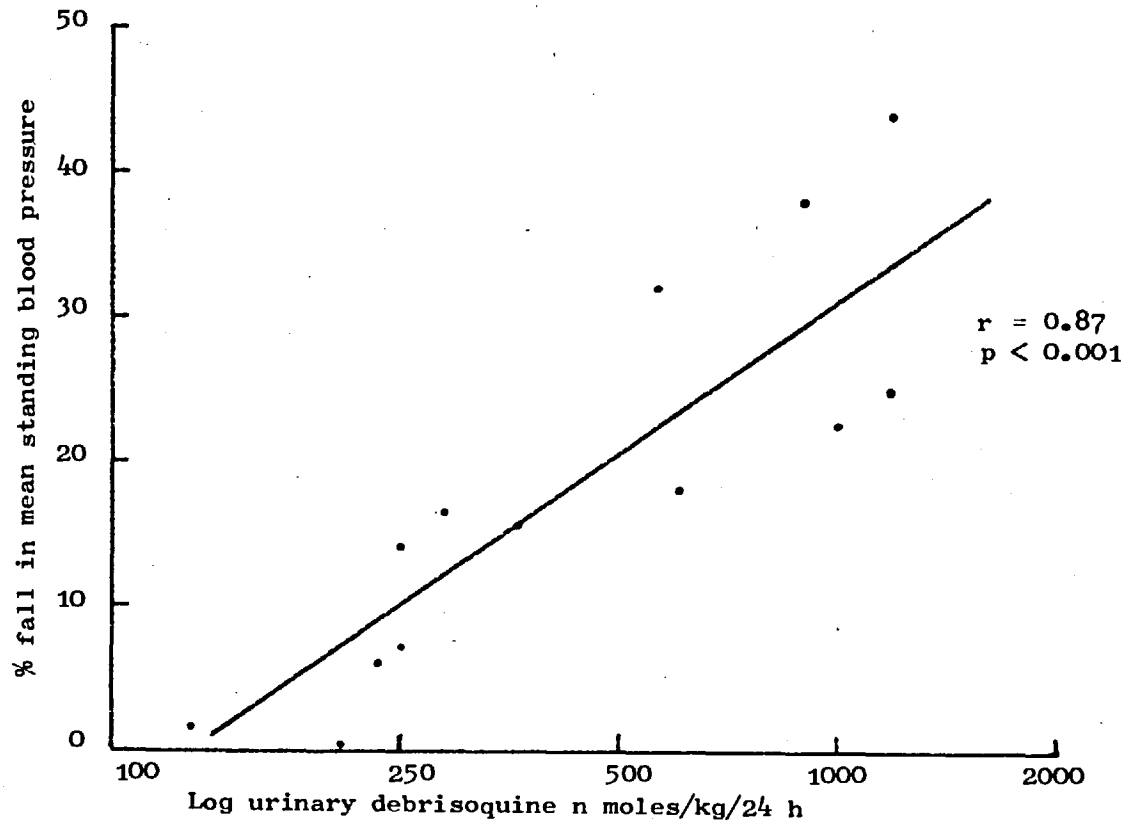
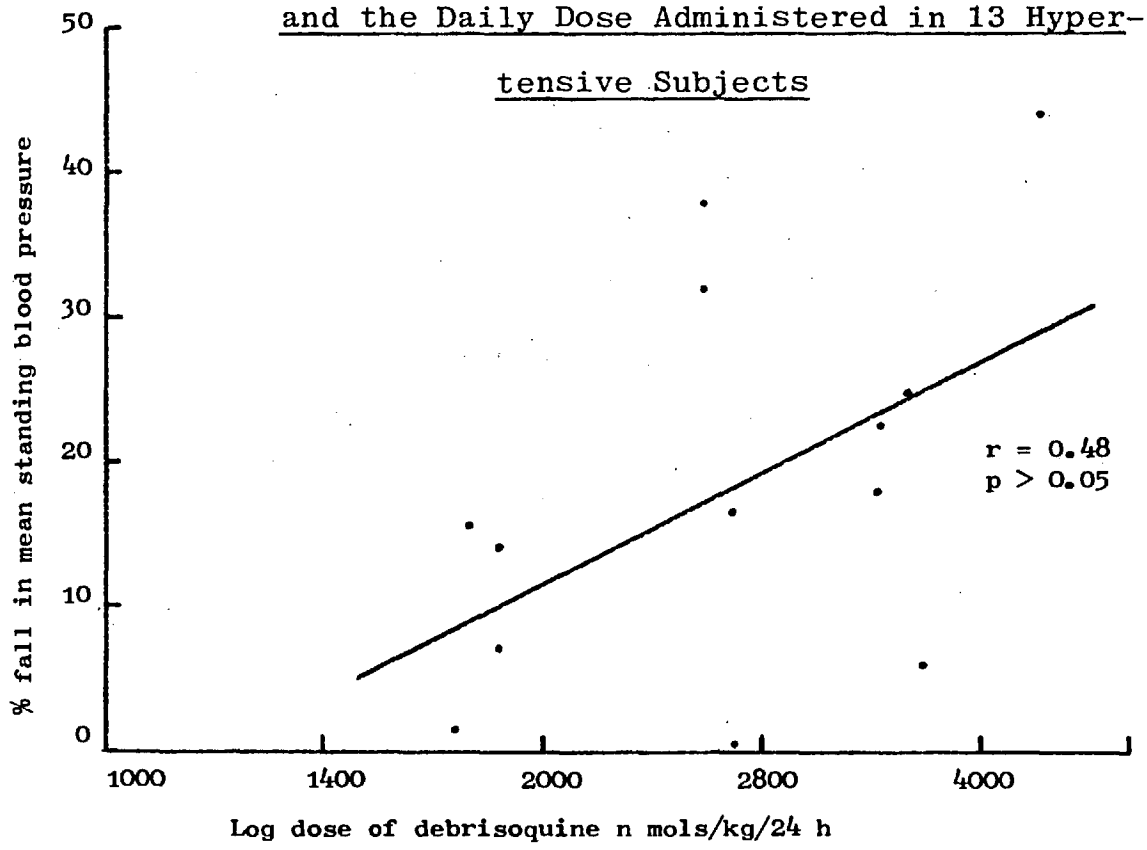


Figure 4.5. Relationship between Response to Debrisoquine and the Daily Dose Administered in 13 Hypertensive Subjects



A Double-Blind Trial of Debrisoquine and Hydrochlorothiazide in Four Normotensive Human Subjects

A carefully controlled pilot study was designed to investigate the therapeutic implications of concomitant debrisoquine and hydrochlorothiazide administration. Four healthy male volunteers (aged 30-37 years; G.K. and T.E., negro; J.M. and G.D., caucasian) with normal renal function, undertook tests on four separate days. Each subject received the following combinations of compounds contained in two unlabelled gelatine capsules: debrisoquine (40 mg) + placebo (lactose), debrisoquine (40 mg) + hydrochlrothiazide (100 mg), hydrochlorothiazide (100 mg) + placebo, and placebo + placebo. The actual drug combination that was dispensed to each subject on a particular day was determined using the latin square method, by a person with no further involvement in the experiment. This information was not disclosed until the end of the study, after the results had been analysed. Measurements of the lying, standing and post-exercise (after a brisk 50 m walk) blood pressures were made hourly for 8 h and then at 24 h, using a random zero sphygmomanometer. The mean blood pressure values were calculated and the figures for the four test days analysed for statistically significant differences using a paired t-test. $\underline{p}$ Values between the test days were thus obtained and the "double-blind" code broken to allow direct comparison of the results. Average 0-24 h "mean blood pressures" (± S.D.) for each test day are given in Table 4.2, together with the corresponding $\underline{p}$ values. An indication of the

Table 4.2 The Effects of Debrisoquine, Hydrochlorothiazide and the Compound Dose on the Mean Lying Standing and Post-Exercise Blood Pressures of Four Normotensive Male Subjects

Subject	Mean Lying Blood Pressure ± S.D.*			
	Placebo	Debrisoquine (40 mg)	Hydrochlorothiazide (100 mg)	Debrisoquine (40 mg) + Hydrochlorothiazide (100 mg)
G.D.	116.2 ± 7.9	110.3 ± 6.6 (+)	110.3 ± 9.0 (-)	117.7 ± 6.7 (-)
J.M.	89.6 ± 6.3	83.8 ± 6.9 (-)	91.2 ± 4.9 (-)	83.2 ± 4.4 (+)
G.K.	97.1 ± 9.2	99.7 ± 8.0 (-)	95.9 ± 7.3 (-)	98.8 ± 8.2 (-)
T.E.	109.8 ± 8.5	103.0 ± 13.3 (-)	104.2 ± 3.7 (-)	107.8 ± 2.8 (-)

* Statistical significance of lowering in blood pressure after treatment, as compared with the placebo in each case, is denoted as:

(-) not statistically significant ($P < 0.05$) (+) $P < 0.05$ (++) $P < 0.025$ (+++) $P < 0.005$

† Statistically significant increase in blood pressure ($P < 0.05$)

Cont/...

Table 4.2. Continued.

Subject	Placebo	Debrisoquine (40 mg)	Hydrochlorothiazide (100 mg)	Debrisoquine (40 mg) + Hydrochlorothiazide (100 mg)
		Mean Standing Blood Pressures ± S.D.*		
G.D.	125.6 ± 7.0	103.8 ± 24.2 (+++)	121.3 ± 7.6 (-)	109.9 ± 15.7 (-)
J.M.	89.9 ± 4.3	94.8 ± 20.8 (-)	86.9 ± 4.2 (-)	80.0 ± 6.7 (+)
G.K.	107.0 ± 3.6	111.4 ± 4.1 †	121.3 ± 7.6 (-)	108.9 ± 7.3 (-)
T.E.	95.8 ± 9.4	95.2 ± 5.9 (-)	93.2 ± 6.4 (-)	99.6 ± 5.8 (-)

Cont/...

