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METABOLIC AND DISPOSITION STUDIES OF QUATERNARY AMMONIUM COMPOUNDS IN MAN AND LABORATORY SPECIES

by

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To

My Parents

with

Love & Gratitude

ABSTRACT

The work described in this thesis deals with two aspects of the biochemical pharmacology of quaternary ammonium compounds.

The first part deals with the disposition of a quaternary ammonium neuromuscular blocking drug, fazadinium bromide. A convenient radiometric assay for this drug in biological fluids was developed, and applied to the study of its disposition in patients with normal and impaired renal function. The second part describes a study of the metabolic N-methylation and hence quaternisation, of ^{14}C-pyridine *in vivo*. The main findings are as follows:-

A) Disposition studies in the dog and man showed that biliary excretion is an important pathway for the elimination of fazadinium.

   i) The ratio of the hepatic to renal excretion being 3:1 in the dog, whilst in man, faecal elimination arising presumably from biliary excretion accounted for over one-third the total elimination.

   ii) Biliary excretion was not augmented to compensate for renal dysfunction after bilateral renal pedicle ligation in the dog; metabolic studies showed the major metabolite in the urine and bile of dogs was 3-methyl-2-phenylimidazole (1,2α) pyridinium, but an "unknown" metabolite in human urine was also detected.

B) Clinical studies demonstrated no significant alterations in the pharmacokinetic or pharmacodynamic properties of fazadinium in patients with normal or impaired renal function.

i) The duration of action of fazadinium was not prolonged in patients undergoing renal transplantation, nor were the plasma distribution or elimination half-lives changed.

ii) A steep dose-response relationship was obtained with fazadinium, the mean plasma concentrations at 10% and 50% recovery from neuromuscular blockade being 1.39 $\mu g\ ml^{-1}$ and 0.88 $\mu g\ ml^{-1}$ respectively. Similar studies in patients with renal failure showed no change in the sensitivity of these patients to fazadinium.

C) Biological methylation and hence the quaternisation of ^{14}C-pyridine in vivo has been observed for man and seven animal species. A species variation was found, the metabolic process being poor (5-12%) in the rat, mouse, and man, but relatively high (20-40%) in the gerbil, guinea pig, hamster, rabbit and cat. The possible pharmacological and toxicological implications of this metabolic route is discussed.

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Introduction

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1.1.1 Importance of Nitrogen

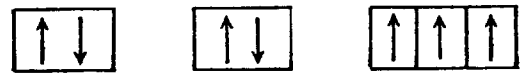
Nitrogen, the most abundant gas in the atmosphere (75% by volume) was prominent ever since the beginnings of chemical thought. Many famous early chemists such as Scheele, Lavoisier, Priestley, Cavendish, Rayleigh and Ramsey have greatly contributed to our present knowledge of this element.

Although molecular nitrogen (N_2) is extremely stable, the nitrogen atom may combine in a variety of redox states, with atoms such as hydrogen, oxygen and carbon to give an enormous number of molecules of chemical and biological importance. Nitrogen is essential for living organisms and several million tons per year are hydrogenated to give ammonia (NH_3) by "nitrogen fixing" organisms in the soil. Some plants have evolved elaborate symbiotic arrangements with bacteria in order to secure nitrogen for their own metabolism from the air.

In addition to ammonia, nitrogen-oxygen compounds are also important. In the form of nitrates (NO_3^-), plants husband this element reprocessing compounds of all sorts including those amino acids (a variety of carbon-nitrogen compounds containing both carboxyl (-COOH) and amino ($-NH_2$) groups) which are most essential to animals. Nitrogen therefore cycles through the living organisms of the biosphere and the assimilation of it by biological systemsinto macromolecules such as vitamins, hormones and proteins is essential for growth.

1.1.2 Derivatives of Nitrogen

Nitrogen having atomic number 7, is the lightest element of the fifth periodic group and so has 5 valence electrons. It has the electronic configuration $1s^2 2s^2 2p^3$, the three 2p electrons being disposed singly in the $2p_x$, $2p_y$ and $2p_z$ orbitals. The ground state of a nitrogen atom is therefore represented as follows:-

Nitrogen 

Hence the nitrogen atom can form three covalent bonds with hydrogen atoms resulting in the stable ammonia molecule. Derivatives of ammonia in which one or more hydrogen atoms have been replaced by carbon-containing groups are called amines. Thus there are four such classes:

(i) primary amines (RNH_2)

(ii) secondary amines (R_1R_2NH)

(iii) tertiary amines ($R_1R_2R_3N$), and

(iv) quaternary ammonium compounds $(R_1R_2R_3R_4\overset{+}{N})\overset{-}{X}$

where $\overset{-}{X}$ is an anion such as halide, nitrate, hydroxide, sulphate perchlorate, etc.

Furthermore, the groups attached to the nitrogen atom (R, R_1, R_2, R_3, R_4) may be the same or different, and may also contain other functional groups so that a very large variety of aliphatic, alicyclic, aromatic and heterocyclic compounds are known. Many of these occur in nature and play an important role in the structure and function of living organisms (table 1.1)

Table 1.1 Structure and function of some amines found in the body

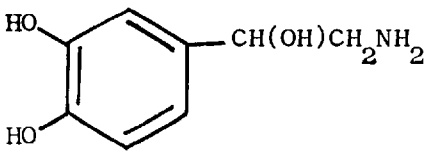
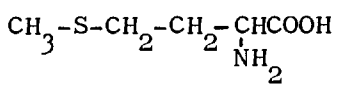
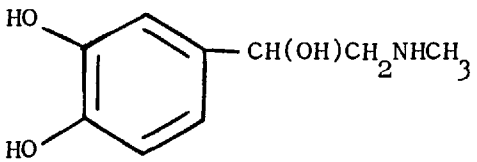
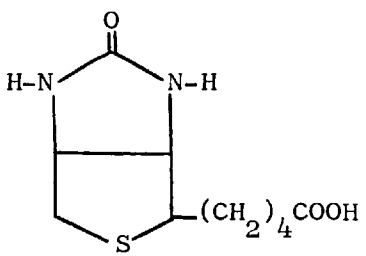
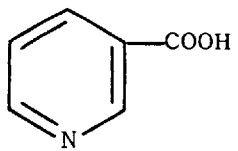
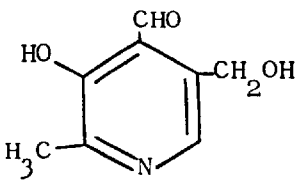
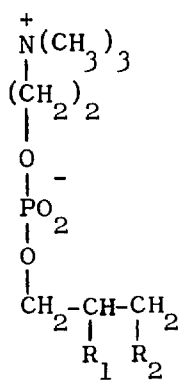
AMINES	Structure	Function
Primary amines		
Noradrenaline		Transmitter at adrenergic neurones, regulates heart rate and blood pressure
Methionine		Essential amino acid involved in many methyl transfer reactions
Secondary amines		
Adrenaline		Formed by N-methylation of noradrenaline, also regulates heart rate and blood pressure
Biotin		Vitamin, concerned in carboxyl transfer reactions

Table 1.1 Structure and function of some amines found in the body (cont'd)

Tertiary amines		
Nicotinic acid (niacin)		Forms part of coenzymes (I and II) concerned with energy-producing metabolic reactions
Pyridoxal		Coenzyme responsible for decarboxylase and transaminase reactions in fat and amino acid metabolism
Quaternary amines		
Choline	$HO\text{-}CH_2\text{-}CH_2\text{-}\overset{+}{N}(CH_3)_3$	Methyl donor. Its acetic acid ester, acetylcholine, is a chemical transmitter at cholinergic nerve endings
Phosphatidylcholine		Major component of cell membranes

$R_1 = O\text{-}\overset{O}{\overset{\|}{C}}\text{-}(C_{17}H_{35})$

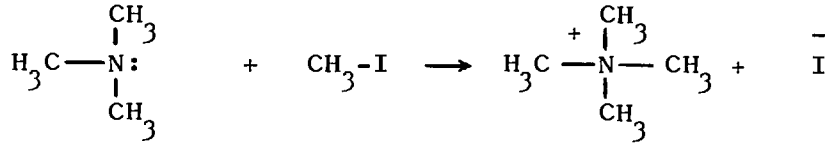
$R_2 = O\text{-}\overset{O}{\overset{\|}{C}}\text{-}(C_{17}H_{33})$

Most drugs too have their origins in nitrogen-containing plant products such as the alkaloids (compounds synthesised by plants and distinguished by their powerful effects on the physiology of animals). These may contain nitrogen in pyridine, pyrrolidine, piperidine, quinoline and isoquinoline ring systems; some examples are given in table 1.2

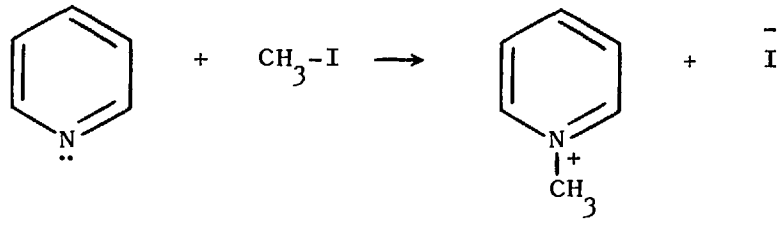
1.1.3 Quaternary ammonium compounds

When a tertiary nitrogen atom donates its unshared electron pair to an atom other than hydrogen (usually a carbon) thus forming a covalent bond, the nitrogen atom is then said to be quaternised and consequently acquires a positive charge.

For instance, methyl iodide readily reacts with trimethylamine to give tetramethylammonium iodide and with pyridine it gives N-methylpyridinium iodide. These reactions take place vigorously with the evolution of heat:-

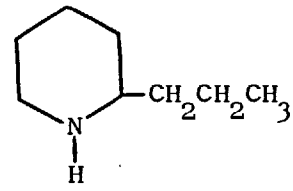
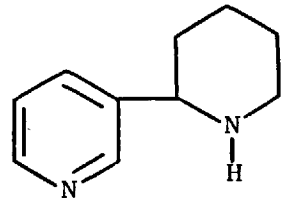
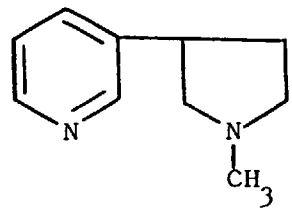
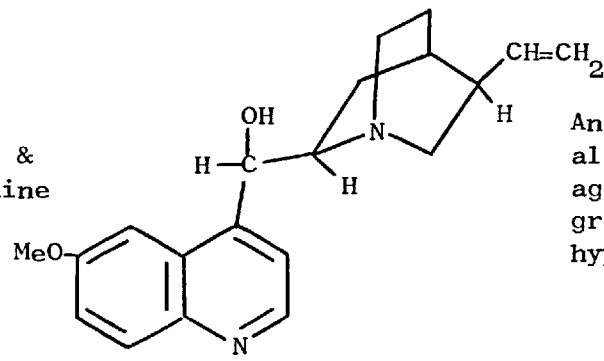
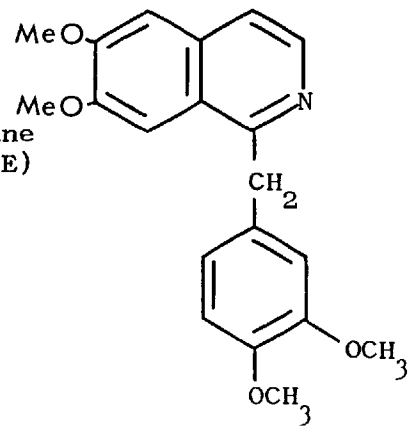


trimethylamine tetramethylammonium iodide



pyridine N-methylpyridinium iodide

Table 1.2 Some alkaloids employed in medicine

Nucleus	Structure	Medical Use
Piperidine (CONIINE)		Used in small doses for tetanus and acute mania
Pyridine (ANABASINE)		Insecticide
Pyrrolidine (NICOTINE)		Agricultural insecticide
Quinoline & Quinuclidine (QUININE)		Antimalarial agent, also a diagnostic agent for myaesthenia gravis and hyperthyroidism
Isoquinoline (PAPAVERINE)		Non-specific smooth muscle relaxant (antispasmodic)

Such compounds readily occur in nature (table 1.3) and the many quaternary ammonium compounds that have been synthesised have wide applications in medicine (table 1.4).

These compounds are not bases (as they are erroneously termed) but are permanent cations since they remain positively charged irrespective of pH. Consequently, they have the physical properties of salts, being readily soluble in water giving conducting solutions.

In contrast, tertiary nitrogen compounds are lipid soluble and have a well defined pKa, usually near the physiological range. A large fraction of these molecules however, may be present as cations at pH 7.4 but they are always in equilibrium with some non-ionised free base.

This inherent physicochemical difference between tertiary and quaternary amines has important pharmacological implications, particularly with regard to their passage across biological membranes and to the manner with which they interact with receptors in the body.

Table 1.3 Naturally occuring quaternary ammonium compounds

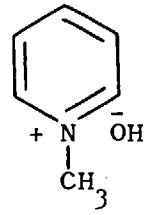
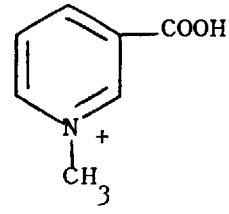
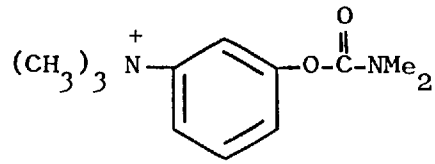
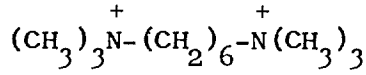
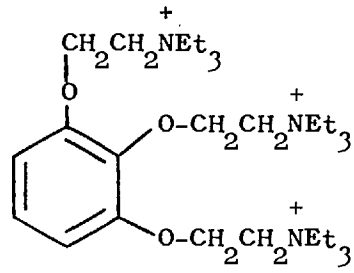
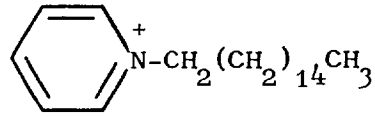
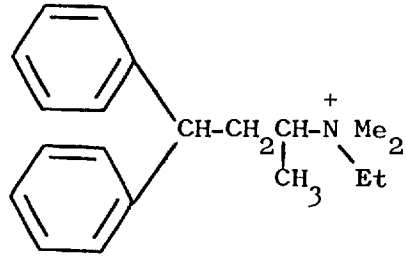
Name	Structure	Occurence
Tetramethyl-ammonium hydroxide	$(CH_3)_4\overset{+}{N}\ \overset{-}{OH}$	Sea anemone (Actinia Equina)
N-Methylpyridinium hydroxide		Sea anemone , crabs mussels, actinozoans
Betaine	$(CH_3)_3\overset{+}{N}CH_2\overset{-}{C}O_2$	Sugar beet, plant juices, crustacea, muscle tissues
Choline	$(CH_3)_3\overset{+}{N}CH_2CH_2OH$	all living cells
Trigonelline		Plant seeds, sea urchins, jelly fish

Table 1.4 Some synthetic quaternary ammonium compounds employed in medicine

Name	Structure	Medical Use
Neostigmine		Anticholinesterase
Hexamethonium		Antihypertensive
Gallamine		Competitive-type neuromuscular blocking drug
Cetylpyridinium		Antibacterial agent
Cetiprin		Antispasmodic agent

1.2 Neuromuscular blocking drugs - The Muscle Relaxants

1.2.1 History and Discovery of Curare

Curare is a generic term for various South American arrow poisons. The first account of these poisons was written by D'Angler (1516) in his book entitled, "De Orbo Novo" where he recorded stories of explorers who visited the 'New World' shortly after Columbus. Curare is involved with a long and romantic history; reports of early travellers and the complex problems associated with this deadly plant extract, have been presented in excellent monographs by McIntyre (1947), Bovet and others (1959) and by Bryn (1964).

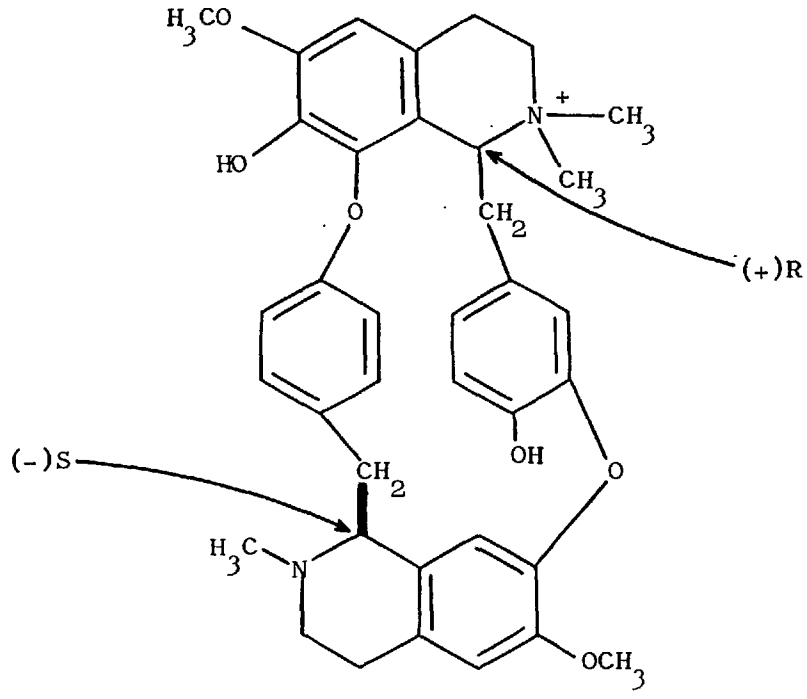
The South American Indians employed curare for centuries to kill wild animals for food - when applied in minute quantities to the tips of hunters' arrows, it produced rapid death of the quarry from respiratory paralysis, without tainting the flesh. From this same poison was isolated (+)-tubocurarine, a quaternary ammonium neuromuscular junction blocker that has had a tremendous influence on the practice of anaesthesia, and also in our understanding of neuromuscular transmission.

In 1805 Humbolt presented the first authentic account of the preparation of this poison. According to where the Indians lived, he noted that some species of Strychnos and several species of Chondrodendron were used in the preparation of curare. Scientific curiosity about the nature of its action was mainly prompted by a desire to find an effective antidote to the poison which was often used to ward off these early explorers.

Sir Benjamin Brodie (1811, 1812) demonstrated for the first time, the use of artificial ventilation in the treatment of curare paralysis when he kept an ass alive for two hours until recovery was complete. But it was Claude Bernard's classical studies on the frog which finally elucidated the nature of curare poisoning, when he traced the site of action to the neuromuscular junction. However, it is to Kuhne (1862), a former pupil of Bernard's that the credit goes for concentrating interest on the neuromuscular junction as we know it today. Later Boehm (1886) classified the curares into tube curare (tubocurare), pot curare and calabash (gourd) curare, the name signifying the vessel used in the preparation and thus to the locality and tribe of Indians who made it; this also governed the nature of the plants used.

However, the isolation of the active principle of curare was not achieved until 1935, when Harold King working with a crude specimen of curare obtained from the British Museum, established it to be a diquaternary ammonium compound. This structure was recently challenged by Everett and others (1970) and it is now thought to contain one quaternary ammonium nitrogen and one tertiary nitrogen (Fig 1.1). Other neuromuscular junction blocking compounds are also present in smaller amounts. They may also contribute to the activity of native arrow poisons since one, toxiferine, is a more potent neuromuscular junction blocker than tubocurare (Paton and Perry, 1951).

Fig 1.1 Chemical structure of (+)-Tubocurarine



Chemical Name: [Tubocuraranium, 7', 12'-dihydroxy-6,6'-dimethoxy-2, 2', 2'-trimethyl]chloride

Chemical formula: $C_{37}H_{42}Cl_2N_2O_6,5H_2O$

Molecular Wt.: 771.7

(after Everett and others, 1970)

The clinical use of curare dates from 1932, when West used highly purified extracts of curare in patients suffering from tetanus and spastic disorders. Later Bennett (1940) used this drug to diagnose the peripheral effects of electroconvulsive therapy in the treatment of psychiatric disorders; from this time muscle relaxants have played an important role in this treatment. The first clinical trial, was performed with "Intocostrin" prepared by McIntyre from the bark of Chondrodendron tomentosum brought to the Squibb Laboratories in the U.S.A. by Gill (1940).

Griffith and Johnson (1942) introduced this preparation into anaesthetic practice to obtain muscle relaxation in a patient anaesthetised with cyclopropane undergoing appendectomy. This historic occasion at the Homeopathic Hospital of Montreal (23rd January 1942) can now be seen to be the opening of a new chapter in the development of this field of medicine.

1.2.2 Depolarising and Non-depolarising neuromuscular blocking drugs

Much of what is known about the electrical events during chemical neuro-transmission at the myoneural junction stems from the discoveries by Bernard Katz and his co-workers (Katz, 1966; 1969).

Normal propagation of an impulse through a motor nerve results in depolarisation of the cell membrane at the nerve endings. Accordingly, this triggers the release of transmitter substance, acetylcholine, which in turn activates receptors on the postjunctional membrane causing depolarisation, and subsequently initiates the contraction

of muscle. Restitution of the neuromuscular unit is achieved by the enzyme acetylcholinesterase, which destroys acetylcholine. The postjunctional membrane then becomes repolarised and ready for transmission of the next impulse (see Fig 1.2)

Interference with any of these events will cause neuromuscular block and the clinically useful muscle relaxants can interrupt this process in two ways, depending on whether they competitively block the access of acetylcholine to its receptors, or mimic acetylcholine in their action, but produce long-lasting depolarisation of the postsynaptic membrane (see below).

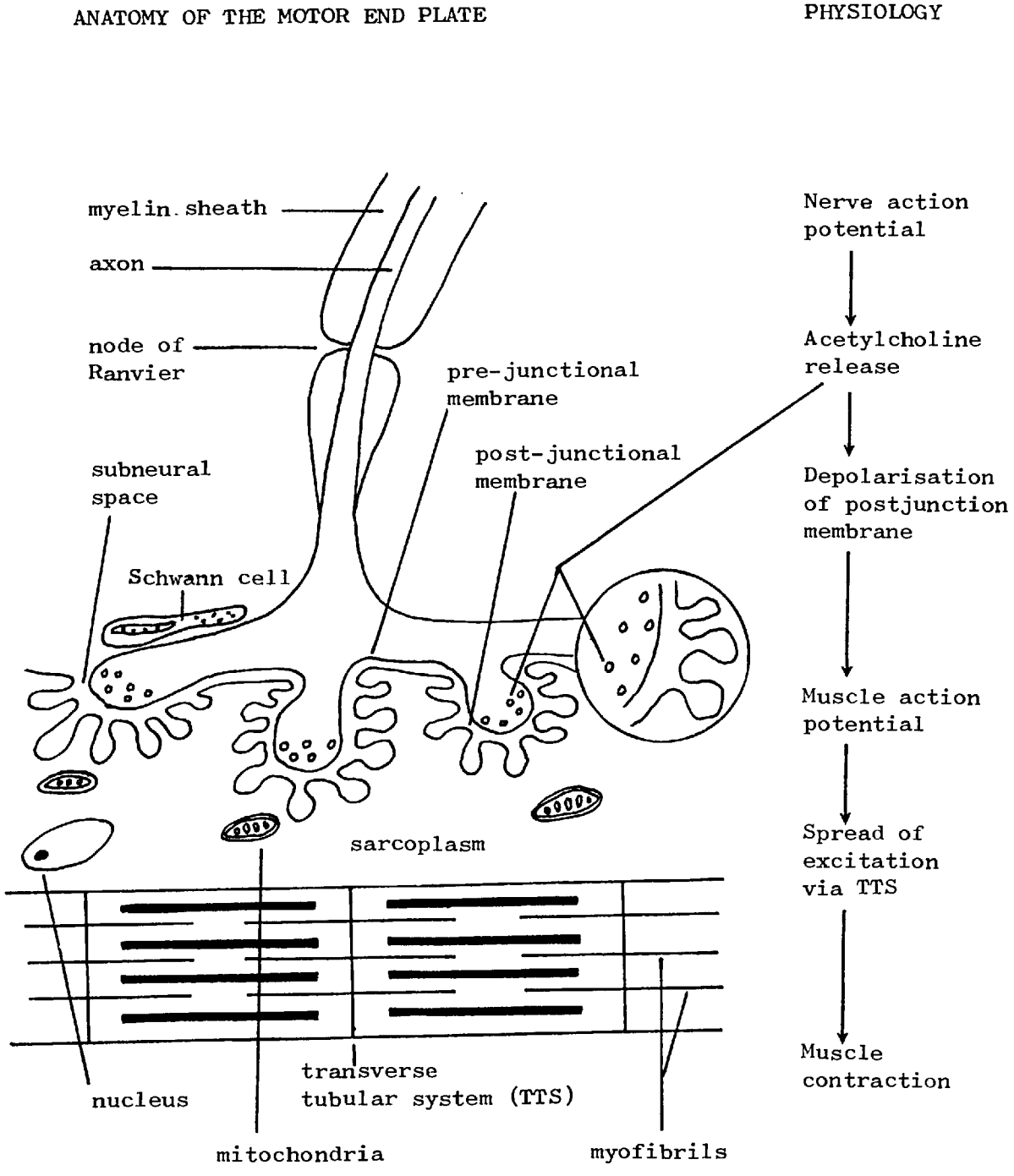
Non-depolarising muscle relaxants

Non-depolarising or competitive muscle relaxants (e.g. (+)-tubocurarine, gallamine, pancuronium, fazadinium) compete with acetylcholine for "occupation" of the receptors on the postjunctional motor end-plate. Acetylcholine normally released from the nerve terminal now finds fewer receptors available for activation and consequently, neuromuscular transmission is blocked. The block can be reversed by increasing the local concentration of acetylcholine which is commonly achieved by using anticholinesterase drugs such as neostigmine, edrophonium or pyridostigmine.

Depolarising muscle relaxants

In contrast, depolarising or non-competitive muscle relaxants (e.g. suxamethonium, decamethonium) act by a different but as

Fig 1.2 Schematic representation of the neuromuscular junction



(taken from Goodman and Gilman, 1975)

yet unclear mechanism. They first stimulate and then block response at the myoneural junction (Saverese and Kitz, 1975). By activating the receptors, they appear to lower the transmembrane potential beyond threshold levels such that acetylcholine fails to produce sufficient shift in the ionic balance in order to propagate an impulse through a motor nerve. Their initial effect is to depolarise the membrane in the same manner as acetylcholine but more persistently, which results in a brief period of firing manifested by transient muscular fasciculations. This phase is succeeded shortly by neuromuscular paralysis, the mechanism and even the primary site of which are still uncertain and controversial (Zaimis and Head, 1976). The action of depolarising drugs cannot therefore be reversed by anticholinesterases.

The ideal muscle relaxant

Both types of neuromuscular blocking drugs (competitive and non-competitive) have their drawbacks in clinical usage (Table 1.5) and the competitive neuromuscular blockers appear to be preferred in this respect. Blogg and others (1973a)listed a number of qualities essential for an ideal muscle relaxant and briefly these are:-

(i) non-depolarising action

(ii) rapid speed of onset

(iii) short duration of action

(iv) either rapidly excreted unchanged, or very quickly metabolised to pharmacologically inactive metabolites

(v) easily reversible with anticholinesterases

Table 1.5 Limitations of neuromuscular blocking drugs

Non-depolarising	Depolarising
(competitive, curare-like)	(non-competitive, phase I)
Slow onset	Fasciculation:
	increase in intragastric pressure
	increase in intra-ocular tension
	increase in extracellular potassium
	muscle pains
Long duration	Prolonged action:
	abnormality or deficiency of
	pseudocholinesterase
	"dual block"
Cardiovascular effects	Cardiac effects with repeated doses
(tachycardia, hypotension)	
Histamine release	Malignant pyrexia
(bronch ospasm)	

(taken from Blogg and others, 1973 a)

(vi) specific action on the neuromuscular junction

(vii) no significant placental or blood-brain transfer

(viii) no profound local or systemic side effects (e.g. muscle pains, hypotension due to ganglion blockade; histamine release leading to acute bronch-ospasm).

An overview of the situation leads to the conclusion that the most useful drug would provide complete neuromuscular blockade in every patient, at sufficiently low concentration with a short duration of action, and therefore would require no antagonist.

Adverse reactions to neuromuscular drugs are almost always due to their clinical overdose or their injudicious use. This results in serious disadvantages such as a greater incidence of unwanted side-effects (histamine release, tachycardia), a prolonged duration of action, or even difficulty with reversing the block. Provided that adequate ventilation is maintained, it is extremely rare for a patients life to be in jeopardy by the use of these agents, particularly since they have a large therapeutic ratio (Feldman, 1973). Up to fifty times the paralytic dose of (+)-tubocurarine was administered to cats in a single dose without killing them.