Table 4.2. Continued

Subject	Placebo	Mean Post-Exercise Blood Pressures ± S.D.* Debrisoquine (40 mg)	Hydrochlorothiazide (100 mg)	Debrisoquine (40 mg) + Hydrochlorothiazide (100 mg)
G.D.	128.1 ± 17.6	108.7 ± 9.2 (++)	121.8 ± 10.7 (-)	118.4 ± 8.0 (-)
J.M.	91.8 ± 11.7	87.0 ± 6.3 (-)	93.3 ± 5.6 (-)	85.4 ± 7.0 (-)
G.K.	104.0 ± 10.7	115.9 ± 8.1 †	110.9 ± 5.9 (-)	105.0 ± 8.8 (-)
T.E.	106.1 ± 12.5	108.8 ± 12.5 (-)	100.0 ± 10.2 (-)	103.4 ± 13.8 (-)

Cont/...

efficacy of acute hydrochlorothiazide treatment was obtained by a comparison of the urinary volumes on the four experimental days (see Table 4.3). The effects of debrisoquine, hydrochlorothiazide and the compound dose upon the lying, standing and post-exercise blood pressure will be discussed separately.

Effect on lying blood pressure.

Of the four normotensive subjects, only G.D. showed a statistically significant fall in blood pressure on treatment with debrisoquine alone. G.D. exhibited no fall in lying blood pressure on hydrochlorothiazide treatment alone or the compound treatment. Interestingly, the lying blood pressure after hydrochlorothiazide alone or debrisoquine alone was lower than after compound dosing. Neither hydrochlorothiazide nor debrisoquine lowered the lying blood pressure in the case of J.M., the compound dose however proved effective in this volunteer. Lying blood pressure measurements showed a remarkable similarity after all four treatments in G.K. and T.E.

Effect on standing blood pressure

Again, G.D. was the only subject who showed a decrease in blood pressure with debrisoquine, subjects J.M. and T.E. showing no differences and G.K. exhibiting a slight increase in blood pressure after debrisoquine treatment. Subject J.M. experienced a significant fall in blood pressure after the compound dose. However, in the case of both G.K. and T.E., neither of the two drugs administered singly or together could be differentiated in its effect from the placebo.

Effect on post-exercise blood pressure

Post-exercise blood pressure was reduced by debrisoquine

Table 4.3. The Urinary Volumes Excreted After Placebo, Debrisoquine, Hydrochlorothiazide and the Compound Dose by Four Normotensive Subjects

Treatment	Urinary volumes excreted by the four subjects (in ml)			
	G.D.	J.M.	G.K.	T.E.
Placebo	2100	2242	860	1010
Debrisoquine (40 mg)	2400	2580	1100	1000
Hydrochlorothiazide (100 mg)	1680	4780	2730	1690
Debrisoquine (40 mg) + Hydrochlorothiazide (100 mg)	2200	3645	1940	2210

alone in G.D. but in no other subject. None of the treatments had an effect on the post-exercise blood pressures of J.M. and T.E. Surprisingly, G.K.'s post-exercise blood pressure was increased ($p < 0.05$) on debrisoquine treatment. Neither hydrochlorothiazide nor compound dosing evoked any significant change in the post-exercise blood pressure of any subject.

The results of this trial are summarized in Table 4.4

Table 4.4 The Effects of Various Treatments on the Blood Pressures of Four Normotensive Volunteers

Subject	Blood Pressures		
	Lying	Standing	Post-Exercise
G.D.	Debrisoquine↓	Debrisoquine↓	Debrisoquine↓
J.M.	Compound dose↓	Compound dose↓	n.s.d.
G.K.	n.s.d.	Debrisoquine↑	Debrisoquine↑
T.E.	n.s.d.	n.s.d.	n.s.d.

Compound dose = debrisoquine + hydrochlorothiazide treatment

↓= a statistically significant decrease compared to placebo

↑= a statistically significant increased compared to placebo

n.s.d. = no statistical difference.

CHAPTER FIVE

DISCUSSION

Contents

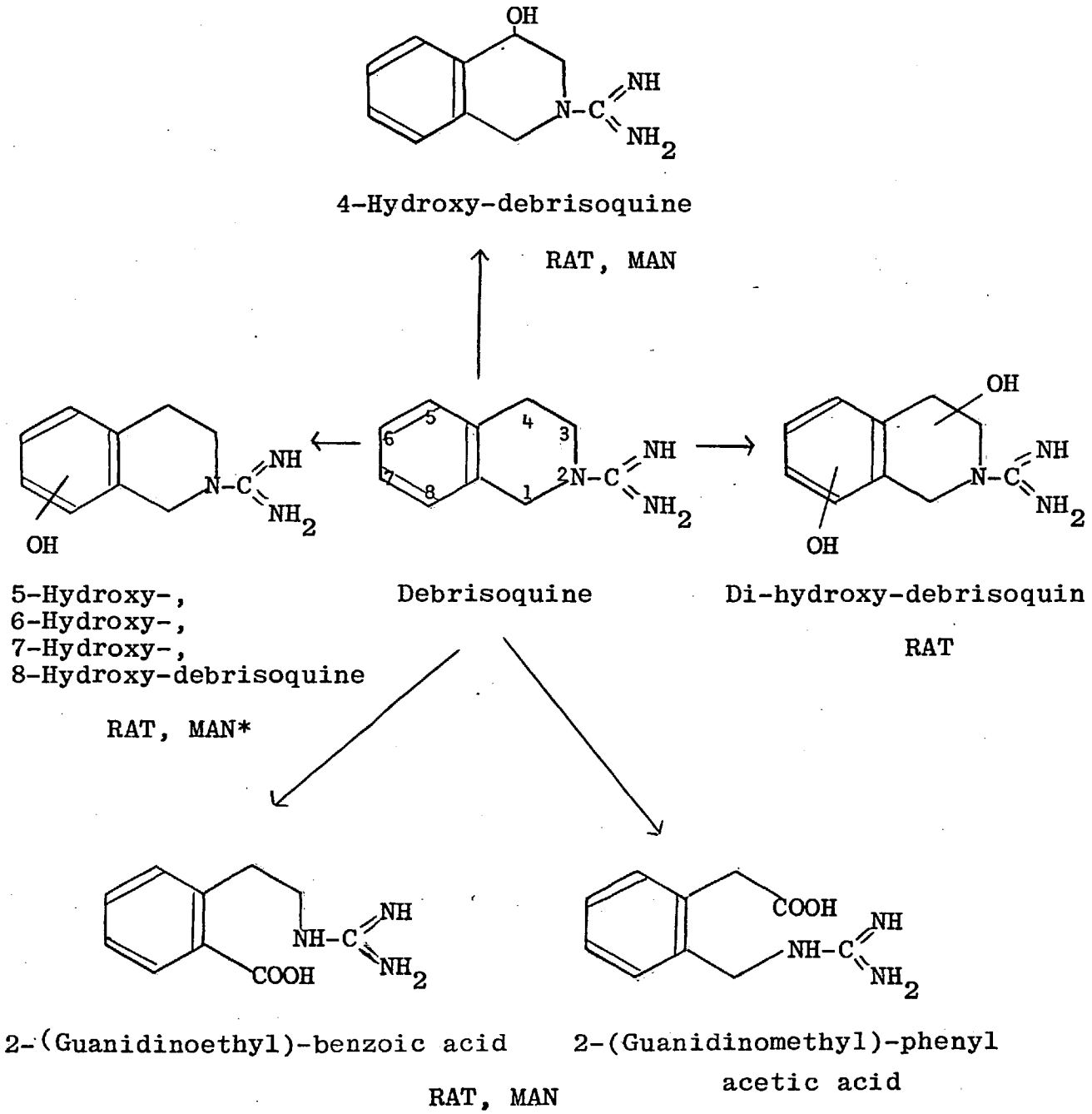
This investigation has contributed towards establishing the metabolic fate of debrisoquine and the therapeutic implications associated with its biotransformation.

Studies with the rat as an experimental species have shown that orally administered debrisoquine is largely excreted in the urine as its oxidation products, the unchanged drug accounting for only a small proportion of the dose. The metabolites have been identified as: chiefly 4-hydroxy-debrisoquine, all four phenolic compounds with a predominance of 6-hydroxy- and 8-hydroxy- debrisoquine, a di-hydroxylated metabolite thought to be oxidized on both the aromatic and heterocyclic rings and excreted as the glucuronide, and two acidic compounds arising from ring cleavage.

These studies have been extended to normal human volunteers and the metabolic pattern appears to be much the same as in the rat; the presence of 4-hydroxy-debrisoquine and the four phenols, mainly 7-hydroxy-debrisoquine, was revealed. However, no di-hydroxy-debrisoquine could be found in the urine of the subjects under investigation, and furthermore, no glucuronide or sulphate conjugates were detected. Although suggestive evidence for the presence of acidic metabolites in human urine was obtained, this is by no means unequivocal.

Corroboration of these findings (summarized in Fig. 5.1) was furnished by Allen et al. (1975) who showed the pattern of debrisoquine metabolism to be quantitatively similar in man and rat with the exception of di-hydroxy-debrisoquine production. These workers (Allen et al., 1976) were also able to provide positive evidence by g.l.c.-m.s. and n.m.r., for the presence of the two acids resulting from cleavage of

Figure 5.1. Metabolic Fate of Debrisoquine in Rat and Man



* Rat produces predominantly 6-hydroxy- and 8-hydroxy-debrisoquine. Man produces mainly 7-hydroxy-debrisoquine.

the tetrahydroisoquinoline ring at the 1- and 3- positions. However, no quantitative data was obtained by these investigators.