1.2.3 Anaesthetic practice and the use of muscle relaxants

In anaesthetic practice analgesia, anaesthesia and muscle relaxation are important requisites in the management of patients for most surgical procedures (Gray, 1954). Ether and chloroform were the first agents employed, but these always resulted in unwanted side-effects.

During surgery respiratory inadequacy and hypotension often occurred; postoperatively too, complications such as nausea, vomiting, perspiration and fluid loss were common. Later cyclopropane and thiopentone came into use but they had relatively weak muscle relaxant properties, and it was not surprising that Griffith (1942), one of the foremost exponents of cyclopropane anaesthesia, introduced curare into anaesthesia.

Nowadays anaesthesia employs specific drugs for analgesia (opiate drugs), anaesthesia (inhalation anaesthetics e.g. nitrous oxide, halothane) and muscle relaxation (neuromuscular blocking drugs). The specific indications for the last group of drugs fall in three categories (Churchill-Davidson, 1978)

i) To relax the muscle for surgical, anaesthetic and investigational procedures.

ii) To facilitate the control of respiration.

iii) To limit the amount of general anaesthetic required during surgery.

Despite its side-effects (see table 1.6), (+)-tubocurarine has remained the standard muscle relaxant for many years; in short operations, suxamethonium is used because it is rapidly inactivated by plasma pseudocholinesterases. Most recent drugs are synthetic and hence purity, solubility in various media and chemical stability can accurately be described.

Many of these synthetic muscle relaxants have been introduced, but relatively few have stood the test of time. The introduction

Table 1.6 Muscle relaxants used in anaesthetic practice

Relaxant	Dose	Duration (min)	Predominant side-effects	References
Depolarising				
suxamethonium (Thesleff, 1951)	1 mg/kg	2-4	increase in intra-ocular tension cardiac dysrrhythmias muscle pain	Eakins and Katz (1966) Dowdy and Fabian (1963) Churchill-Davidson (1954)
decamethonium (Paton and Zaimis, 1949)	2-4 mg	15-20	phase II block	Organe (1956)
Non-depolarising				
(+)-tubocurarine (Griffith and Johnson, 1942)	0.1-0.6 mg/kg	30-50	hypotension due to ganglionic blockade	Bono and Mapelli (1960)
gallamine (Mushin and others, 1949)	80-140 mg	20-35	increase in blood pressure induces tachycardia	Kennedy and Farman (1968) Riker and Wescoe (1951)
pancuronium (Baird and Reid, 1967)	0.04-0.08	25-40	**	
fazadinium (Simpson et al, 1972)	0.5-1.0	24-40	**	

** no predominant side-effects observed

of pancuronium bromide presented the anaesthetists with an agent that did not cause hypotension (Loh, 1970) as is observed with (+)-tubocurarine.

Fazadinium is the latest newcomer, being introduced primarily due to its advantages for tracheal intubation. For instance, fazadinium (1.5 mg/kg dose) causes rapid onset of complete neuromuscular block in man such that intubation of the trachea can be carried out as rapid as is possible with suxamethonium (Brittain and Tyers, 1973) However, fazadinium does not cause muscle fasciculations as does suxamethonium, and thus does not cause vomiting by increasing the intra-abdominal and intragastric pressure (Anderson, 1962; Roe, 1962). Fazadinium is therefore a non-depolorising alternative for "crash intubation" of the trachea. Furthermore, suggestions have been made by Churchill-Davidson and others (1967) that the non-depolorising agents with the exception of gallamine, are reasonably safe in patients with renal failure. This is dealt with in section 1.3.

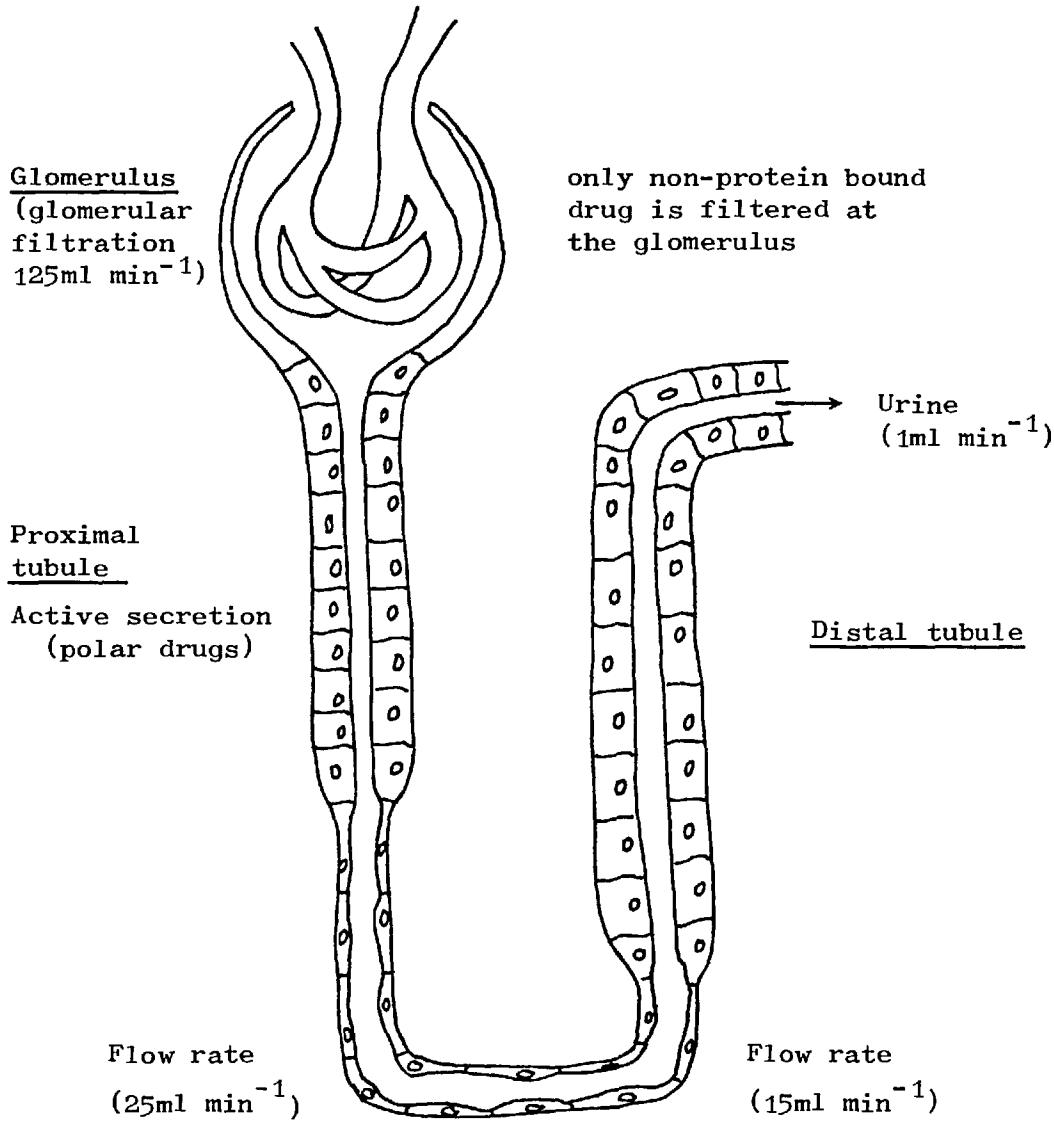
1.3 Drug action and the influence of renal failure on neuromuscular blocking activity

Pharmacologists have attempted to develop neuromuscular blocking drugs which provide adequate muscle relaxation during surgery, are free of unwanted side-effects, and whose effects may be easily reversed in the post-operative period. For the predictable and safe use of these agents, it is necessary to be aware of their pharmacodynamic properties, i.e. the consequence of drug-receptor interaction; their pharmacokinetic properties, i.e. the quantitative characterisation of drug absorption, distribution, metabolism and excretion; and the influence of disorders of homeostasis on the pharmacodynamic and pharmacokinetic properties of these drugs. One such example in the latter case, is the influence of renal failure.

1.3.1 Functions of the kidney and renal disease

The major functions of the kidney are the removal of nitrogenous waste, mostly in the form of urea, maintenance of electrolyte and water balance and the regulation of acid-base status. The kidneys therefore play an essential role in the maintenance of the mileu interieur. The excretory function of the kidneys is a consequence of the production of urine, and many drugs are eliminated in this way. Their renal excretion can involve one or more of the processes of glomerular filtration, tubular reabsorption and tubular secretion (see Fig 1.3).

Fig 1.3 Diagram of a single kidney nephron illustrating renal transport of various types of drugs



Lipid soluble drugs are passively re-absorbed throughout the nephron as urine is concentrated

Glomerular filtration

The glomerulus of the kidney is a capillary bed and filtration is subject to the same physical laws that govern transport across capillary membranes. The only restriction is one of molecular weight - compounds of molecular weight greater than 60,000 -70,000 are unable to pass into the glomerular space, with the result that the glomerular filtrate is essentially free of plasma proteins. Hence, only the non-protein bound drug is available for filtration, the filtering force being provided by the hydrostatic pressure of the blood derived from the work of the heart.

Tubular reabsorption

Once present in the glomerular filtrate, a compound may be reabsorbed across the tubular epithelium and subsequently, back into the blood. The process of reabsorption is regarded mainly as a passive process however, active reabsorption mechanisms exist in the tubules for small endogenous molecules such as sugars, amino-acids and water in order to maintain the composition of the extracellular fluid.

Tubular secretion

Some organic compounds can be actively transported from the plasma to the lumen of the proximal tubules. Separate "pumps" exist for organic anions and cations, and only the unbound drug in the plasma of the peritubular capillaries is available for secretion. Although drugs that are actively secreted are also filtered at the glomerulus, secretion is the more important process and hence these drugs are "excreted by active secretion".

Reabsorption and secretion therefore refer to the direction of net transport. Drugs with relatively high lipid solubility (e.g. phenobarbitone) are readily reabsorbed, whereas polar compounds (e.g. neuromuscular blocking drugs) are actively secreted unless they are also reabsorbed by a carrier transport system.

The physiology of renal disease can be classified into four different categories (Guyton, 1976)

1) Acute renal failure, in which the kidneys lose most of their function.
2) Chronic renal failure, in which progressively more nephrons are destroyed such that the kidney cannot perform all its vital functions.
3) Hypertensive renal disease, in which vascular or glomerular lesions can cause hypertension (possibly due to the diminished vascular supply and glomerular filtration) but not renal failure.
4) Specific tubular abnormalities, in which disorders occur for the transport of substances through the tubular membrane, and essentially all these disorders are hereditary.

The most important effect of renal failure however, appears to be the inability of the kidneys to cope with the large "loads" of electrolytes and other substances that must be excreted. Under normal conditions, homeostasis may be maintained with only one-third of the nephrons functioning, but death ensues when the number of working nephrons fall below 10-20% of normal.

In patients with advanced renal failure, the major problems are those of uraemia (accumulation in the blood of nitrogenous products of protein metabolism), anaemia, hypertension, infection and electrolyte disorders, especially hyperkalaemia, although these factors can be controlled to a great extent by haemodialysis.

1.3.2 Pharmacodynamics of neuromuscular blocking drugs

The direct and rapid reversibility of neuromuscular blockade by anticholinesterase drugs, suggests that a given intensity of effect is associated with a particular relaxant concentration at cholinergic receptors. Whether the concentration at the receptor is in turn related to drug plasma levels is a matter of dispute.

There are a number of instances in which plasma levels of drugs do not correlate with their pharmacological effect:

i) The assay method may not be sensitive enough to give an indication of the amount of drug in an appropriate pool e.g. guanethidine which is stored in adrenergic neurones (Oates and others, 1971).

ii) The plasma levels of drugs that are bound irreversibly to their receptors will not correlate with their effect since they may still be tightly bound to receptors when their plasma levels are very low e.g. reserpine, alkylating agents and monoamine oxidase inhibitors (Brodie and Mitchell, 1973).

iii) Some drugs may act via active metabolites which often have different properties to that of the parent compound e.g. after the administration of α-methyldopa, α-methylnoradrenaline (an active metabolite) persists in the adrenergic neurones long after the parent drug has disappeared (Muscholl and Maitre, 1963).

Using the technique of temporarily isolating the circulation of the blood to the arm by means of a tourniquet, and monitoring neuromuscular function by measuring the force of contraction of the adductor pollicis following stimulation of the ulnar nerve (see section 1.4.), Feldman and Tyrrell (1970) observed a rapid onset of action of two competitive neuromuscular blocking drugs, (+)-tubocurarine and gallamine. By releasing the tourniquet, thus restoring the circulation of blood to the forearm, they found that recovery from neuromuscular blockade was relatively very slow (15-20 min). Similar observations were also encountered with other muscle relaxants such as fazadinium (Simpson and others, 1972) and pancuronium (Heneghan and others, 1978).

Based on results like these, Feldman (1975) postulated that the primary factor controlling the recovery from neuromuscular blockade was the rate of dissociation of the relaxant receptor complex since if the effect of these drugs were related to plasma concentration, recovery would be faster after releasing the tourniquet. He added that the plasma concentration of the drug only

affected the intensity of effect in a secondary manner, by decreasing the concentration gradient that allowed the drug to leave the receptor area.

Due to the abundant blood supply to the neuromuscular junction, the primary distribution equilibrium between plasma and the neuromuscular function will be achieved rapidly. Consequently, it should be possible to correlate the plasma levels of muscle relaxants to their pharmacological effect. In cats and dogs, Paton and Waud (1967) showed that 75-80% of the cholinergic receptors have to be occupied by (+)-tubocurarine before there is any reduction in neuromuscular function. As the dose of relaxant is increased beyond this level, the size of twitch is directly related to receptor occupancy, but when 90-95% of the receptors are occupied, neuromuscular transmission is completely blocked.

The presence of this narrow margin for neuromuscular transmission together with the analytical problems of developing sensitive and specific chemical assays for muscle relaxants (see section 1.4), has made it difficult for any attempts at relating plasma levels to the intensity of neuromuscular blockade. For instance, Cohen and others (1957) using a spectrophotometric method for (+)-tubocurarine failed to demonstrate any relationship between plasma levels and neuromuscular blockade for this drug. However, Matteo and others (1974) using a specific radio-immunoassay method demonstrated a significant linear correlation

between the serum concentration of (+)-tubocurarine and neuromuscular blockade in man, and this was confirmed by Ham and others (1979), using the same analytical technique. A similar relationship was also observed for pancuronium (Agoston and others, 1977) using a spectrofluorimetric assay.

In contrast to the hypothesis of Feldman (1975), these results appear to suggest that the recovery from neuromuscular blockade is primarily dependant upon the decrease in plasma relaxant concentration. The situation however, is more complex and both the mechanisms function as the rate-determining factor in the termination of action of non-depolarising muscle relaxants (Shanks and others, 1978; Agoston and others, 1979).

Patients with renal failure often suffer other illness and it is well known that treating these patients with the customary dose regimes frequently result in serious toxic manifestations (Bennett and others, 1970). However, these patients react abnormally and unpredictably to many drugs and such patients are also prone to adverse drug reactions compared to patients with normal renal function. This problem was seriously investigated for the first time by Kunin and Finland (1959a) in a series of studies on the behaviour of antibiotics administered to patients with acute renal failure. Retention in the plasma of drugs such as penicillin, streptomycin and kanamycin was

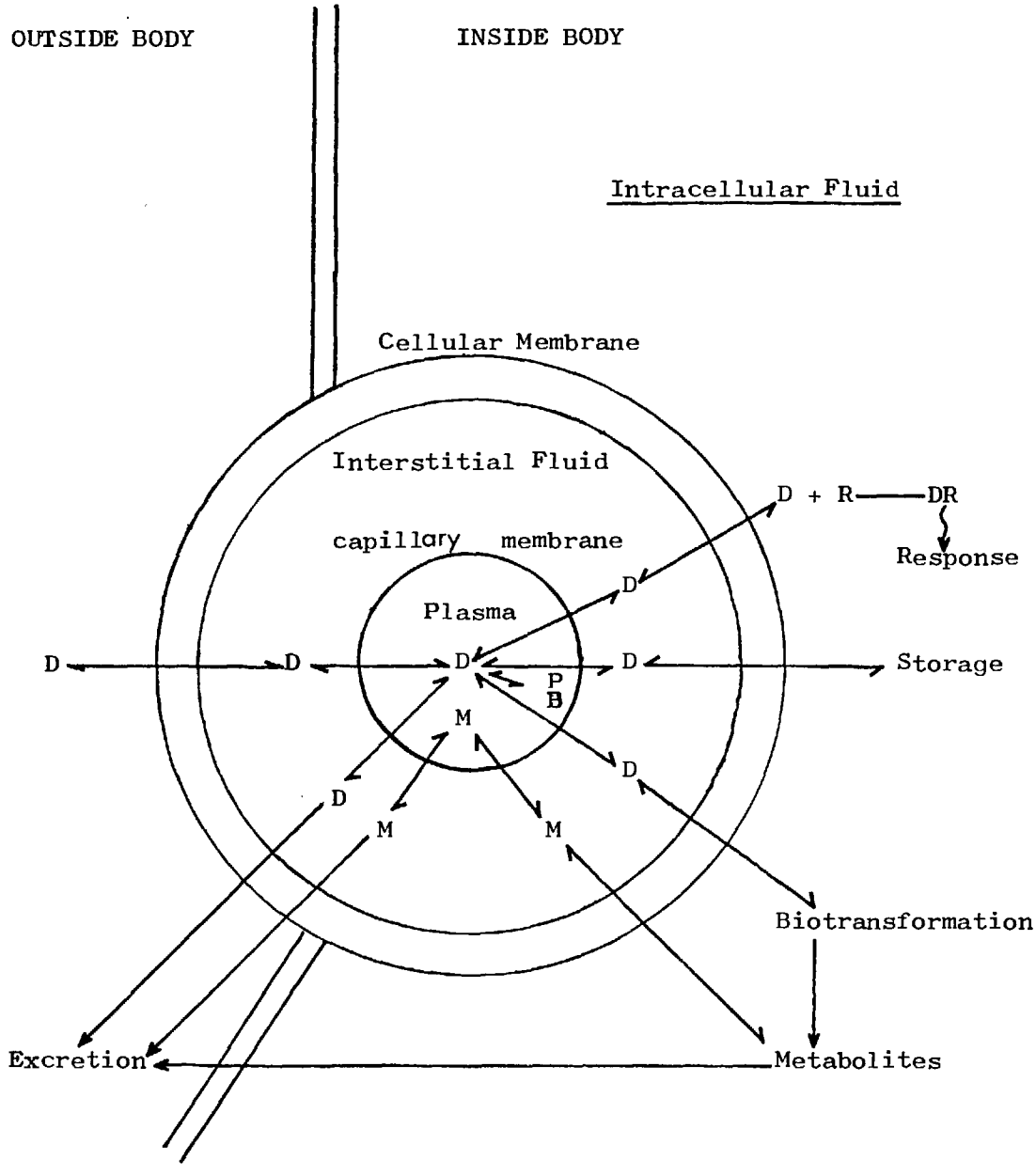
observed (Kunin and Finland, 1959b) due to the reduced elimination of these drugs by the kidney. Frequently, there is also an increase in sensitivity of drugs when used in patients with renal failure. Several examples, with thiopentone (Dundee and Richards, 1954) Phenobarbitone or chloropromazine (Fabre and others, 1967) demonstrate that this phenomenon exists independently of drug retention probably involving functional or morphological modifications of the drug receptors.

Precise information is still lacking on the behaviour of neuromuscular blocking drugs in the presence of renal failure. These drugs are generally excreted extensively by the kidney and many examples of prolongation of effect have been reported (see section 1.3.5)

1.3.3 Pharmacokinetics of neuromuscular blocking drugs

As indicated in Fig 1.4, the concentration of drug (D) in plasma plays a central role in the disposition of drugs throughout the body. The drug is absorbed into the plasma from enteral or parenteral sites of administration or can be placed directly into plasma by intravenous injection. Plasma in the circulating blood then delivers the drug to its site of action, storage, metabolism and excretion. Metabolites formed may also enter plasma and be distributed throughout the body.

Fig 1.4 Disposition of a drug(D) in the body



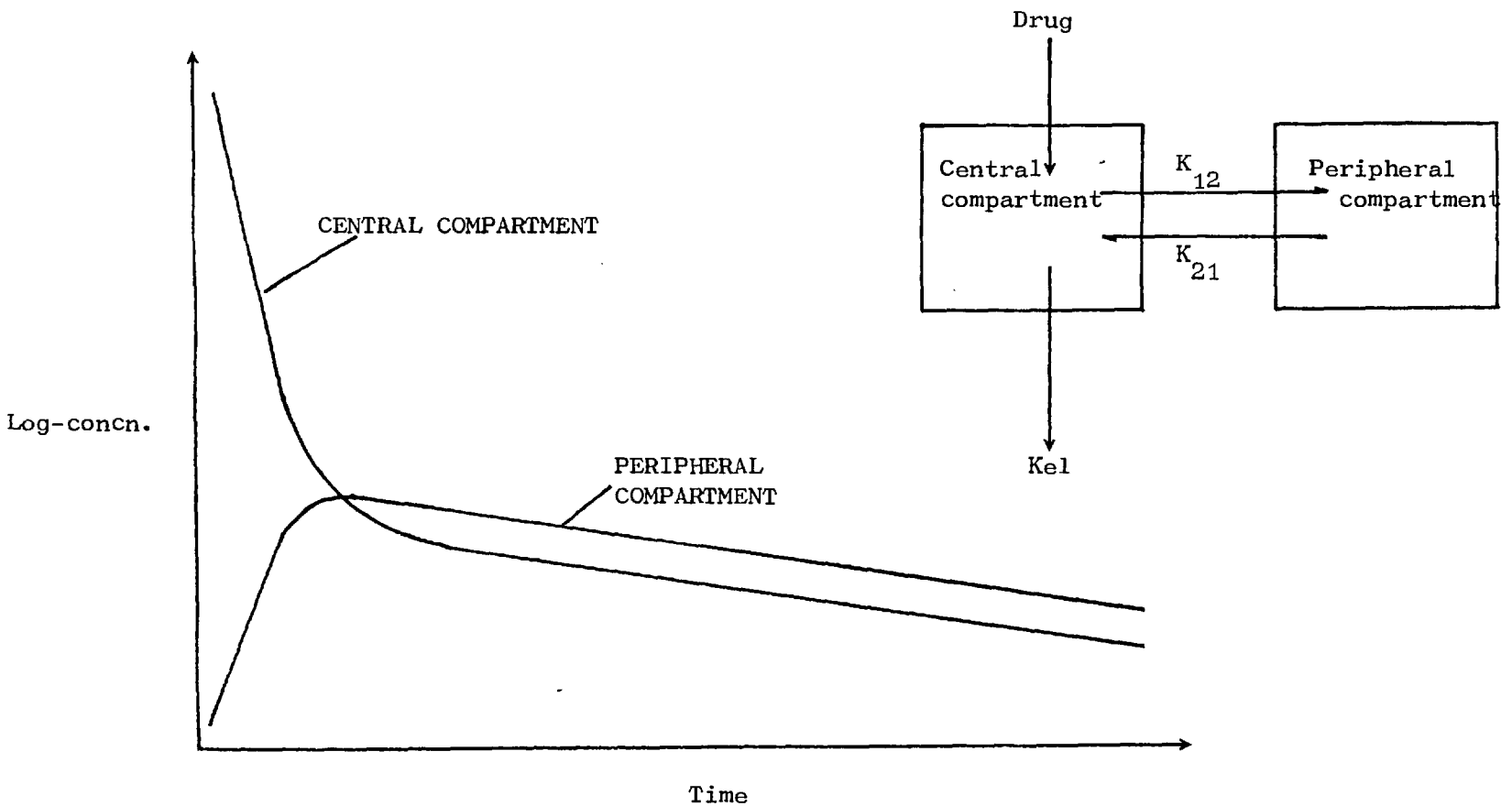
Key: R = receptor at site of action
M = metabolite(s) of drug, which can enter the same disposition processes as shown for D.
P = Protein
B = Red blood cells

(taken from Hug, 1978)

The term "pharmacokinetics" was introduced by Dost (1953) to describe the mathematical analysis of drug quantity and activity within the body. Consequently, the use of mathematical models to describe the processes of drug absorption, distribution, metabolism and excretion has allowed for the prediction of concentrations in various parts of the body as a function of dosage, route of administration and time (Wagner, 1971). Many drugs behave as though the body consists of two compartments but sometimes drug disposition is more complex, and hence three or more compartments are required to explain the observed data.

The two compartment open model (Riggs, 1963) has been shown to be applicable to most neuromuscular blocking drugs such as (+)-tubocurarine (Cohen and others, 1965), pancuronium (McLeod and others, 1976; Somogyi and others, 1976) and fazadinium (Duvaldestin and others, 1978). This model considers the body to be composed of two compartments, one central and one peripheral. The drug is administered into the central compartment and exchange of the drug between the two compartments take place by a first order process. Elimination occurs solely from the central compartment and also by a first order process. The model is summarised in Fig 1.5 (for mathematical treatment and limitations, see Appendix I). In the case of suxamethonium, because the drug is so rapidly hydrolysed only a single compartment is appropriate to describe the pharmacokinetic data (Levy 1967).

Fig 1.5 Schematic representation of the two compartment open model with the corresponding theoretical concentration-time profile of a typical drug in the two compartments



The pharmacokinetic approach can therefore be used to predict distinctive aspects of the pharmacodynamics of neuromuscular blocking drugs.

Absorption

Functional and anatomical changes to the gastrointestinal tract caused by chronic uraemia may affect to some degree the absorption of drugs given orally. The clinical practice of anaesthesia however, has bypassed the question of drug absorption into the circulation since neuromuscular blocking drugs are usually administered intravenously. The possible influence of renal failure on drug absorption will thus not be considered here.

Distribution

Once the drug has been injected into the circulation it may gain access to a variety of "compartments" within the body. The rate and extent of drug distribution depends principally on:-

i) The physicochemical properties of the drug.

ii) The regional distribution of blood flow to the various tissue and organs of the body.

Neuromuscular blocking drugs are all quaternary ammonium compounds of comparatively high molecular weight (>450); they are also highly ionised, water soluble and only possess limited lipid solubility. Due to these physiocochemical properties we would expect neuromuscular blocking drugs to distribute rapidly throughout the water compartments of the body, but very slowly penetrate lipid

membrane barriers. Consequently, they are poorly absorbed from the gastrointestinal tract and do not cross the placenta or the blood-brain barrier.

Marsh (1952) was the first to suggest that the duration of action of (+)-tubocurarine, after a single bolus injection, depends mainly on the rate of redistribution of the drug from the circulation into various tissues within the extracellular compartment. Studies with isotope-labelled compounds have shown that the muscle relaxants are localised in muscle and especially concentrated in the junctional area, but they are also found in the liver, kidney and spleen (Cohen and others, 1965; Waser and Luthi, 1968; Shindo and others, 1974).

An important pharmacokinetic parameter which provides a useful assessment of the degree of localization of drugs in tissues is the apparent volume of distribution, Vd (see Appendix I). When discussing Vd, it should be recognised that this pharmacokinetic parameter is a theoretical expression and is part of a mathematical approach to physiological problems (Riggs,1963). It symbolises the space in which the drug will instantaneously distribute for an uniform concentration throughout the body compartments. Vd is called "apparent" because in practice not all the body compartments in which the drug distributes have the same concentration at any one time.

Patients with renal failure often show alterations in the apparent volume of distribution. For example, oedema considerably disturbs the behaviour of drugs such as the sulphonamides or thiocyanate, whose apparent volume of distribution approximately equals the extracellular space. On the other hand, dehydration will tend to increase the plasma levels of most drugs (Fabre and Balant, 1976). Modification of the red blood cell concentration can also disturb the Vd, thus anaemia is accompanied by decreased binding of gentamicin to red blood cells, and consequently plasma concentrations are much higher than in patients with normal renal function (Riff and Jackson, 1971).

Metabolism

Most drugs that are administered to man are metabolised to some extent, and this usually involves two phases (Williams 1959):

Drug $\xrightarrow[\text{enzymes}]{\text{Phase I}}$ oxidation, reduction, hydrolysis, metabolites $\xrightarrow[\text{enzymes}]{\text{Phase II}}$ conjugated metabolites

(After Williams, 1959)

In the initial phase (Phase I), the compound may undergo reactions classified as oxidations, reductions and hydrolysis. As a result of these reactions, groups such as OH, NH_2, COOH and SH are introduced into the drug molecule. In the second phase (Phase II), the phase I metabolite is linked with endogenous substances such as sugars (e.g. glucuronic acid) or amino acids (e.g. glycine, glutamine) to yield conjugates.