An interesting result of our study was the observation of major quantitative differences in the proportions of the metabolites found in human and rat urine (see Table 5.1). Furthermore, whereas all four rats studied were found to excrete fairly consistent proportions of the various metabolites, notable quantitative variations were observed between human subjects (Table 5.1). Inter-individual differences in the plasma debrisoquine and metabolite levels were also observed (2 h debrisoquine levels varied between 5-300 ng/ml). However, any pharmacokinetic analysis on the basis of the present results must be regarded as tentative, since the sensitive hexafluoroacetylacetone plasma assay was not available at the time and consequently only a small number of subjects were studied. Nonetheless, the available data support the view that orally-administered debrisoquine is subject to a major first-pass effect by hepatic extraction.

The structural congeners of debrisoquine, namely guanethidine and guanoxan, also undergo metabolism chiefly by oxidation (see Fig 1.10). An investigation of the fate of orally-administered guanoxan in five hypertensive subjects (Jack et al., 1972) showed marked individual differences in the extent of metabolism of the drug. The first day urine of these subjects contained non-conjugated 6-hydroxy- and 7-hydroxy- guanoxan in amounts ranging from 12 to over 50% of the daily dose. It is interesting to speculate that

Table 5.1. Metabolites of [^{14}C]Debrisoquine in Man and Rat, expressed as a % of the Dose in 0-24 h Urine

Metabolite R_F†	Probable identity	Human subjects				Rat¶
		R.L.	G.D.	R.S.	A.L.	
0.00)) 0.14)	m-1 (see text)	5.8	9.1	1.5	11.2	11.0 (8.8-13.6)
0.33	4-hydroxy-debrisoquine	24.2	16.7	5.4	15.1	34.9 (29.9-40.1)
0.58	Phenolic band*	26.8	25.2	7.3	32.7	11.5 (7.8-13.4)
0.73	Debrisoquine	18.8	23.2	54.0	18.6	8.8 (6.9-12.2)

† Descending paper chromatography on Whatman No. 1 paper with butan-2-ol:formic acid:water (100:12:10 v/v)

* Presence of the phenols in the m-3 radioactive band has only been shown qualitatively. No quantitative figures are available for the various phenols.

¶ Mean values for 3 rats with range in parentheses

guanoxan may be the active agent; since of the three patients being maintained on various doses of the drug (50, 120 and 200 mg/day), only the subject who required the lowest dosage did not produce any phenolic metabolites whatsoever. However, no formal investigations have, as yet, been carried out to establish the therapeutic implications of guanoxan metabolism.

The biological consequences of metabolism are manifold and may result in an increase or decrease in pharmacological activity or toxicity of the drug. The classical example of the formation of an active metabolite is the insecticide parathion which requires oxidation to paraoxon to evoke its powerful anticholinesterase activity (Gage, 1953). Conversely, amongst the barbiturate sedatives are examples of metabolism resulting in either inactive or equally-active products. Thus, whilst hexobarbitone is metabolized to inactive products resulting in a short pharmacological half-life (Tsukamoto et al., 1956), prominal (methyl phenobarbitone) is N-demethylated to phenobarbitone, a long-acting drug of lesser intensity (Butler and Bush, 1939). The extent of oxidative metabolism of debrisoquine was found to be inversely related to the percentage fall in mean blood pressure in both normotensive and hypertensive subjects (Angelo et al., 1975). Data presented in Figures 4.4 and 4.5 indicate that it is debrisoquine per se which is the active agent, since any attempt to correlate the administered dose with the therapeutic response proved unsuccessful. Furthermore, results of the debrisoquine plasma assays provide more direct evidence for the concept that variations in systemic availability of the drug may explain differences in response, and consequently

provide an explanation for the clinically observed variations in dose requirement of the drug.

Similar conclusions were reached by Lennard *et al.* (1976), whose investigation involved measurements of blood pressure along with both plasma and urinary debrisoquine and 4-hydroxy-debrisoquine levels in thirteen hypertensive patients. They also found a positive correlation between urinary recovery of unchanged debrisoquine (showing an inter-individual range of 9-80%) and the fall in standing diastolic blood pressure ($r = 0.86$; $P < 0.001$), which was largely independent of the dose. No correlation was observed between urinary 4-hydroxy-debrisoquine levels (inter-individual range of 0-30%) and fall in blood pressure. Moreover, the daily urinary recovery of debrisoquine and 4-hydroxy-debrisoquine, was shown by these workers to correlate well with the peak and trough plasma values.

In recent years, a positive attempt has been made to elucidate the underlying reason for observed individual variations in the extent of drug metabolism. Environmental factors are generally known to alter the rates at which human subjects metabolize certain drugs. These include exposure to inducers of drug-metabolizing enzymes, the hormonal and nutritional status of the subjects and their general physiological condition. However, comparative pharmacological studies involving identical and fraternal twins, have shown that the wide differences in drug metabolism by hepatic microsomal oxidative enzymes, may be attributed to genetic factors. In these studies of the metabolism of bis-hydroxy-coumarin, phenylbutazone and antipyrine (Vesell and Page, 1968*a*,*b*,*c*), as well as ethanol (Vesell *et al.*, 1971), halothane

(Cascorbi et al., 1971) and nortryptyline (Alexanderson et al., 1969), large inter-individual differences in half-life were shown to vanish within a set of identical twins, but to persist within a set of fraternal twins. Nevertheless, the mode of inheritance associated with observed variations in drug metabolism has only been elucidated in a number of cases. The involvement of genes at a single locus in the acetylation of isoniazid, has been ascertained by means of distribution studies in a series of identical and fraternal twins (Bönicke and Lisboa, 1957). A discontinuous distribution curve was found. Alternatively, similar studies with other drugs reveal a unimodal (Gaussian) distribution of nortryptyline steady state blood levels (Alexanderson et al., 1969) and antipyrine half-lives (Vesell and Page, 1968b), suggesting polygenic control of these variations.

The pharmacological significance of these differences in metabolism, in terms of drug response and drug toxicity have been investigated to a certain extent. With the example of isoniazid acetylation, some degree of controversy exists as to whether it is the fast or slow acetylators that are vulnerable to the greater toxic effects. The original belief that slow acetylators experience hypovitaminosis due to elevated blood levels of the parent compound (Kalow, 1962) is now being replaced by the finding that fast acetylators produce high levels of acetylhydrazine, a toxic metabolite that may cause serious hepatitis (Mitchell et al., 1975; Black et al., 1975). In practical terms, however, large variations in drug metabolism indicate the difficulty of predicting an optimum dose of a compound. On administration of the same dose of a drug to a normal population, some people will exhibit an

ineffective plasma concentration, others will be in the therapeutic dose range, and still others will be in the toxic range. Such is the case with diphenylhydantoin, where seizure control is most effective with blood concentrations of the drug between 10 and 20 μg/ml and where levels of 40 μg/ml or over cause nystagmus, ataxia and lethargy (Kutt, 1971).

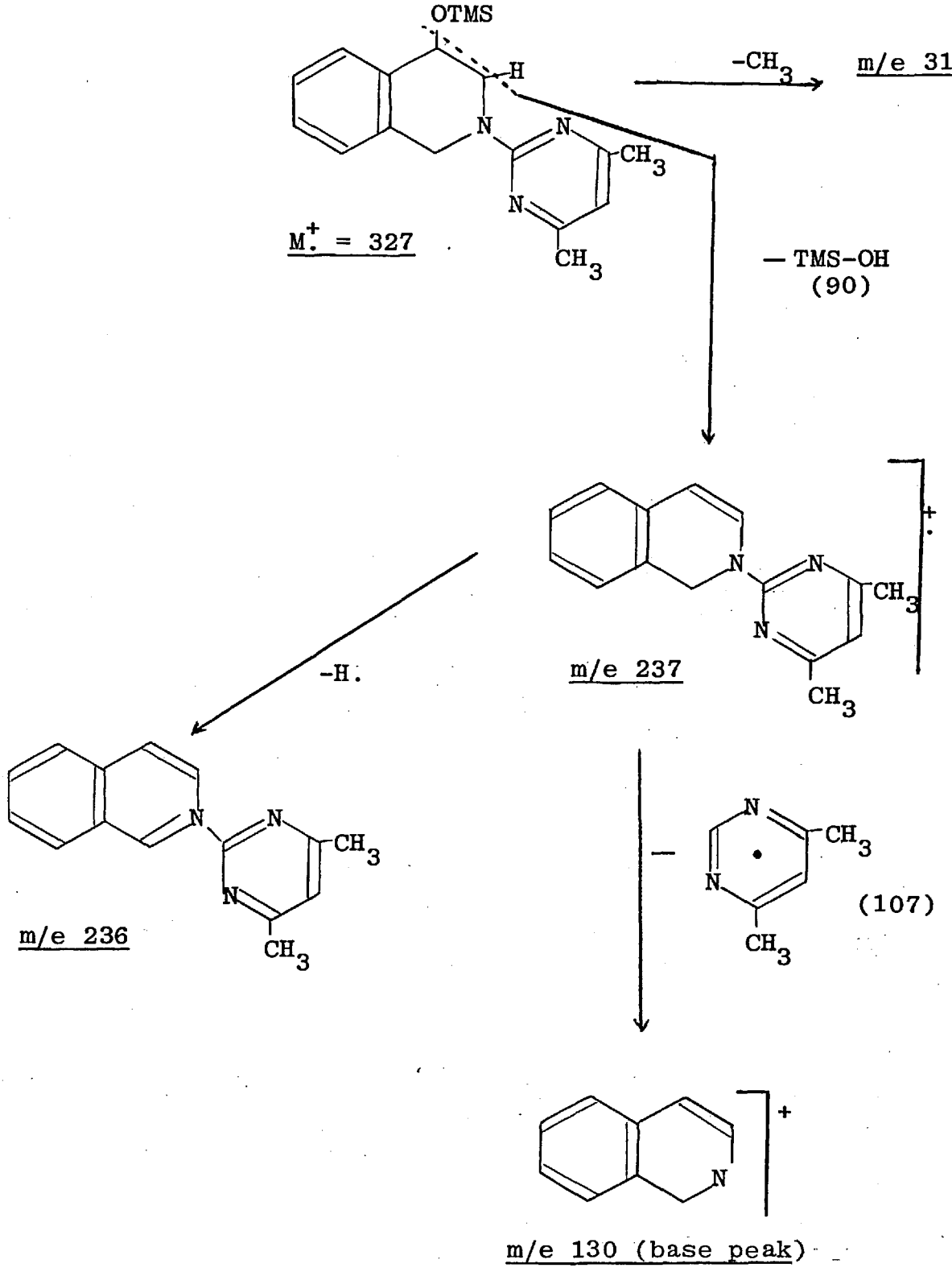
Debrisoquine is often simultaneously prescribed with a diuretic and therefore special attention must be paid to the question of their interaction. From our pilot double-blind study we were fortunate enough to see a variety of responses ranging from a statistically significant effect with only the compound dose to no hypotensive effect with any of the treatments (see Table 4.4). However, it is evident that similar studies should be performed with large populations to evaluate the therapeutic efficacy of concommitant debrisoquine and diuretic treatment. In general, drug interactions have many manifestations which include the displacement of one drug by another from its plasma protein binding sites, and interference with the metabolism or renal excretion of one drug by another. An understanding of such interactions may be important for safe and effective therapy.

Data presented in this thesis would suggest that debrisoquine metabolism lends itself ideally to pharmacogenetic analysis. For this type of study, the need to perfect the available techniques for measuring debrisoquine and its metabolites in plasma samples from a larger population, is evident. Thereafter, familial and twin studies may be employed to define the mode of inheritance involved.

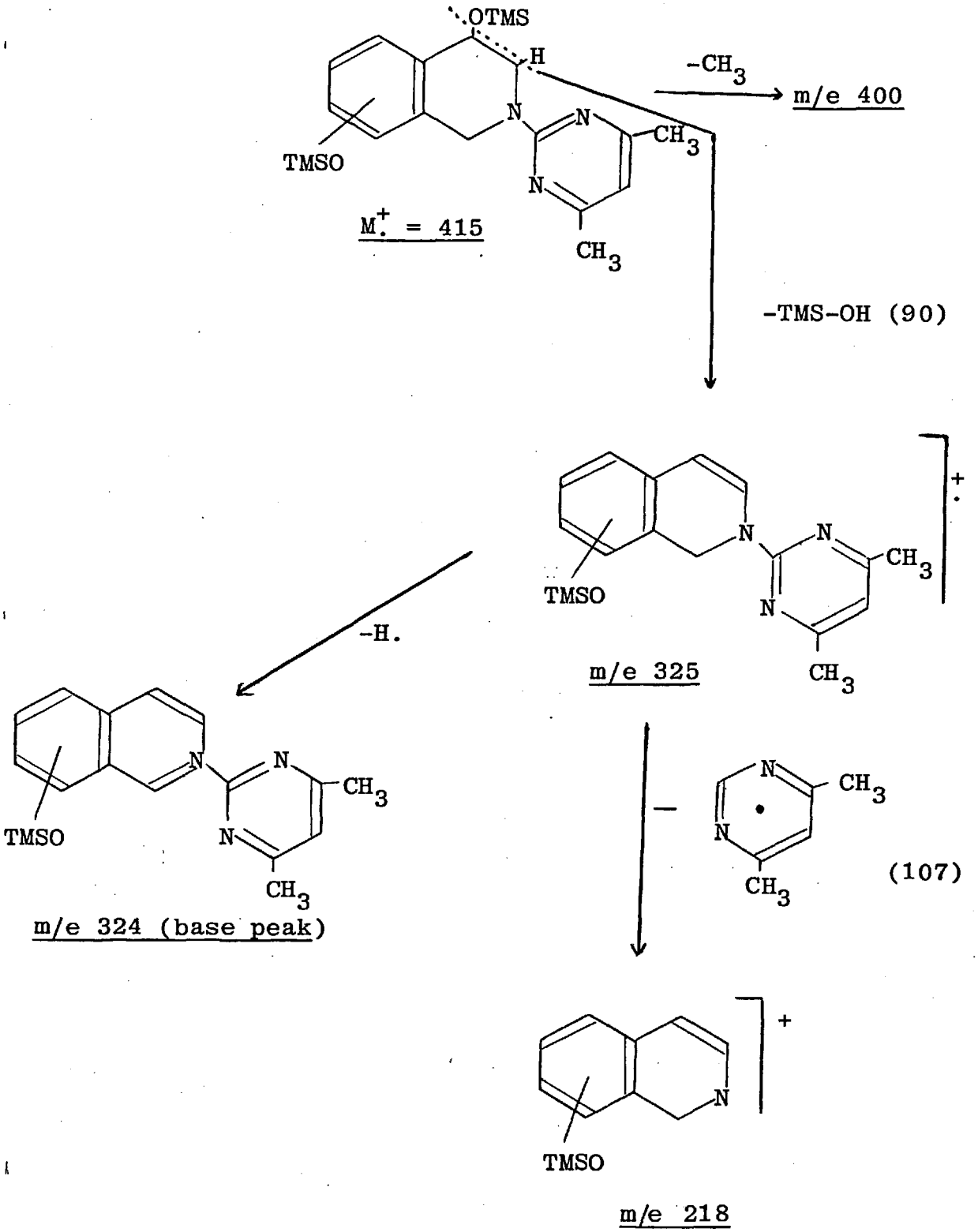
APPENDIX

PROPOSED FRAGMENTATION PATTERNS

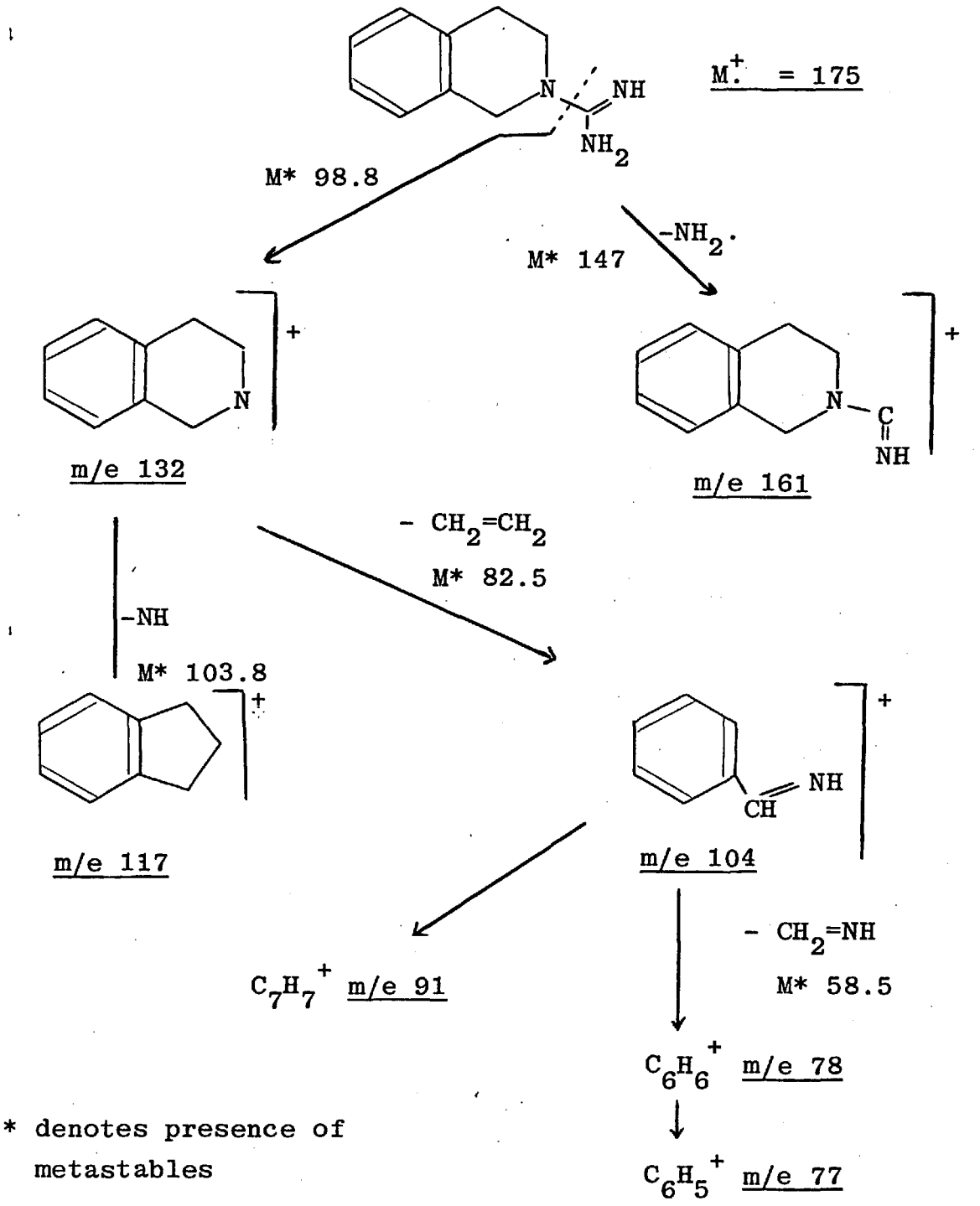
Contents



Proposed Fragmentation Pattern of 4-Hydroxy-debrisoquine trimethylsilyl-4,6-dimethylpyrimidine derivative



Proposed Fragmentation Pattern of Di-hydroxy-debrisoquine trimethylsilyl-4,6-dimethylpyrimidine derivative



* denotes presence of metastables

Proposed Fragmentation Pattern of Debrisoquine

Evidence for Hydroxylation of Debrisoquine on Both Rings

Comparison of the mass spectra of the derivatized di-hydroxy-debrisoquine isolated from rat urine and of derivatized 4-hydroxy-debrisoquine would suggest that the dihydroxylation had occurred on both the aromatic and heterocyclic rings. A prominent feature of both fragmentation patterns was the M-90 transition which corresponded to the loss of TMS-OH from the molecular ions. A large ion at M-90 was not, however, a mass spectral characteristic of the derivatized phenols (see Figs. 3.6 and 3.9).