Not all drugs, however are metabolised in this biphasic fashion. (+)-Tubocurarine is excreted unchanged in the urine, ethanol undergoes solely oxidative metabolism, while paracetamol is conjugated directly with glucuronic acid and sulphate.

The majority of these processes are carried out in the liver by relatively non-specific enzymes located in the smooth endoplasmic recticulum of the hepatocytes. Metabolism may also occur to varying extents in other parts of the hepatocyte, in other tissues (e.g. skin, kidney) and also by bacterial flora of the gastrointestinal tract (see Table 1.7). The metabolites formed are not necessily less pharmacologically active than the parent compound since some active products can also be formed (e.g. the 3-desacetyl metabolite of pancuronium).

Renal failure can affect drug metabolism in a number of ways, the effect appears to depend upon the specific pathway of drug metabolism being studied (see Table 1.8). Whereas hydrolyses and some acetylation reactions are slowed in patients with renal failure, conjugation is generally normal and oxidation may even be accelerated. Patients with the nephrotic syndrome (protein loss via the kidneys with subsequent hypoproteinaemia and dysproteinaemia) can have low plasma cholinesterase concentrations, which would explain the reason for the delayed hydrolyses of drugs such as procaine. The mechanism of the accelerated oxidation of drugs in renal failure has not been elucidated.

Table 1.7 Tissue distribution of some drug metabolising enzymes

	Phase I enzymes		Phase II conjugating systems	
Tissue	3,4-Benzypyrene hydroxylase	N-Methylaniline N-demethylase	Methylation	Glucuronide formation
Liver	+	+	+	+
Kidney	+	+	+	+
Lung	+	+	+	+
Gut	+	+	+	+
Spleen	+		+	+
Brain		+	+	+
Skin	+		+	+
Adrenals	+		+	+
Thyroid	+			
Placenta	+			+
Bladder		+		+
Testis	+			
Blood	)		+	
Epidydymal fat	)	Chlorpromazine sulfoxide		
Thymus	)			+
Heart	)			
Skeletal muscle	)	Niridazole nitroreductase		

(taken from Testa and Jenner, 1976)

Table 1.8 Apparent elimination rate of drugs in patients with renal failure

DRUG	PLASMA ELIMATION HALF LIFE	REFERENCE
Oxidations		
Quinidine	normal	Kessler and others, 1974
Tolbutamide	normal	Reidenberg, 1972
Phenacetin	normal	Prescott, 1969
Antipyrine	normal/shortened	Maddocks and others, 1975
Propranolol	normal/shortened	Bianchetti and others, 1976; Lowenthal and others, 1974a
Phenytoin	shortened	Odar-Cederlof and Borga, 1974
Reductions		
Cortisol	prolonged	Englert and others, 1958
Hydrolysis		
Insulin	prolonged	Rabkin and others, 1970
Procaine	prolonged	Reidenberg and others, 1972
Conjugations		
Chloramphenicol	normal	Kunin and others, 1959
Acetaminophen	normal	Lowenthal & others, 1976
Salicylate	normal	Lowenthal & others, 1974b
p-Aminosalicylate	prolonged	Ogg and others, 1968
Isoniazid	normal/prolonged	Reidenberg and others, 1973; Dettli and Spring, 1973

(taken from Reidenberg and Drayer, 1978)

With respect to neuromuscular blocking drugs, gallamine (Feldman, 1969) and (+)-tubocurarine (Cohen, 1967) are almost completely excreted unchanged whereas pancuronium (Agoston and others 1973a, 1973b) undergoes some deacetylation. Fazadinium can undergo degradation by azo-reduction (Bell and Martin, 1979) with subsequent hydroxylation and glucuronidation. Suxamethonium however, is completely hydrolysed by plasma pseudocholinesterase. Whether the rate of metabolism of muscle relaxants is altered by renal failure is yet unknown.

Most metabolites are more polar and less lipid soluble than the parent compound and hence excreted more readily by the kidney. During renal failure, such drug metabolites may accumulate and in some cases may precipitate adverse effects (Drayer, 1976). For example norpethidine formed from pethidine, and hydroxyamylobarbitone, from amylobarbitone, contribute to excessive excitatory and depressant effects, respectively, in patients with renal failure (see Table 1.9)

Excretion

By virture of their lipid insolubility and high ionisation, neuromuscular blocking drugs are not reabsorbed by the kidney tubules and hence undergo extensive renal elimination. Furthermore due to their high molecular weight, compensatory pathways of drug elimination may also be available for these compounds. The membrane of the hepatic parenchyma contains aqueous pores large enough to admit a number of lipid insoluble substances (Schanker, 1962a). It also possesses

Table 1.9 Clinical consequences of accumulation of active drug metabolites

Drug	Accumulated metabolite	Effect
Procainamide	N-Acetylprocainamide	enhanced pharmacologic response (Drayer and others, 1977)
Pethidine	Norpethidine	siezures (Szeto and others, 1977)
Clofibrate	Chlorophenoxyisobutyric acid	muscle damage (Pierides and others, 1975)
Allopurinol	Oxypurinol	side effects (Elion and others, 1968)
Sulfadiazine	Acetylsulfadiazine	nausea, vomiting, rash (Adam and Dawborn, 1970)
Amobarbital	3-Hydroxyamobarbital	CNS depression (Balasubramaniam and others, 1972)

special transport mechanisms for certain organic cations which are actively transported into the bile despite their low lipid solubility (Schanker, 1962b).

Hughes and others (1973) reported that in the rat, urinary excretion was greatest for those cations of low molecular weight which decreased as the molecular weight of the cation was increased; in the latter case, biliary excretion predominated. They concluded that only those cations above a threshold molecular weight (500-600) were appreciably excreted ($>10\%$ of dose) in the bile of rats.

In the rat also, a similar situation was found for organic anions (Hirom and others, 1976). A wide range of compounds could be classified into three groups with regard to their pattern of elimination and molecular weight (see Table 1.10). The first group (I) of compounds (m.w. 250-350) are those with a high affinity for urinary excretion and low affinity for biliary excretion; their biliary elimination is not increased by ligation of the renal pedicles. Group II compounds (m.w. 450-850) have a high affinity for biliary excretion and low affinity for urinary excretion; in this case their urinary excretion is not increased on bile duct ligation. Both group I and group II compounds therefore do not possess compensatory mechanisms for excretion. Group III compounds (m.w. 350-450) however, are excreted by both routes of drug elimination and when one pathway is occluded, the other compensates by increasing its excretion.

Table 1.10 Urine and bile as complementary pathways for drug elimination in the rat

Group	% Dose excreted in Urine	% Dose excreted in Bile	% Dose excreted in Bile in absence of renal function	Examples
I (Mw 250-350)	40-90	1-10	4-30	Sulphathiazole Phthalylsulphacetamide
II (Mw 450-850)	0-2	70-90	70-90	Bromophenol blue Bromocresol green
III (Mw 350-450)	20-60	25-60	50-80	Glutarylsulphathiazole Tartrazine

(taken from Hirom and others, 1976)

The possible inter-relationship between these two pathways of drug elimination is very important especially in patients with disorders that can lead to the impairment of drug excretion by either of these routes. Usually it is not possible to demonstrate an increase in the biliary excretion in patients with renal failure, but only by an increased fraction of the drug eliminated extrarenally. Thus for digoxin (Bloom and Nelp, 1966), the amount of drug found in the urine is reduced, whereas the quantity in the stools is greatly increased in patients with renal failure compared to those with normal renal function.

Clinically, impairment of renal function occurs more often than impairment of hepatic excretion. The duration of action of drugs in patients with non-functional kidneys depends on the efficacy of redistribution, metabolic destruction and biliary excretion.

1.3.4 Protein binding in Renal failure

Many drugs circulate in the blood partly bound to a protein, particularly albumin. Abnormal serum protein concentration such as when fluid balance is disturbed in renal failure, will make it difficult to predict changes in the serum concentration of drugs.

Only the unbound fraction of the drug in plasma is freely accessible to its receptor site. Any variation in the plasma protein binding will influence the free concentration of the drug and hence markedly alter the magnitude and duration of pharmacological response (Solomon, 1973).

Furthermore, analytical measurements determine only the total (free and bound) drug concentration in plasma. If protein binding is constant, it follows that the measured concentration will reflect the intensity of effect. However, any change in the plasma protein binding will not only alter the intensity of effect but will also affect the pharmacokinetics of the drug since it is the unbound fraction of the drug that is available for metabolism or excretion. This is illustrated by the differing rates of elimination of digitoxin ($T\frac{1}{2}$ 100-200h) and digoxin ($T\frac{1}{2}$ 30-40h); both are bound exclusively to albumin , but at therapeutic plasma levels the unbound fraction is 5% for digitoxin and 77% for digoxin. As a result digitoxin is less available for metabolism and therefore has a longer half life (Lukas and De Martino, 1969).

The situation is however more complex in patients with renal failure who often have reduced amounts of serum albumin (hypoalbuminaemia) (Gugler and Azarnoff, 1976), and the binding capacity of their albumin is also unusually low (Reidenberg and Affrime, 1973). In these patients, it was found that for some acidic drugs (e.g. sulfonamides, phenytoin), the binding to plasma proteins was reduced, whereas, most basic drugs (e.g. (+)-tubocurarine, quinine) did not show any difference in their protein binding (see Table 1.11). For hypoproteinaemic conditions (e.g. nephrotic syndrome), a rise in the unbound fraction in blood could lead to changes in the efficacy and toxicity of a given dose. Clearly, however, such influences are likely to be important only for drugs that are extensively bound to plasma proteins and which have a small apparent volume of distribution.

Table 1.11 Binding of drugs to plasma proteins in patients with renal failure

DRUGS	BINDING	REFERENCE
Acidic drugs		
Sulfonamides	decreased	Anton and Corey, 1971
Phenytoin	decreased	Reidenberg and others, 1971
Clofibrate	decreased	Farrell and others, 1972
Benzylpenicillin	decreased	Farrell and others, 1972
Basic drugs		
Desmethylimipramine	normal	Reidenberg and others, 1971
Quinidine	normal	Lunde, 1973
Dapsone	normal	Reidenberg and Affrime, 1973
Triamterene	normal	Reidenberg and Affrime, 1973
(+)-Tubocurarine	normal	Ghoneim and others, 1973

Fazadinium is only 17% bound to human plasma proteins (Maclagan, 1976), whereas 44% of (+)-tubocurarine is plasma protein bound (Ghoneim and others, 1973). It is now thought that pancuronium is over 87% protein bound (Thompson, 1976) and if binding of this drug were reduced then an increase in the amount of free drug in plasma would obviously result.

1.3.5 Prolonged action of neuromuscular blocking drugs

It has frequently been observed that patients with impaired renal function develop prolonged duration of action after the administration of neuromuscular blocking drugs (Popescu, 1972).

Of the clinically used relaxants, only suxamethonium has its pharmacological activity terminated exclusively by metabolic breakdown (Glick, 1941) and hence its urinary elimination is unimportant. Occasionally, some patients are unable to metabolise suxamethonium due to a genetic absence of the enzyme, plasma pseudocholinesterase. Kalow and Davies (1958) showed that in man, there were two types of this enzyme - a "normal" and an "atypical" one. Individuals with the normal enzyme hydrolyse the drug rapidly within one minute (Pantuck, 1967) however, those possessing the atypical enzyme are unable to do this and consequently the effects of the drug are markedly prolonged.

Prolonged paralysis has also been described when non-depolarising agents have been administered to severely ill patients. In these cases, the main causes have been attributed to drug overdose (Feldman, 1973);

hypothermia (Zaimis and Cannard,1958); hypoproteinaemia (Aladjemoff and others, 1958); electrolyte disturbances (Feldman, 1963) or acid imbalance (Brooks and Feldman, 1962). Most of these can be overcome by the use of regular haemodialysis surgery.

Frequently, reports have appeared also suggesting the prolongation of effect occurring with the non-depolarising neuromuscular blocking drugs (Table 1.12). Most of these reports involve gallamine even though White and others (1971) have described the successful use of this drug in seventeen patients with chronic renal failure undergoing bilateral nephrectomy. In some instances, (+)-tubocurarine and pancuronium have been involved, but in no case has the prolongation of action been as great as that observed with gallamine. This suggests that there may be a fundamental difference in the way the body eliminates gallamine compared to (+)-tubocurarine or pancuronium.

Gallamine is excreted unchanged, principally by the kidneys (Mushin and others, 1949; Agoston and others, 1978). Furthermore, Feldman and others (1969) showed that negligible amounts of gallamine were excreted in the bile of dogs whose renal pedicles were ligated, resulting in sustained blood levels and prolonged paralysis.

Pancuronium (Agoston and others, 1973) and (+)-tubocurarine (Fleischli and Cohen, 1966) are also largely excreted unchanged via the kidney. In contrast to gallamine, the action of (+)-tubocurarine is not solely dependent upon urinary elimination. Studies in the dog confirmed this, when Cohen (1967) demonstrated that in the absence

Table 1.12 Non-depolarising muscle relaxants and the incidence of prolonged paralysis in patients with renal failure

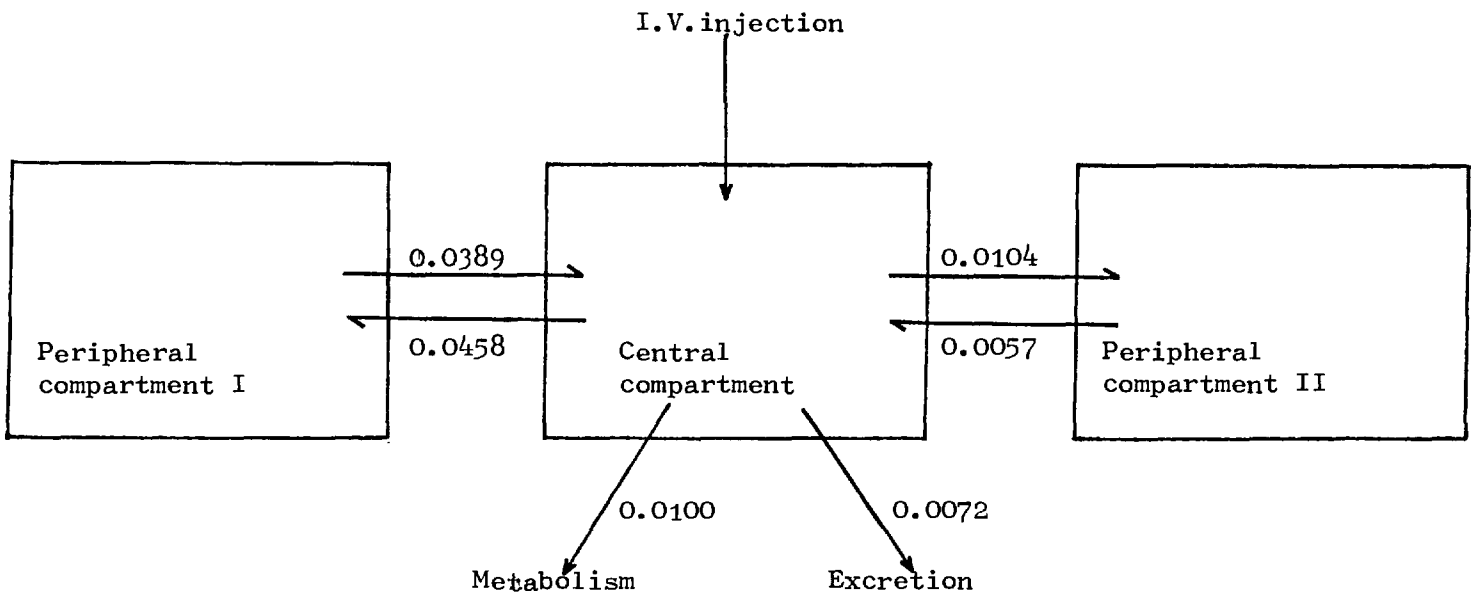
Relaxants	Extent of paralysis	Reference
Gallamine	1hr to 5days, and death reported in one case	Fairley,1950; Joske & others,1954; Montgomery & Bennett-Jones,1956; Jenkins,1961; Feldman & Levy,1963; Churchill-Davidson & others,1967; Lowenstein & others,1970; Singer & others, 1971; McLaughlin & others,1972.
(+)-Tubocurarine	1hr to 30hr	Riordan & Gilbertson,1971; Logan & others,1974; Miller & Cullen,1976.
Pancuronium	20min to 2 days	Miller & others,1973; Abrams & Hornbein,1975; Geha & others,1976; Rouse & others,1977.

Note "Recurarisation" and not prolongation of neuromuscular blockade was observed in some cases; in others the use of antibiotics also appeared to complicate the issue.

of renal function, the liver greatly increased its capacity to transport (+)-tubocurarine into the bile.

However, Riordan and Gilbertson (1971) described a patient with chronic renal failure who showed unusually prolonged neuromuscular blockade after several doses of (+)-tubocurarine, while Churchill-Davidson and others (1967) found that the duration of action of this drug was normal in six patients with terminal renal failure. These different observations concerning the duration of action of (+)-tubocurarine were rationalised by Gibaldi and others (1972a). Using the urinary excretion data from Kalow (1953) and plasma concentration data from Kalow (1959) and Cohen and others (1965), they characterised the distribution and elimination of (+)-tubocurarine on the basis of a three compartment model, with the site of action located in the central compartment (see Fig. 1.6). The effect of renal failure was then simulated by setting the excretion rate constant to zero. Using this model Gibaldi and others (1972b) predicted that renal failure will have little effect on the duration of action of relatively small single doses of (+)-tubocurarine (4 mg/m^2) but that the duration of action of large single doses (e.g. 18 mg/m^2) and multiple doses will be significantly prolonged.

Fig 1.6 Kinetic model for the distribution and elimination of (+)-Tubocurarine in man



Rate constants (min^{-1}) are shown next to the arrows which represent apparent First-order processes for transfer of the drug between the compartments and also for metabolism and excretion from the central compartment.

(taken from Gibaldi and others, 1972b)

Summary

Renal failure can therefore lead to a high incidence of toxic manifestations such as the prolongation of effect of neuromuscular blocking drugs. Many factors may interfere with the disposition of these drugs in renal failure. They include alterations in the rate of drug metabolism, decrease in protein binding leading to an apparent increase in the sensitivity to drugs in these patients, and the availability of compensatory pathways for drug elimination. The most obvious effect being due to the decreased renal excretion of these highly ionised compounds that can lead to the possible accumulation of potentially toxic drugs and metabolites.

1.4 Experimental Techniques

1.4.1 Pharmacodynamic assessment of neuromuscular blockade

When muscle relaxants are administered, it is obviously of great importance to ensure that adequate neuromuscular block has been attained during the operation; and that adequate return of neuromuscular function has also been achieved in the post-operative period. The various techniques that have been developed include simple clinical assessment, observation of ventilatory function, and nerve stimulation methods.

The measurements of head lift and grip strength by Johansen and others (1964) cannot be carried out during anaesthesia; even in conscious patients, their accuracy is severely limited by the lack of reproducibility. This method therefore, only offers a crude index of neuromuscular function. Secondly, observation of ventilatory function (Bendixen and others, 1959) cannot be considered satisfactory since a number of drugs including narcotics, hypnotics and inhalation anaesthetics also depress the respiratory centre.

Stimulation of a nerve with an electrical current, and measurement of the evoked muscle contraction (Katz, 1965; Churchill-Davidson, 1965) appears to be the most satisfactory method. The adductor pollicis muscle of the thumb is normally used for this purpose. Under appropriate conditions (Merton, 1954), it is the only muscle stimulated by the ulnar nerve; hence it approaches the single muscle fibre precision of an isolated nerve-muscle preparation. Stimulating electrodes are placed on the surface of the skin or percutaneously

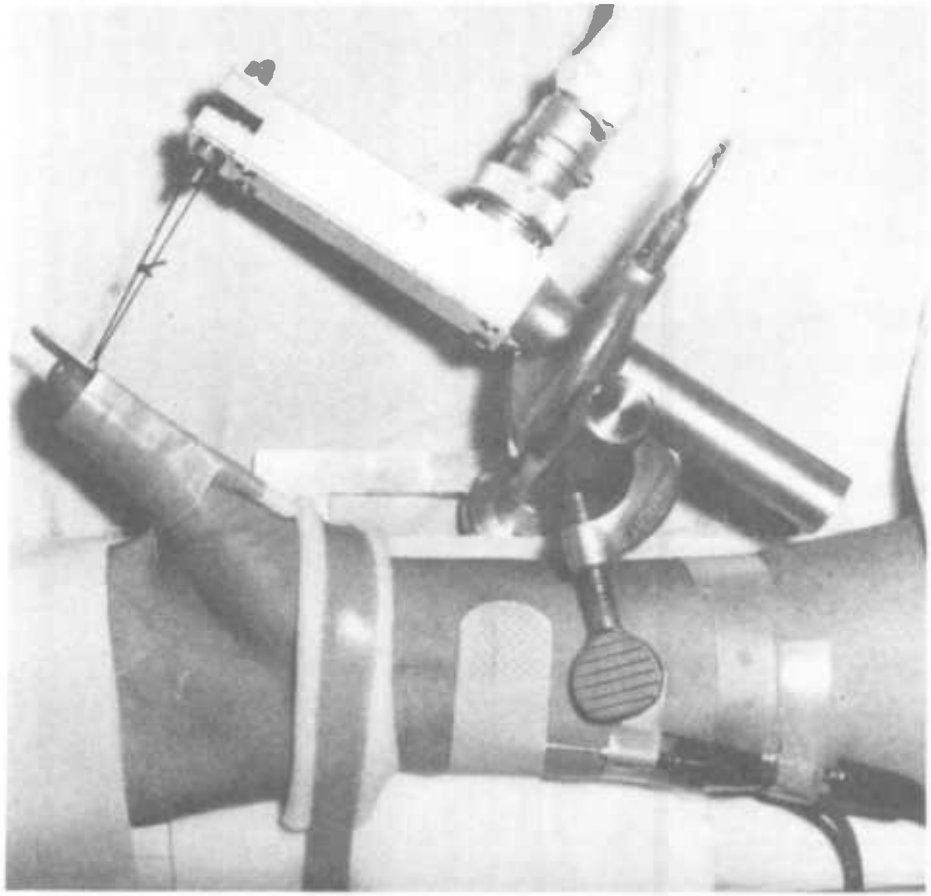
along the ulnar nerve (at the wrist or elbow). The evoked tension is then recorded on a strip chart recorder, using a force-displacement transducer (Grisen, 1973; Walts and Dillon, 1968) (see Fig 1.7)

The margin of safety of neuromuscular transmission is such that only 20-25% of the post-synaptic cholinergic receptor pool is necessary for transmission to all of the fibres in a muscle; and 90-95% need to be "occupied" before transmission fails (Paton and Waud, 1967).

Three modes of nerve stimulation have been used: single twitch, tetanic rates of 30 to 100 Hz, and train-of-four stimulation. Using receptor occlusion techniques in animals, Waud and Waud (1971, 1972 a,b) showed that the three modes of nerve stimulation indicate "normal" function at quite different levels of free receptors, as shown in table 1.13.

Single twitch and sustained tetany at 30 Hz seem to be equally sensitive in detecting the degree of block reversal. The fade in response to a 5-second tetanic stimulus at 100 or 200 Hz is much more sensitive than either single twitch or train-of-four however, high frequency tetanic stimulation (greater than 50 Hz) is unphysiological, since during maximal voluntary effort no more than 50 Hz are required to match a comparable evoked response (Merton, 1954). Clinically, the brief train-of-four twitch at low frequency appears to be a more sensitive index of receptor block. This method was developed by Ali and co-workers (1970, 1971a, 1971b) and is based on the observations of Berry (1966).

Fig 1.7 Photograph illustrating the immobilised forearm for the recording of neuromuscular function



The hand and forearm immobilized for recording of thumb adduction in a special arm board. The transducer is aligned parallel to the fully abducted thumb. The direction of thumb adduction is perpendicular to the transducer and corresponds to the direction of displacement of the transducer cantilever.

(taken from Ali and Saverese, 1976)

Table 1.13 Comparison of indices of recovery from neuromuscular block

Stimulus	% of receptors needed for normal response *
Twitch (0.1-0.2 Hz)	20-25
Train-of-four (2 Hz for 2 seconds, each train repeated every 10 seconds)	25-30
Tetanic-fade ratio (duration of 5 seconds)	
30 Hz	20-25
100 Hz	50
200 Hz	70

* the more receptors needed, the more sensitive the index

(taken from Waud and Waud, 1972a ; and from Zeh and Katz , 1978)

The method utilises a short train of four supramaximal stimuli applied to the ulnar nerve at a frequency of 2 Hz. Each train is repeated not more frequently than once every 10 seconds, either intermittently or continuously. The ratio of the amplitude of the fourth evoked twitch response to the amplitude of the first response in the same train (see Fig 1.8) is used to quantitate the block. While apparently not more sensitive than the single twitch, the train-of-four technique has many advantages:-

i) no pre-block control is required.

ii) provides a means of quantitating residual curarisation of a non-depolarising muscle relaxant.

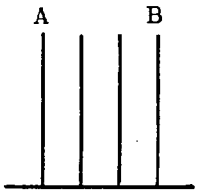
iii) can be applied to situations before and after reversal of block with an anticholinesterase.

iv) tetanic stimulation is painful to the recipient since characteristically the patient may be conscious when measurement is needed.

Recently, Lee (1975) discovered that the disappearance of the 4th, 3rd, 2nd and 1st twitch in the train-of-four corresponded with approximately 75%, 80%, 90% and 100% block, thus allowing simple estimation of neuromuscular blockade.

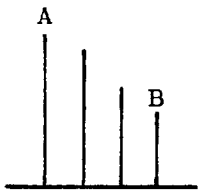
It appears that the great advantage of muscle relaxants compared with most other drugs is that their effects can be monitored continuously. This is particularly useful when relating pharmacological effects to plasma levels.

Fig 1.8 Train-of-four tracings and the estimation of recovery indices from neuromuscular blockade



normal response

Normal response before non-depolarising neuromuscular blocking drug or when the effect of the muscle relaxant has disappeared. The height of B to A expressed as a percentage is 100%



partial block

Response some time after administration of the non-depolarising neuromuscular blocking drug. The ratio of the height of B to A is 50%

Short train-of-four supramaximal stimuli applied to the ulnar nerve at a frequency of 2Hz., each train-of-four repeated every 10 seconds.

1.4.2 Quantitative determination of neuromuscular blocking drugs for Pharmacokinetic studies

Simple and reliable methods for the measurement of the concentration of therapeutic agents in biological media greatly facilitate the study of their disposition upon which sound therapy must be based.

This obtains with muscle relaxants, and the lack of sensitive chemical assays for the quantitative estimation of these quaternary ammonium compounds has seriously hampered the study of their disposition and metabolism in vivo. The major problem is centred around the difficulty in extracting these highly ionised, water soluble compounds from biological fluids.

Initially those quaternary ammonium compounds used as antiseptics and germicides were standardised on the basis of their phenol coefficient or actual bactericidal activity (Elarmann and Wright, 1948; Clausen, 1955).

Bioassay preparations which are currently most frequently used are listed on table 1.14. These bioassays in the case of acetylcholine for example, are very sensitive but time consuming and their reliability has been questioned in view of the fact that their response is not solely due to one compound alone (Hosein and others, 1962; Hosein and Koh, 1965; Hosein and Orzeck, 1966). Furthermore, pharmacological agents used in the experiments are likely to be present in the biological extracts and these may sensitize or desensitize the bioassay preparation (Hanin and Jenden, 1966).

Table 1.14 Some bioassays preparation currently used

Preparation	Agent	Reference
Frog rectus	(+)-tubocurarine, dimethyltubocurarine	Kalow,1954
Cat, sciatic-tibialis	(+)-tubocurarine, dimethyltubocurarine gallamine, suxamethonium, decamothonium	Payne,1958, 1959, 1960
Rabbit, sciatic-gastrocnemius	(+)-tubocurarine, dimethyltubocurarine	Gamstorp & Vinnars, 1961
Cat peroneal tibialis	(+)-tubocurarine, dimethyltubocurarine gallamine, suxamethonium, decamothonium	Katz & others,1963
Cat sciatic-gastrocnemius	(+)-tubocurarine, dimethyltubocurarine gallamine, suxamethonium	Hughes,1970
Man, ulnar-ring finger flexors	(+)-tubocurarine, alcuronium (+)-tubocurarine	Coleman & others,1966 Baraka (1964)
Man, ulnar-adductor policis	(+)-tubocurarine, gallamine	Walts & others,1967

(Table modified and updated from Katz and others, 1963)

Since its introduction by Kapfhammer and Bischoff (1930), precipitation by ammonium reineckate has been a common method for the isolation and separation of quaternary ammonium compounds from interfering substances in tissue extracts (Shaw, 1938). Several such methods exist (table 1.15) which involve precipitation of the quaternary ammonium compound as an insoluble salt or complex, followed by filtration and determination of excess precipitant, or by gravimetric estimation of the precipitate. Although the ferricyanide method has been extensively used, being the U.S.P. method since 1945, these methods lack the required sensitivity for pharmacokinetic analyses.