REFERENCES

References

Aars, H. (1968) Acta Physiol. Scand. 72, 298-309.

Abbs, E.T. (1966) Br. J. Pharmacol. 26, 162-171.

Abeloud, J.E. & Bardier, E. (1908) C.R. Seances Soc. Biol. Ses Fil. 64, 848-849

Abercrombie, G.F. & Davies, B.N. (1963) Br. J. Pharmacol. 20, 171-177

Abrams, A. (1967) in Baroreceptors and Hypertension (Kezdi, P., ed.) pp. 273-291, Pergamon Press, New York.

Abrams, W.B., Pocelinko, R., Klausner, M., Hanauer, L. & Whitman, E.N. (1964) J. New Drugs 4, 263-283

Addis, G.J. & MacDougall, A.I. (1969) in Catapres in Hypertension (Conolly, M.E., ed.) pp. 145-153, Butterworths, London.

Adi, F.C., Eze, C.J. & Anwunah, A. (1975) Br. Med. J. 1; 482-485.

Ahlquist, R.P. (1948) Am. J. Physiol. 153, 586-600.

Alarcon-Segovia, D., Wakim, K.G., Worthington, J.W. & Ward, L.E. (1967) Medicine (Baltimore) 46, 1-33.

Aleksandrow, D., Wysznacka, W. & Gajewski, J. (1959) N. Engl. J. Med. 260, 51-55.

Alexander, R.S. (1946) J. Neurophysiol. 9, 205-217.

Alexanderson, B., Price Evans, D.A. & Sjöqvist, F. (1969) Br. J. Med. 4: 764-768.

Allum, W., Aminu, J., Bloomfield, T.H., Davies, C., Scales, A.H. & Vere, D.W. (1974) Br. J. Clin. Pharmacol. 1, 51-57.

Amery, A. & Deloof, W. (1970) Lancet 2: 613.

Angell-James, J.E. (1973) Circ. Res. 32, 149-161.

Angelo, M., Dring, L.G., Lancaster, R., Latham, A. & Smith, R.L. (1975) Br. J. Pharmacol. 55, 264P

Athanassiadis, D., Cranston, W.I., Juel-Jensen, B.E. & Oliver, D.O. (1966) Br. Med. J. 2: 732-735.

Au, W.Y.W., Dring, L.G., Grahame-Smith, D.G., Isaac, P. & Williams, R.T. (1972) Biochem. J. 129, 1-10.

Ayman, D. (1934) Arch. Intern. Med. 53, 792-802.

Baer, H.H. & Achmatowicz, B. (1964) J. Org. Chem. 29, 3180-3185.

Baer, J.E., Leidy, H.L., Brooks, A.V. & Beyer, K.H. (1959) J. Pharmacol. Exp. Ther. 125, 295-302.

Ball, J.D. (1950) Lancet 2: 650.

Bamberger, E. & Dieckmann, W. (1893) Ber. Dtsch. Chem. Ges. 26, 1205-1221.

Barlow, R.B. & Ing, H.R. (1948) Br. J. Pharmacol. 3, 298-304.

Barnett, A.J. & Cantor, S. (1968) Med. J. Aust. 1, 87-91.

Bath, N.M., Gunnels, J.C. & Robinson, R.R. (1968) Am. J. Med. 45, 381-390.

Bayer, B.L., Mentz, P. & Foerster, W. (1972) Arch. Int. Pharmacodyn. Ther. 200, 341-347.

Bayliss, W.M. (1902) J. Physiol. 28, 220-231.

Bechgaard, P. (1946) Acta Med. Scand. Suppl. 172, 1-358.

Bein, H.J. (1956) Pharmacol. Rev. 8, 435-483.

Belle, M.S., Gilmore, H.R., Keyes, M.H. & Sanford, H. (1968) J. Fl. Med. Assoc. 55, 1081-1084.

Bentley, G.A. & Li, D.M.F. (1968) Eur. J. Pharmacol. 4, 124-134.

Beyer, K.H. & Baer, J.E. (1961) Pharmacol. Rev. 13, 517-562.

Bing, J. & Poulson, K. (1970) Acta Pathol. Microbiol. Scand. 78A, 6-18.

Black, M., Mitchell, J.R., Zimmerman, H.J., Ishak, K. & Epler, G.R. (1975) Gastroenterology 69, 289-302.

Bohlme, P., Fuxe, K. & Lidbrink, P. (1972) Res. Commun. Chem. Pathol. Pharmacol. 4, 657-697.

Bohr, D.R. (1974) Fed. Proc. Fed. Am. Soc. Exp. Biol. 33, 127-132.

Bönicke, R. & Lisboa, B.P. (1957) Naturwissenschaften 44, 314.

Boston Collaborative Drug Surveillance Progam (1974) Lancet 2: 669-671.

Boura, A.L.A. & Green, A.F. (1965) Annu. Rev. Pharmacol. 5, 183-212.

Boyd, G.W., Landon, J. & Peart, W.S. (1967) Lancet 2: 1002-1005.

Boyd, G.W., Adamson, A.R., Fitz, A.E. & Peart, W.S. (1969) Lancet 1: 213-218.

Boynton, R.E. & Todd, R.L. (1948) Am. J. Med. Sci. 216, 397-402.

Bravo, E.L., Tarazi, R.C. & Dustan, H.P. (1974) J. Lab. Clin. Med. 83, 119-128.

Bridges, J.W., Davies, D.S. & Williams, R.T. (1967) Biochem. J. 105, 1261-1267.

Brobeck, J.R. (1973) ed. Best and Taylor's Physiological Basis of Medical Practice, 9th ed., chap. 3, pp. 164-166, Williams & Wilkins, Baltimore.

Brown, J.J., Davies, D.L., Lever, A.F. & Robertson, J.I.S. (1965) Br. Med. J. 2: 1215-1219.

Brown, J.J., Davies, D.L., Lever, A.F. & Robertson, J.I.S. (1966) Br. Med. J. 1: 505-508.

Brown, J.J., Lever, A.F., Robertson, J.I.S. & Schalekamp, M.A. (1976) Lancet 1: 1217-1219.

Brunner, H.R., Kirshman, J.D., Sealey, J.E. & Laragh, J.H. (1971) Science, 174, 1344-1346.

Brunner, H.R., Laragh, J.H., Baer, L., Newton, M.A., Goodwin, F.T., Krakoff, L.R., Bard, R.H. & Bühler, F.R. (1972) N. Engl. J. Med. 286, 441-449.

Bryant, J., Schvartz, N., Fertig, H., Fletcher, L.Jr. & Quan, R.B.F. (1964) J. New Drugs 4, 221.

Bühler, F.R., Laragh, J.H., Vaughn, E.D., Brunner, H.R., Gavras, H. & Baer, L. (1973) Am. J. Cardiol. 32, 511-522.

Bumpus, F.M., Sen, S., Smeby, R.R., Sweet, C., Ferrario, C.M. & Khosla, M.C. (1973) Circ. Res. Suppl. 32, 150-158.

Burn, J.H. & Dale, H.H. (1915) J. Pharmacol. Exp. Ther. 6,, 417-438.

Butler, T.C. & Bush, M.T. (1939) J. Pharmacol. Exp. Ther. 65, 205-213.

Carlsson, A. & Lindqvist, M. (1962) Acta Physiol. Scand. 54, 87-94.

Cascorbi, H.F., Vesell, E.S., Blake, D.A. & Hebrick, M. (1971) Clin. Pharmacol. Ther. 12, 50-55.

Cass, R., Kuntzman, R. & Brodie, B.B. (1960) Proc. Soc. Exp. Biol. Med. 103, 871-872.

Celander, O. (1954) Acta Physiol. Scand. 32, Suppl. 116.

Chalmers, J.P. (1975) Circ. Res. 36, 469-480.

Chalmers, J.P. & Reid, J.L. (1972) Circ. Res. 31, 789-804.

Chalmers, J.P. & Wurtman, R.J. (1971) Circ. Res. 28, 480-491.

Christlieb, A.R., Biber, T.U.L. & Hickler, R.B. (1969) J. Clin. Invest. 48, 1506-1518.

Cohen, S.I., Young, M.W., Lau, S.H., Haft, J.I. & Damato, A.N. (1968) Circulation 37, 738-746.

Cole, T.O. & Adadevoh, B.K. (1970) Br. J. Clin. Pract. 24, 245-249.

Constantine, J.W. & McShane, W.K. (1968) Eur. J. Pharmacol. 4, 109-123.

Conway, J. & Palmero, H. (1963) Arch. Intern. Med. 111, 203-207.

Couper, I.M. & Turner, P. (1970) Br. J. Pharmacol. 38, 463P-464P.

Dahl, L.K. & Love, R.A. (1954) Arch. Intern. Med. 94, 525-531.

Dahl, L.K. & Love, R.A. (1957) J. Am. Med. Assoc. 164, 397-400.

Dahl, L.K., Heine, M. & Tassinari, L. (1962) Nature, 194, 480-482.

Daniels, E.G., Hinman, J.W., Leach, B.E. & Muirhead, E.E. (1967) Nature 215, 1298-1299.

Day, M.D. & Rand, M.J. (1963) J. Pharm. Pharmacol. 15, 221-224.

Day, M.D. & Rand, M.J. (1964) Br. J. Pharmacol. 22, 72-86.