With the advent of highly sensitive and specific physicochemical instrumentation, many methods based on gas chromatography (g.c.) and gas-chromatography coupled to mass-spectrometry (g.c.m.s.) have increasingly found much application in the pharmacokinetic evaluation of drugs. For quaternary ammonium compounds, most of these methods measure the N-demethylation products (see table 1.16), a method first described by Hofmann (1851a, 1851b)

Gas chromatographic techniques appear to be sensitive but their specificity is limited to the simple quaternary ammonium compounds. More complex molecules such as propantheline give a mixture of tertiary amines. Hofmann degradation of large molecules such as cholestanyltrimethyl ammonium salts give a mixture of tertiary amines as well as olefins (Wall and McKenna, 1970). Hence the quantitative estimation of complex molecules such as the diquaternary muscle

Table 1.15 Precipitation agents employed for quaternary ammonium compounds

Precipitant	Reference
Ferrocyanide	Lottermoser and Stendel (1938)
Dichromate	Flotow (1942)
Ferricyanide	Wilson (1946)
Reineckate	Wilson (1952a); (1952b); (1954)
Phosphotungstate	Yoshima and Morita (1955a) Lincoln and Chinnick (1956)
Phosphomolybdate	Yoshima and Morita (1955b)
Tetraphenylboron	Kirsten and others (1958)

Table 1.16 Gas chromatographic methods used for the analysis of quaternary ammonium compounds

Compound	Procedure	Reference
alkylbenzyldimethyl ammonium	(i) thermal degradation (ii) measurement of tertiary amine using flame ionisation detector	Warrington (1961)
dialkyldimethyl ammonium, alkyltrimethyl ammonium	(i) thermal degradation (ii) measurement of tertiary amine using flame ionisation detector	Metcalfe (1963)
choline esters	(i) precipitation using ammonium reineckate (ii) debenzylation using sodium benzene thiolate (iii) measurement of tertiary amine using flame ionisation detector	Hanin and Jenden (1969)
neostigmine pyridostigmine	(i) ion-pair extraction with KI (ii) measurement of ion-pair using nitrogen detector	Chan and others (1976)
acetylcholine neostigmine	(i) thermal degration (ii) measurement of CH_3I released by electron capture detector	Pohlmann (1977)
propantheline	(i) thermal degradation (ii) calculation of % peak area ratio after analysis by mass spectrometry	Ford and others (1977)

relaxants (fazadinium, pancuronium) and triquaternary compounds (e.g. gallamine) remains difficult. No chromatographic methods have yet been published for these compounds.

Enzymic radioassay methods have also been cited in the literature. For example Reid and others (1971) described a method for measuring nanogram quantities of acetylcholine or choline in as little as 10 mg of brain tissue. The procedure involves the isolation of acetylcholine (or choline) by electrophoresis, alkaline hydrolysis of acetylcholine to choline, quantitative conversion of choline to a radioactive derivative, P^{32}-phosphorylcholine in the presence of choline kinase and adenosine triphosphate - γ - P^{32} (ATP^{32}), and finally isolation of P^{32}-phosphorylcholine by column chromatography. Horowitz and Spector (1973) developed a radio-immunoassay technique for the estimation of (+)-tubocurarine. Antibodies specific for (+)-tubocurarine were elicited by immunizing rabbits with an antigen consisting of the drug coupled to bovine serum albumin. The method depends upon the competition between unlabelled drug and a standard of tritium-labelled drug for specific antibodies present in the rabbit antisera. As little as 5 ng per ml is detected and the assay only requires 10μl of serum or urine sampled.

Both these methods are extremely sensitive and specific but they are very laborious. Laboratories therefore, are still in need of a reliable, rapid and sensitive method for quantitatively measuring neuromuscular blocking agents.

These claims appear to be justified by the dye extraction methods (table 1.17) which are based on Prudhomme's discovery (1938), that alkaloids form lipid-soluble complexes with acid dyes such as eosin; furthermore, the reaction was shown to be quantitative (Prudhomme, 1940).

In this method, a buffered solution of the quaternary ammonium compound and dye is extracted with an organic solvent. The dye-complex so formed is quantitatively extracted without contamination from other substances or any unreacted dye. Some physical property of the dye complex such as optical density or fluorescence is measured which then enables estimation of the amount of quaternary ammonium compound, by comparing previously derived calibration curves. These methods give reproducible results so long as the laws governing the optical density measurements and fluorescence measurements with respect to drug concentrations, are obeyed. Fluorescence assays have recently appeared to offer much promise since it is inherently a much more sensitive tool than colorimetry (Modin and Schill, 1967). This method is in fact employed at present for the assay of pancuronium (Kersten and others, 1973).

Table 1.17 Dye extraction methods employed for quaternary ammonium compounds

Reference	Indicator	pH	Solvent	Method of clarifying	Applicability
Auerbach (1943	Bromophenol blue (BPB)	11.5	Ethylene dichloride	Anhydrous sodium sulphate	$[R_1R_2R_3R_4.\overset{+}{N}\ \overset{-}{X}]$ where R = benzyl or butyl of derivative thereof
Auerbach (1944)	BPB	11.5	Benzene	Centrifuging	DITTO
Cochin and Woods (1951)	Bromocresol purple (BCP)	7.0	Chloroform	Centrifuging	tetraethylammonium bromide
Mitchell and Clark (1952)	BPB	9.0	Ethylene dichloride	Centrifuging	hexamethonium, tetraethylammonium bromide, neostigmine and (+)-tubocurarine
Gottilieb(1953)	Bromothymol blue (BTB)	8.4	Chloroform	Filter paper	quaternary ammonium and amine salts
Cohen (1963)	Rose bengal * (R.B.)	7.5 to 8.5	Chloroform + 2.5% phenol	Centrifuging	(+)-tubocurarine

Table 1.17 Dye extraction methods employed for quaternary ammonium compounds (cont'd)

Reference	Indicator	pH	Solvent	Method of clarifying	Applicability
Kersten and others (1973)	R.B. *	7.8	Chloroform + 2.5% phenol	Centrifuging	pancuronium and mono- and bis-quaternaries mw > 200 and mostly containing ring structures
Tanaka and others (1974)	B.P.B.	-	Ethylene dichloride	Centrifuging	pancuronium or generally quaternaries with 10+ carbon atoms
Stevens and Moffat (1974)	B.T.B.	7.5	Ditto	Filtering	suxamethonium
Buzello (1974)	B.P.B.	9.8	Chloroform	Centrifuging	pancuronium
Michoel (1976)	B.T.B.	9.0	Chloroform	Centrifuging	pancuronium

*Fluorescence measurements

All others are colorimetric measurements

1.5 Objectives of the present study

The aim of the present study is to evaluate the disposition and clinical pharmacokinetics of the competitive neuromuscular blocking drug, fazadinium bromide, in order to ascertain certain features of the drug in the context of an assessment of its use for patients with renal failure. Also included in this study is an investigation of the metabolic N-methylation and quaternisation of ^{14}C-pyridine *in vivo* in man and several animal species.

CHAPTER TWO

Assay of Fazadinium bromide in plasma and urine

Contents

List of Tables

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Introduction

Chemistry of fazadinium

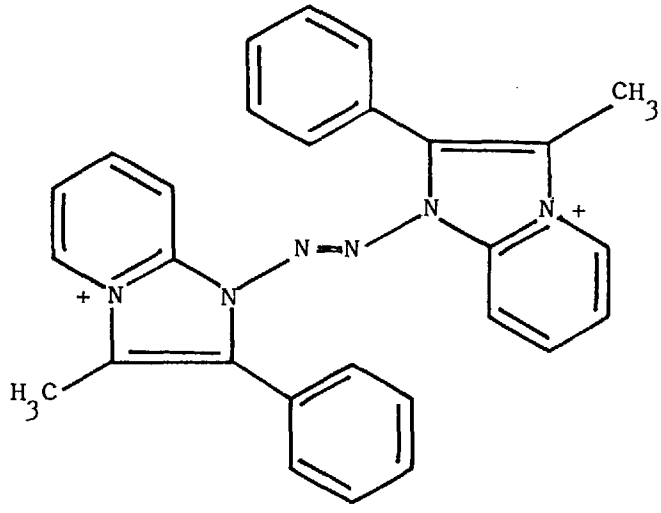
Fazadinium (Fig 2.1) is the most potent of a series of azo-bis-arylimidazo-(1,2 α) pyridinium salts (Bolger and others,1972) which possesses non-depolarising neuromuscular blocking activity. The molecule comprises of two identical halves, each with a quaternary ammonium group and linked together by a nitrogen chain (or tetrazine link). The dibromide salt is a yellow crystalline powder, soluble in water giving a clear yellow solution.

Metabolism of fazadinium

Fazadinium differs from other clinically used muscle relaxants such as pancuronium and (+)-tubocurarine (see table 2.1) in its chemical configuration which makes it more susceptible to metabolic breakdown. It is reduced under anaerobic conditions by liver microsomal azoreductases which require reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. Each molecule of Fazadinium gives rise to two molecules of 3-methyl-2-phenylimidazo-(1,2 α)-pyridinium (MIP) and one molecule of nitrogen, by scission of the tetrazene link. (Blogg and others, 1973b)

Using ^{3}H- and ^{14}C- Fazadinium, Bell and Martin (1979) showed that this drug is extensively metabolised by rat and rabbit but not by dog and man. The main metabolite in man was found to be MIP but in the rat and rabbit an unidentified metabolite was found (possibly a glucuronide of hydroxylated MIP) and this accounted for an increasing proportion of excreted radioactivity with time after dosing (Fig 2.2).

Fig. 2.1 Structure of Fazadinium bromide (Fazadon ^{R})



Chemical name: 1,1'-azo-bis [3 methyl-2 phenyl-1H-imidazo (1,2α) pyridinium] dibromide

Empirical formula: $C_{28}H_{24}N_6Br_2$

Molecular weight: 604.38

Table 2.1 Chemical structures of quaternary ammonium compounds

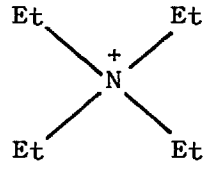
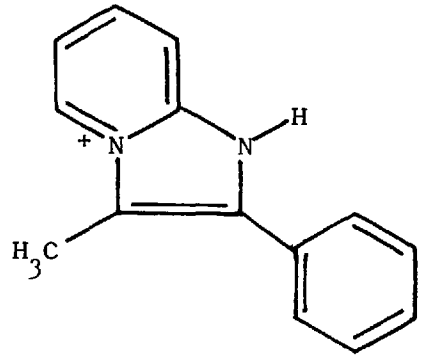
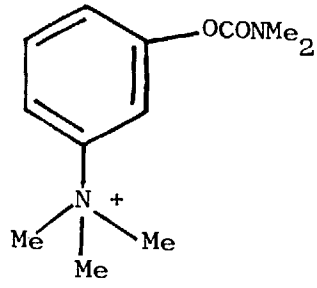
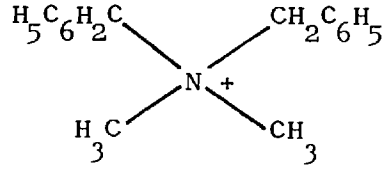
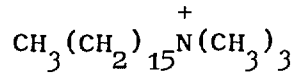
A Monoquaternaries	Molecular weight of the cation
(1) tetraethylammonium 	136
(2) 3-methyl-2 phenylimidazole (1,2α) pyridinium (MIP) 	210
(3) neostigmine 	223
(4)dibenzyldimethylammonium 	226

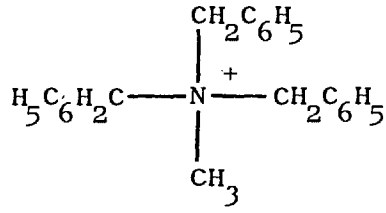
Table 2.1 Chemical structures of quaternary ammonium compounds(cont'd)

(5) cetyltrimethylammonium



285

(6) tribenzylmethylammonium



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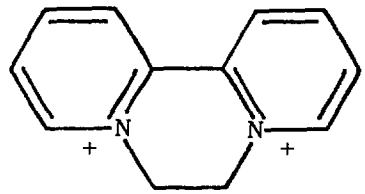
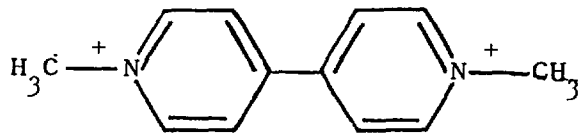
B Bis-quaternaries

(7) diquat



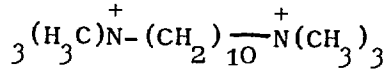
184

(8) paraquat



186

(9) decamethonium



258

(10) suxamethonium

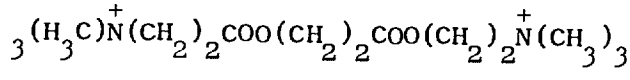


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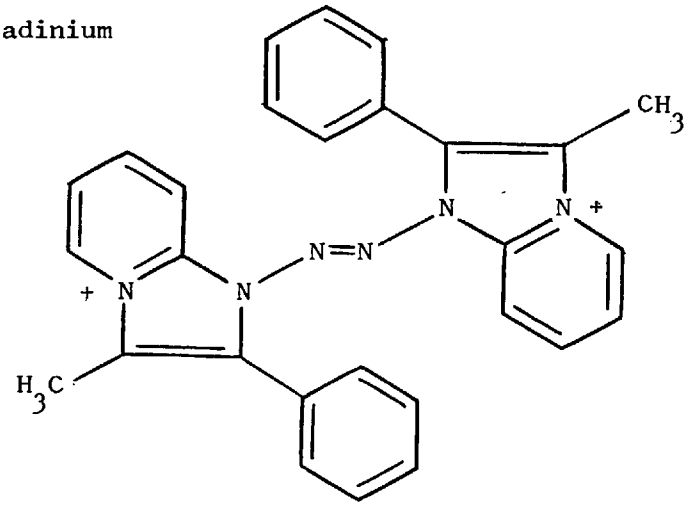
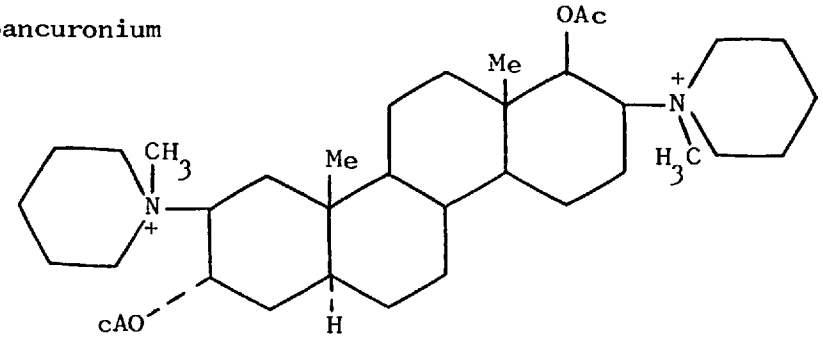
Table 2.1 Chemical structures of quaternary ammonium compounds (cont'd)