Day, M.D. & Roach, A.G. (1973) Fed. Proc. Fed. Am. Soc. Exp. Biol. 32, 724.

Dawber, T.R., Kannel, W.B., Kagan, A., Donabedian, R.K., McNamara, P.M. & Pearson, G. (1967) in The Epidemiology of Hypertension, (Stamler, J., Stamler, R. & Pulman, T.N., eds.), p. 255, Grune & Stratton, New York.

DeChamplain, J., Farley, L., Cousineau, D. & Van Ameringen, M.-R. (1976) Circ. Res. 38, 109-114.

Dollery, C.T., Emslie-Smith, D. & Milne, M.D. (1960) Lancet 2: 381-387.

Dollery, C.T., Lewis, P.J., Myers, M.G. & Reid, J.L. (1973) Br. J. Pharmacol. 48, 343P.

Douglas, W.W. (1970) in The Pharmacological Basis of Therapeutics, (Goodman, L.S. & Gilman, A., eds.), 4th ed., p. 668, Macmillan, London & Toronto.

Dunleavy, D.L.F., MacLean, A.W. & Oswald, I. (1971) Psychopharmacologia 21, 101-110.

Dunman, E.W. & Zimmerman, B.G. (1970) Am. J. Physiol. 219, 1279-1285.

Durant, G.J., Roe, A.M. & Green, A.L. (1970) Prog. Med. Chem. 7, 124-213.

Edwards, F., McKeown, T. & Whitfield, A.G.W. (1959) Clin. Sci. 18, 289-300.

Ehinger, B., Falck, B. & Sporrong, D. (1966) Bibl. Anat. 8, 35-45.

Engleman, K. & Portnoy, B. (1970) Circ. Res. 26, 53-57.

Erdmansky, P. & Goehl, T.J. (1975) J. Anal. Chem. 47, 750-752.

Esler, M.D. & Nestel, P.J. (1973) Br. Heart J. 35, 469-474.

Evans, G.H., Nies, A.S. & Shand, D.G. (1973) J. Pharmacol. Exp. Ther. 186, 114-122.

Evans, G.H., Wilkinson, G.R. & Shand, D.G. (1973) J. Pharmacol. Exp. Ther. 186, 447-454.

Falck, B., Hillarp, N.-Å., Thieme, G. & Torp, A. (1962) J. Histochem. Cytochem. 10, 348-354.

Ferrario, C.M., Gildenberg, P.L. & McCubbin, J.W. (1972) Circ. Res. 30, 257-262.

Ferriera, S.H. & Vane, J.R. (1967) Nature 216, 868-873.

Fishberg, A.M. (1939) Hypertension and Nephritis, 4th ed. Lea & Fiberg, Philadelphia.

Fitzgerald, J.D. & O'Donnel, S.R. (1971) Br. J. Pharmacol. 43, 222-235.

Folkow, B. (1975) in Pathophysiology and Management of Arterial Hypertension (Berglund, G., Hansson, L. & Werko, L., eds.), p. 95, Göteburg.

Folkow, B. & Rubinstein, E.H. (1966) Acta Physiol. Scand. 68, 48-57.

Folkow, B., Grimby, G. & Thulesius, O. (1958) Acta Physiol. Scand. 44, 255-272.

Folkow, B., Hallbäck, M., Lundgren, Y. & Weiss, L. (1970) Acta Physiol. Scand. 80, 93-106.

Friedman, S.M., Nakashima, H. & Friedman, C.L. (1963) Circ. Res. 13, 223-231.

Fuxe, K. (1965) Z. Zellforsch. Mikrosk. Anat. 65, 573-596.

Gage, J.C. (1953) Biochem. J. 54, 426-430.

Geffen, L.B., Rush, R.A., Louis, W.J. & Doyle, A.E. (1973) Clin. Sci. 44, 617-620.

General Practitioner Clinical Trials (1969) Practitioner 202, 294-297.

Gent, A.E. & Bacon, A.P.C. (1967) Practitioner 198, 673-679.

Giachetti, A. & Shore, P.A. (1967) Biochem. Pharmacol. 16 237-238.

Gifford, R.W.Jr., Mattox, V.R., Orvis, A.L., Sones, D.A. & Rosevar, J.W. (1961) Circulation 24, 1197-1205.

Goldblatt, H. (1938) Bull. N.Y. Acad. Med. 14, 523-553.

Goldblatt, H., Lynch, S., Hanzel, R.F. & Summerville, W.W. (1934) J. Exp. Med. 59, 347-379.

Goormaghtigh, N. (1945) J. Pathol. Bacteriol. 57, 392-393.

Graff, S., Engleman, M., Gillespie, H.B. & Graff, A.M. (1951) Cancer Res. 11, 388-392.

Green, A.F. (1962) Adv. Pharmacol. 1, 161-225.

Green, M.A., Friedlander, R., Boltax, A.J., Hadjigeorge, C.G. & Lustig, G.A. (1966) Proc. Soc. Exp. Biol. Med. 121, 580-585.

Greenblatt, D.J. & Koch-Weser, J. 1973) Am. Heart J. 81, 473-484.

Greenblatt, D.J. & Shader, R.I. (1972) Curr. Ther. Res. 14, 615-625.

Gribbin, B., Pickering, T.G., Sleight, P. & Peto, R. (1971) Circ. Res. 29, 424-431.

Grollman, A. (1970) Proc. Soc. Exp. Biol. Med. 134, 1120-1122.

Grollman, A. & Krishnamurty, V.S.R. (1971) Am. J. Physiol. 221, 1499-1506.

Guyton, A.C., Jones, C.E. & Coleman, T.G. (1973) Cardiac Output and its Regulation, 2nd ed., pp. 323-352, W.B. Saunders, Philadelphia.

Haeusler, G., Thoenen, H., Haefely, W. & Huerlimann, A. (1968) Helv. Physiol. Acta, 26, 352-354.

Haeusler, G., Haefely, W. & Huerlimann, A. (1969a) Naunyn-Schmiedeberg's Arch. Pharmacol. 265, 260-277.

Haeusler, G., Haefely, W. & Huerlimann, A. (1969b) Naunyn-Schmiedeberg's Arch. Pharmacol. 264, 241-243.

Haeusler, G., Lorez, H.P., Bartholini, G., Kettler, R. & Tranzer, J.P. (1974) J. Pharmacol. Exp. Ther. 189, 646-658.

Hamet, P., Kuchel, O., Cuche, J.L., Boucher, R. & Genest, J. (1973) Can. Med. Assoc. J. 109, 1099-1103.

Hamilton, M., Pickering, G.W., Roberts, J.A.F. & Sowry, G.S.C. (1954) Clin. Sci. 13, 273-304.

Hansson, L. (1973) Acta Med. Scan. Suppl. 550, 1-40.

Hansson, L., Hunyor, S.N., Julius, S. & Hoobler, S.W. (1973) Am. Heart J. 85, 605-610.

Harris, R.E., Sokolow, M., Carpenter, L.G.Jr., Freedman, M. & Hunt, S.P. (1953) Circulation 7, 874-879.

Hayes, A. & Cooper, R.G. (1971) J. Pharmacol. Exp. Ther. 176, 302-311.

Healey, L.A., Magid, G.D. & Decker, J.L. (1959) N. Engl. J. Med. 261, 1358-1362.

Heffernan, A.G. & Carty, A.T. (1970) Int. J. Med. Sci. 3, 37-43.

Heffernan, A., Carty, A., O'Malley, K. & Bugler, J. (1971) Br. Med. J. 1: 75-78.

Hengestmann, J.H., Falkner, F.C., Watson, J.T. & Oates, J. (1974) Anal. Chem. 46, 34-39.

Henning, M. (1969) Acta Physiol. Scan. Suppl. 332, 1-37.

Henning, M. (1973) in Frontiers in Catecholamine Research, (Usdin, E. & Snyder, S., eds.), p. 595, Pergamon Press, New York.

Henning, M. & Rubenson, A. (1970a) J. Pharm. Pharmacol. 22 241-243.

Henning, M. & Rubenson, A. (1970b) J. Pharm. Pharmacol. 22, 535-560.

Henning, M. & Rubenson, A. (1971) J. Pharm. Pharmacol. 23, 407-411.

Henning, M. & Van Zwieten, P.A. (1968) J. Pharm. Pharmacol. 20, 409-417.

Henry, J.P., Meehan, J.P. & Stephens, P.M. (1967) Psychosom. Med. 29, 408-432.

Heymans, C. & De Vleeschhouwer, G.R. (1950) Arch. Int. Pharmacodyn. Ther. 84, 401-408.

Heymans, C. & Neil, E. (1958) Reflexogenic Areas of the Cardiovascular System, J. & A. Churchill, London.

Hickler, R.B., Lauler, D.P., Saravis, C.A., Vagnucci, A.E., Steiner, G. & Thorn, G.W. (1964) Can. Med. Assoc. J. 90, 280-287.

Hilgenberg, F. (1967) in Baroreceptors and Hypertension, (Kezdi, P., ed.), Pergamon Press,

Hoefke, W. & Kobinger, W. (1966) Arzneim.-Forsch. 16, 1038-1050.

Hofman, E. & Wunsch, A. (1958) Naturwissenschaften 45, 388.

Iggo, A. & Vogt, M. (1960) J. Physiol. 150, 114-133.

Jack, D.B., Stenlake, J.B. & Templeton, R. (1972) Xenobiotica 2, 35-43.

Jagger, P.I. & Braunwald, E. (1974) in Harrison's Principles of Internal Medicine (Wintrobe, M.M., Thorn, G.W., Adams, R.D., Braunwald, E., Isselbacher, K.J. & Petersdorf, R.G., eds.), p. 1238, McGraw-Hill, New York.

Jarische, A. & Richter, H. (1939) Arch. Exp. Pathol. Pharmakol. 193, 355-371.

Jeffay, H. & Alvarez, J. (1961) Anal. Chem. 33, 612-615.

Johnson, G. (1868) Med.-Chir. Trans. 51, 57.

Jouvre, A. & Tassy, J. (1974) Epidemiologie de l'Hypertension Arterielle, p. 72, Merck, Sharp & Dohme, France.

Juhasz-Nagy, A. & Szentivanyi, M. (1961) Arch. Int. Pharmacodyn. Ther. 131, 39-53.

Kagawa, C.M., Sturtevant, F.W. & Van Arman, C.G. (1959) J. Pharmacol. Exp. Ther. 126, 123-130.

Kakaviatos, N., Tuckman, J., Chupleovich, V. & Finnerty, F.A. (1964) Am. J. Cardiol. 13, 111-112.

Kalow, W. (1962) Pharmacogenetics: Heredity and Response to Drugs, p. 95, W.B. Saunders, Philadelphia & London.

Kannel, W.B. (1974) Prog. Cardiovasc. Dis. 17, 5-24.

Karvonen, M., Orma, E., Keys, A., Fidanza, F. & Brozek, J. (1959) Lancet 1: 492-494.

Kettler, R. & Tranzer, J.P. (1974) J. Pharmacol. Exp. Ther. 189, 646-658.

Kincaid-Smith, P. (1975) Med. J. Aust. Special Suppl. 1, 7-9.

Kitchen, A.H. & Turner, R.W.D. (1966) Br. Med. J. 2: 728-731.

Kobinger, W. & Walland, A. (1967) Eur. J. Pharmacol. 2, 155-162.

Kuntzman, R. & Jacobson, M.M. (1963) Ann. N.Y. Acad. Sci. 107, 945-950.

Kutt, H. (1971) Ann. N.Y. Acad. Sci. 179, 704-722.

Langren, S. (1952) Acta Physiol. Scand. 26, 1-34.

Laverty, R. (1973) Br. Med. Bull. Suppl. on Catecholamines, p. 152.

Ledingham, J.M. (1971) Practitioner, 207, 5-19.

Lee, J.B. (1967) N. Engl. J. Med. 277, 1073-1079.

Lee, J.B., Attalla, A., Vance, V.K., Elwood, C. & Prezyna, A. (1973) J. Clin. Invest. 52, 50a.

Leishman, A.W.D. (1959) Br. Med. J. 1: 1361-1373.

Lennard, M.S., Malcolm, S.L., Marten, T.R., Silas, J.H., Smith, A.J.,& Tucker, G.T. (1976) Abstract of the British Pharmacological Society Meeting - In press.

Liddle, G.W. (1961) Metabolism,10, 1021-1030.

Loewi, O. (1921) Pfügers Arch. Ges. Physiol. 189, 239-242.

Louis, W.J., Doyle, A.E. & Anavekar, S.M. (1973) N. Engl. J. Med. 288, 599-601.

Louis, W.J., Doyle, A.E., Anavekar, S.M., Johnston, C.I., Geffen, L.B. & Rush, R. (1974) Circ. Res. Suppl. 34, 57-61.

Louis, W.J., Doyle, A.E. & Anavekar, S.M. (1975) Clin. Sci. Mol. Med. 48, 239s-242s.

Lups, S. & Francke, C. (1947) Acta Med. Scand. 126, 449-458.

Luria, M.H. & Freis, E.D. (1965) Curr. Ther. Res. 7, 289-296.

Maas, A.R., Jenkins, B., Shen, Y. & Tennenbaum, P. (1969) Clin. Pharmacol. Ther. 10, 366-371.

Macleod, V.H. (1972) Br. J. Pharmacol. 45, 194P-195P.

Malmfors, T. & Abrams, W.B. (1970) J. Pharmacol. Exp. Ther. 174, 99-110.

Margolius, H.S., Geller, R.G., de Jong, W., Pisano, J.J. & Sjoerdsma, A. (1972) Circ. Res. 31, Suppl. 2, 125-131.

Marin-Grez, M., Cottone, P. & Carretero, O.A. (1972) Am. J. Physiol. 223, 794-796.

Marshall, C.R. (1913) Trans. R. Soc. Edinb. 1, 17-40.

McCubbin, J.W., Green, J.H. & Page, I.H. (1956) Circ. Res. 4, 205-210.

McGiff, J.C. & Itskovitz, A.D. (1973) Circ. Res. 33, 479-488.

McGiff, J.C., Terragno, N.A., Strand, J.C., Lee, J.B. & Lonigro, A.J. (1969) Nature 223, 742-745.

McGiff, J.C., Crowshaw, K., Terragno, N.A. & Lonigro, A.J. (1970) Circ. Res. Suppl. 27, 121-130.

McIsaac, W.M. & Kanda, M. (1964) J. Pharmacol. Exp. Ther. 143, 7-13.

McMartin, C. (1969) Biochem. Pharmacol. 18, 238-243.

Medina, M.A., Giachetti, A. & Shore, P.A. (1969) Biochem. Pharmacol, 18, 891-901.

Meneely, G.R. & Dahl, L.K. (1961) Med. Clin. North. Am. 45, 271-283.

Merguet, P., Heimsoth, V., Murata, T. & Bock, K.D. (1968) Pharmacol. Clin. 1, 30-37.

Miall, W.E. (1959) Br. Med. J. 2: 1204-1210.

Miall, W.E. & Oldham, P.D. (1955) Clin. Sci. 14, 459-488.

Miall, W.E. & Oldham, P.D. (1958) Clin. Sci. 17, 409-444.

Miall, W.E. & Oldham, P.D. (1963) Br. Med. J. 1: 75-80.

Milne, G.E. & Oleesky, S. (1951) Br. Med. J. 2: 177.

Mitchell, J.R. & Oates, J.A. (1970) J. Pharmacol. Exp. Ther. 172, 100-106.

Mitchell, J.R., Thorgeirsson, U.P., Black, M., Timbrell, J.A., Snodgrass, W.R., Potter, W.Z., Jallow, D.J. & Keiser, H.R. (1975) Clin. Pharmacol. Ther. 18, 70-79.

Moe, R.A., Bates, H.M., Palkoski, Z.M. & Banziger, R. (1964) Curr. Ther. Res. 6, 299-317.

Mohammed, S., Fasola, A.F., Privitera, P.J., Lipicky, R.J., Martz, B.L. & Gaffney, T.E. (1969) Circ. Res. 25, 543-548.

Mudge, G.H. (1966) Ann. N.Y. Acad. Sci. 139, 304-311.

Muirhead, E.E., Stirman, J.A., Lesch, W. & Jones, F. (1956) Surg. Gynecol. Obstet. 103, 673-686.

Muirhead, E.E., Jones, F. & Stirman, J.A. (1960) J. Lab. Clin. Med. 56, 167-180.

Muirhead, E.E., Daniels, E.G. & Hinman, J.W. (1966) in L'Hypertension Artérielle (Milliez, P. & Tcherdakoff, P. eds.), pp. 177-187, Expansion Scientifique Francaise, Paris.

Muirhead, E.E., Leach, B.E., Brooks, B., Shaw, P., Brosius, W.L.Jr. Daniels, E.G. & Hinman, J.W. (1967) Rev. Fr. Etud. Clin. Biol. 12, 893-898.

Muirhead, E.E., Leach, B.E., Brooks, B., Shaw, P., Brosius, W.L.Jr., Daniels, E.G. & Hinman, J.W. (1968a) Fed. Proc. Fed. Am. Soc. Exp. Biol. 27, 744.

Muirhead, E.E., Leach, B.E., Daniels, E.G.,& Hinman, J.W. (1968b) Arch. Pathol. 85, 72-79.

Muirhead, E.E., Brown, G.B., German, G.S. & Leach, B.E. (1970) J. Lab. Clin. Med. 76, 641-651.

Mulé, S.J., Bastos, M.L., Jukovsky, D. & Saffer, E. (1971) J. Chromatogr. 63, 283-301.

Mulrow, P.J. (1964) Can. Med. Assoc. J. 90, 277-280.

Muscholl, E. & Maître, L. (1963) Experientia,19, 658-659.

Nakoo, H., Yura, Y. & Itō, M. (1966) Sankyo Kenkyusho Nempo. 18, 48-50.

Nayler, W.G., Price, J.M., Swann, J.B., McInnes, I., Race, D. & Lowe, T.E. (1968) J. Pharmacol. Exp. Ther. 164, 45-59.

Nestel, P.J. & Doyle, A.E. (1968) Aust. Ann. Med. 17, 295-299.

Oates, J.A., Gillespie, L., Udenfriend, S. & Sjoerdsma, A. (1960) Science 131, 1890-1891.

Ogilvie, R.I. & Mikulic, E. (1972) J. Pharmacol. Exp. Ther. 180, 368-372.

Okamoto, K. (1969) Int. Rev. Exp. Pathol. 7, 227-270.

Okun, R., Roth, S.E., Gordon, A. & Maxwell, M.H. (1966) Calif. Med. 104, 46-50.

Onesti, G., La Schiazza, D., Brest, A.N. & Moyer, J.H. (1966) Clin. Pharmacol. Ther. 7, 17-20.

Orgain, E.S. & Kern, A. (1970) Arch. Intern. Med. 125, 255-257.

Osikowska, B.A. & Sever, P.S. (1976) Abstract of the British Pharmacological Society Meeting, in press.

Ostfeld, A.M. & Lebovits, B.Z. (1959) Arch. Intern. Med. 104, 43-52.

Pals, D.T., Masucci, F.D., Denning, G.S., Sipos, F. & Fessler, D.E. (1971) Circ. Res. 29, 673-681.

Paton, W.D.M. & Zaimis, E.J. (1948) Nature 161, 718-719.

Paton, W.D.M. & Zaimis, E.J. (1949) Br. J. Pharmacol. 4, 381-400.

Paton, W.D.M. & Zaimis, E.J. (1952) Pharmacol. Rev. 4, 219-253.

Paul, O. & Ostfeld, A.M. (1965) Prog. Cardiovasc. Dis. 8, 106-116.

Peart, W.S. (1966) Pharmacol. Rev. 18, 667-672.

Peart, W.S. (1975) N. Engl. J. Med. 292 , 302-306.

Perry, M.M.Jr., Tan, E.M., Carmody, S. & Sakamoto, A. (1970) J. Lab. Clin. Med. 76, 114-125.

Pettinger, W.A. & Horst, W.D. (1971) Ann. N.Y. Acad. Sci. 179, 310-321

Pettinger, W.A., Korn, A., Spiegel, H., Solomon, H.M., Pocelinko, R. & Abrams, W.B. (1969) Clin. Pharmacol. Ther. 10, 667-674.

Pickering, G.W. (1961) The Nature of Essential Hypertension, Churchill, London.

Pickering, G.W., Cranston, W.I. & Pears, M.A. (1961) The Treatment of Hypertension, Thomas, Springfield, Ill.

Platt, R. (1959) Lancet 2: 55-58.

Platt, R. (1963) Lancet 1: 899-904.

Pocelinko, R. & Abrams, W.B. (1964) J. Newark City Hospital, 1, 57-65.

Powell, C.H. & Slater, I.H. (1958) J. Pharmacol. Exp. Ther. 122, 480-488.

Prasad, C.M., Shah, D.S. & Gulati, O.D. (1973) Jpn. J. Pharmacol. 23, 805-811.

Preble, W.E. (1923) Boston Med. Surg. J. 188, 617-621.

Quetsch, R.M., Achor, R.W.P., Litin, E.M. & Faucett, R.L. (1959) Circulation 19, 366-375.

Ram, N. & Garvey, H.L. (1973) Fed. Proc. Fed. Am. Soc. Exp. Biol. 32, 724.

Record, R.G. & Whitfield, A.G.W. (1964) Clin. Sci. 27, 385-391.

Redleaf, P. & Tobian, L. (1958) Circ. Res. 6, 185-193.

Rehbinder, D. (1970) in Catapres in Hypertension (Conolly, M.E. ed.), pp. 227-233, Butterworths, London.

Reid, J.L., Briant, R.H. & Dollery, C.T. (1973) Life Sci. 12, 459-467.

Rocha e Silva, M., Beraldo, W.T. & Rosenfeld, G. (1949) Am. J. Physiol. 156, 261-273.

Rosendorff, C., Marsden, C.D. & Cranston, W.I. (1968) Arch. Intern. Med. 122, 487-490.

Rush, R.A. & Geffen, L.B. (1972) Circ. Res. 31, 444-452.

Saker, B.M., Mathew, T.H., Eremin, J. & Kincaid-Smith, P. (1968) Med. J. Aust. 1, 592-593.

Schmitt, H., Schmitt, H., Boissier, J.R., Giudicelli, J.F. & Fichelle, J. (1968) Eur. J. Pharmacol. 2, 340-346.

Schmitt, H., Schmitt, H. & Fénard, S. (1971) Eur. J. Pharmacol. 14, 98-100.

Schmitt, H., Schmitt, H. & Fénard, S. (1972) Eur. J. Pharmacol. 17, 293-296.

Schmitt, H., Schmitt, H. & Fénard, S. (1973) Arzneim.-Forsch. 23, 40-45.

Schultz, B.F. (1966) J. Med. Assoc. State Ala. 36, 147-153.

Shah, D.S., Premanand, N., Jariwala, N.U. & Gulati, O.D. (1974) Res. Commun. Chem. Pathol. Pharmacol. 7, 41-50.

Shand, D.G., Nuckolls, F.M. & Oates, J.A. (1970) Clin. Pharmacol. Ther. 11, 112-120.

Shapiro, A.P., Benedek, T.G. & Small, J.L. (1961) N. Engl. J. Med. 256, 1028-1033.

Shaw, J., Hunyor, S.N. & Korner, P.I. (1971) Eur. J. Pharmacol. 15, 66-78.

Shemyakin, M.M., Ovchinnikov, Yu.A., Vinogradova, E.I., Feigina, M.Yu., Kiryushkin, A.A., Aldanova, N.A., Alakhov, Yu.B., Lipkin, V.M. & Rosinov, B.V. (1967) Experientia 23, 428-430.

Sheppard, Mowles, T.F., Bowen, N., Renzi, A.A. & Plummer, A.J. (1960) Toxicol. Appl. Pharmacol. 2, 188-194.

Short, D. (1966) Br. Heart. J. 28, 184-192.

Short, J.J. & Johnson, H.J. (1939) Am. J. Med. Sci. 198, 220-224.

Sjoerdsma, A., Vendsalu, A. & Engleman, K. (1963) Circulation 28, 492-502.

Skinner, C., Coull, D.C. & Johnston, A.W. (1969) Lancet 2: 564-566.

Smirk, F.H. (1957) High Arterial Pressure, Blackwell, Oxford.

Smirk, F.H. & Hall, W.H. (1958) Nature 182, 727-728.

Søbye, P. (1948) cited by Pickering (1968)

Sommers, K., Sood, N.K. & Brenton, D.P. (1968) Br. J. Clin. Pract. 22, 387-391.

Sparks, H.V. & Bohr, D.F. (1962) Am. J. Physiol. 202, 835-840.

Spector, S., Fleisch, J.H., Malin, H.M. & Brodie, B.B. (1969) Science 166, 1300-1301.

Starke, K. & Altmann, P. (1973) Neuropharmacology 12, 339-347.

Stocks, P. (1930) Ann. Eugen. 4, 49-108.

Stokes, G.S., Weber, M.A. & Thornell, I.R. (1974) Br. Med. J.1: 60-62.

Symonds, B. (1923) J. Am. Med. Assoc. 80, 232-236.

Taggart, P. (1975) Medicine - proceedings of a sponsored meeting on symptomless hypertension, p. 12.

Tarazi, R.C. & Dustan, H.P. (1972) Am. J. Cardiol. 29, 633-640.

Terry, A.H.Jr. (1923) J. Am. Med. Assoc. 81, 1283-1284.

Thirwell, M.P. & Zsoter, T.T. (1972) Am. Heart J. 83, 512-517.

Tobian, L. (1967) Annu. Rev. Pharmacol. 7, 399-408.

Tobian, L.Jr. & Binion, J.T. (1952) Circulation 5, 754-758.

Tobian, L.Jr. & Chesley, G. (1966) Proc. Soc. Exp. Biol. Med. 121, 340-343.

Tomlinson, D.R. & Mayor, D. (1973) Eur. J. Pharmacol. 21, 161-170.

Toxic Substances List 1973 ed. p. 547, U.S. Dpt. of Health, Education and Welfare, National Institute for Occupational Safety and Health.

Trinker, F.R. (1971) J. Pharm. Pharmacol. 23, 306-308.

Tsukamoto, H., Yoshimuri, H. & Toki, S. (1956) Pharm. Bull. 4, 368-371.

United States National Health Survey (1964) Vital and Health Statistics, Series 11, No. 5. U.S. Dpt. of Health, Education and Welfare, Public Health Service.

van Zwieten, P.A. (1973) J. Pharm. Pharmacol. 25, 89-95.

Verschuer, O.von & Zipperlen, V. (1929) Z. Klin. Med. 112, 69-92.

Vesell, E.S. & Page, J.G. (1968a) Science 159, 1479-1480.

Vesell, E.S. & Page, J.G. (1968b) Science 161, 72-73.

Vesell, E.S.,& Page, J.G. (1968c) J. Clin. Invest. 47, 2657-2663.

Vesell, E.S., Page, J.G. & Passananti, G.T. (1971) Clin. Pharmacol. Ther. 12, 192-201.

Veterans Administration Coorporative Study Group on Antihypertensive Agents (1967) J. Am. Med. Assoc. 202, 1028-1034.

Veterans Administration Coorporative Study Group on Antihypertensive Agents (1970) J. Am. Med. Assoc. 213, 1143-1152.

Vetter-Diechtl, H., Vetter, W., Richter, W. & Biemann, K. (1968) Experientia 24, 340-341.

Vogel, A.I. (1956) Practical Organic Chemistry, 3rd ed. pp. 971-972, Longmans, London.

von Euler, U.S. (1946) Acta Physiol. Scand. 12, 73-97.

Walle, T. & Gaffney, T.E. (1972) J. Pharmacol. Exp. Ther. 182, 83-92.

Weitz, W. (1923) Z. Klin. Med. 96, 151-181.

Wenner, W. (1965) J. Med. Chem. 8, 125-126.

Wickström, A. & Salvesen, B. (1952) J. Pharm. Pharmacol. 4, 631-635.

Williams, R.T. (1947) Detoxication Mechanisms, 1st ed. p. 99, Chapman & Hall, London.

Williams, R.T. (1959) Detoxication Mechanisms, 2nd ed. p. 176, Chapman & Hall, London.

Wilson, I.M. & Freis, E.D. (1959) Circulation 20, 1028-1036.

Winer, N. (1974) Clin. Res. 22, 15A

Wood, R.A., Forrester, T.M., Johnston, A.W. & Palmer, K.N.V. (1973) Clin. Trials J. 10, 53-58.

World Health Organization (1962) W.H.O. Technical Report Series 231, World Health Organization, Geneva.

Zacest, R. (1975) Med. J. Aust. Suppl. 1, 4-7.

Zacest, R. & Koch-Weser, J. (1972) Clin. Pharmacol. Ther. 13, 420-425.

Zaimis, E.J. (1950) Br. J. Pharmacol. 5, 424-430.

Zusman, R.M., Caldwell, B.V., Mulrow, P.J. & Speroff, L. (1973) Prostaglandins 3, 679-690.

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