(11) fazadinium



444

(12) pancuronium



572

(13) (+)-tubocurarine

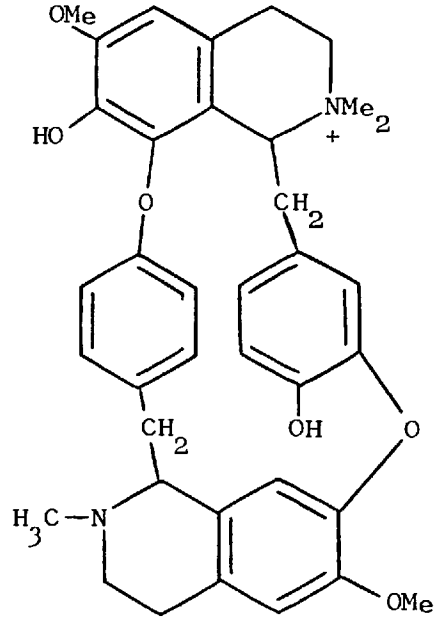


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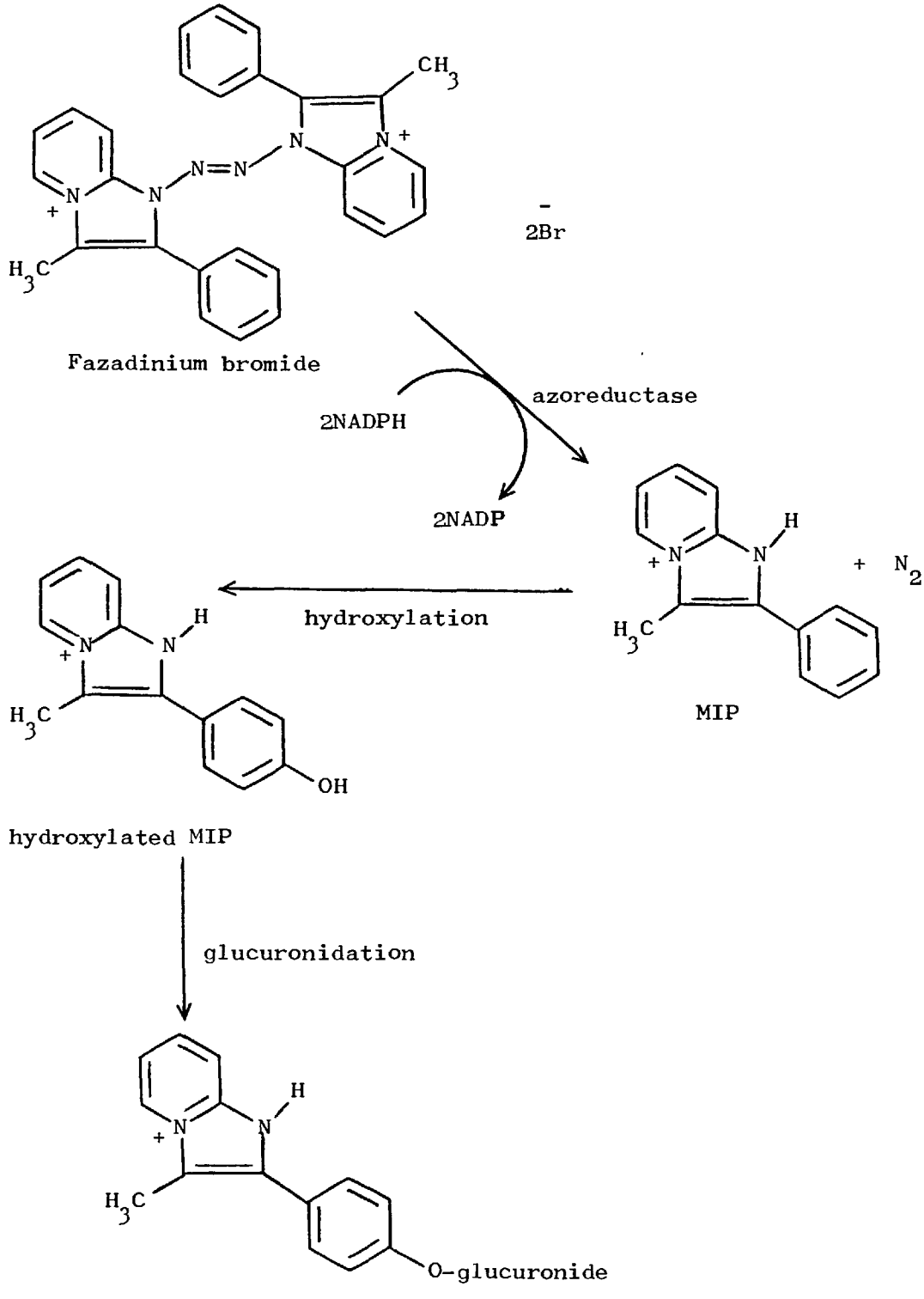
Fig 2.2 Metabolism of fazadinium bromide



Assay of fazadinium

Metabolites usually differ from the parent compound both in polarity and molecular weight; and consequently also in their pharmacological activity and disposition. It is therefore crucial in the analysis of such compounds to describe and understand what a particular method is measuring. The metabolites of fazadinium mentioned above, for example, have no neuromuscular blocking activity (Bolger and others, 1972) but the 3-deacetylated metabolite of pancuronium (its major metabolite) has a neuromuscular blocking activity which is similar to that of the parent compound (Kersten and others, 1973). The analytical procedure employed for a drug should therefore be able to distinguish between the unchanged drug and its metabolites.

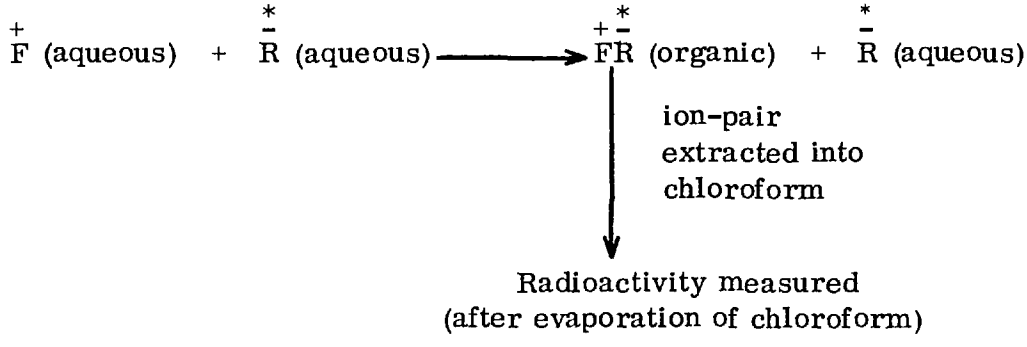
Various methods have been described for the quantitative estimation of quaternary ammonium compounds in biological fluids (see chapter one). Some of these methods include radioimmunoassay, gas chromatography, combined gas-chromatography mass-spectrometry and spectrophotometric and spectrofluorimetric procedures (see chapter one for references). Of these it is the spectrophotometric and spectrofluorimetric methods involving ion-pair extractions which have been most widely used.

In the original Rose Bengal ion-pair complexing method of Cohen (1963) for (+)-tubocurarine, the drug interacts with Rose Bengal to form a lipid soluble ion pair which is then readily extractable into an organic solvent and whose fluorescence is measureable. The procedure however, suffers from

the disadvantage of instability of the fluorescent complex though this can be improved by adding acetone before final reading of fluorescence; the acetone is used to enhance the fluorescence and stability of the complex. Kersten and others (1973) adopted this method for the diquaternary compound pancuronium (see table 2.1). Furthermore, Watson and McLeod (1977) suggested the use of ethanol instead of acetone to improve the stability of the fluorescent complex. However, difficulties may also be experienced when drug concentrations are low and only small volumes of biological fluids are available for the assay.

The method has now been improved by the use of ^{125}I-Rose Bengal as the complexing agent instead of unlabelled Rose Bengal. The radiolabelled Rose Bengal interacts with fazadinium to form a radiolabelled ion-pair complex, readily extractable into an organic solvent, whose ^{125}I content can be determined by liquid scintillation counting (see scheme 2.1)

Scheme 2.1 : Diagrammatic representation of the Radiometric procedure



Key:

$\overset{+}{F}$ = Fazadinium

$\overset{*}{\underset{-}{R}}$ = ^{125}I - Rose Bengal

Materials and Methods

Compounds and Radiochemicals

Rose Bengal (Fig 2.3)

^{125}I-Rose Bengal (specific activity 0.7 mCi mg^{-1}) was purchased as a sterile aqueous solution of its disodium salt (pH 7.5 to 8.5 containing 1% benzyl alcohol as a bactericide) from the Radiochemical Centre, Amersham, UK. Radiochemical purity was 96.5% as tetra[^{125}I]iodo Rose Bengal by paper chromatography on Whatman No.3 paper using the solvent system 0.15N NH_4OH saturated with isoamyl alcohol (lower phase). 0.9% of the ^{125}I was present as the iodide.

Rose Bengal (m.p. > 300^{o}C)was obtained from BDH Chemicals,Poole, U.K. This was purified by the method of Cohen (1963) prior to use.

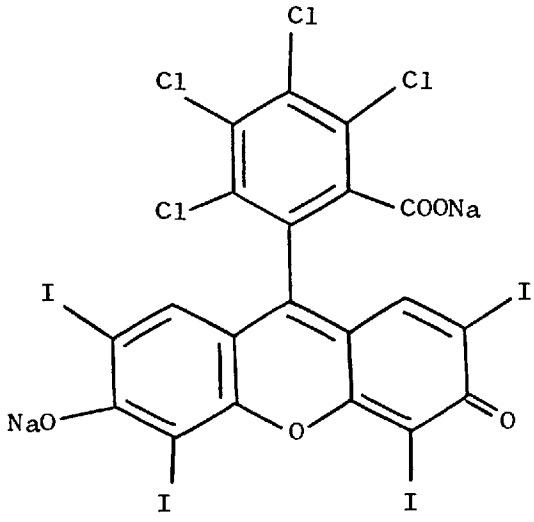
Fazadinium bromide (Fazadon ^{R})

Fazadinium bromide (m.p. 215^{o}C uncorrected) and 3-methyl-2-phenyl-imidazo (1-2α) pyridinium (MIP) (m.p. 241^{o}C uncorrected) were gifts from Allen and Hanburys Research Ltd., Ware, Herts, U.K.

Pancuronium bromide (Pavulon ^{R})

Pancuronium bromide (m.p. 214-217^{o}C uncorrected), 3-desacetyl, 17-desacetyl-and 3,7-didesacetyl-pancuronium (m.p. 230^{o}C, 235^{o}C, 245^{o}C, and code nos. NE35, NB68, NA96, respectively) were gifts from NV Organon, Oss, The Netherlands.

Fig 2.3 Chemical structure of Rose Bengal



Chemical name: 4,5,6,7-tetrachloro-2',4',5',7'-tetraiodo-fluorescein sodium

Empirical formula: $C_{20}H_4Cl_4I_4O_5$

Molecular weight: 973.72

Colour index: 45440

^{125}I Rose Bengal: 96.5% as tetra[^{125}I]iodo Rose Bengal. (equal chance of ^{125}I incorporation expected)

Other quaternary ammonium compounds

Tetraethylammonium bromide, neostigmine chloride, suxamethonium chloride, decamethonium iodide, paraquat diiodide, diquat dibromide (+)-tubocurarine chloride, dibenzyldimethylammonium iodide, cetyltrimethylammonium bromide and tribenzylmethylammonium iodide were samples from previous studies in this department (Hughes, 1973) (Their chemical structures are shown in table 2.1)

Preparation of ^{125}I Rose Bengal solution

A stock solution of unlabelled Rose Bengal (110μ g ml^{-1}) was prepared in 0.45M K_2HPO_4. For the assay, the ^{125}I-Rose Bengal solution supplied was diluted with this carrier Rose Bengal solution and 0.45M K_2HPO_4 so as to provide a stock ^{125}I reagent solution of 100μg ml^{-1}, 2 μCi ml^{-1}. The radioactive solutions were stored in glass bottles housed in lead containers.

Preparation of 0.2M phosphate buffer

Phosphate buffer (pH 7.8) was prepared from 0.2M KH_2PO_4 (25 ml) and 0.2M NaOH (22.6 ml) made up to 100 ml with distilled water.

Counting of ^{125}I

^{125}I was determined by liquid scintillation counting of its secondary β-emissions using a liquid scintillation spectrometer (Packard Tri-carb model 3385). The scintillation cocktail was Triton X-100 (p-isooctylphenoxypolyethoxyethanol, 3.3 litres); POPOP (1,4-di-2-(5-phenoxyloxazolyl)-benzene, 1g); PPO (2,5-diphenyloxazole, 55g)

made up to 10 litres with toluene. (Similar results were obtained using a gamma counter (Packard model 578)).

The efficiency of counting ^{125}I was 80% as determined by spiking samples with known amount (DPM) of ^{125}I-Rose Bengal. For the assay however, only relative counts per minute (CPM) were compared.

Determination of fazadinium in human plasma and urine

A standard calibration curve for fazadinium was prepared by adding an aqueous solution of fazadinium bromide to plasma or urine samples, so as to provide concentrations in the range 0.01-100 $\mu g\ ml^{-1}$

To each sample (0.5 ml in triplicate) in a 15 ml stoppered extraction tube was added 0.2M phosphate buffer 7.8 (1 ml) and ^{125}I-Rose Bengal solution (0.5 ml; containing approximately 2×10^6 CPM). The solution was mixed by vortexing (30 seconds) and then extracted with chloroform (5 ml; 15 minutes) on a rotary mixer, to avoid troublesome emulsions. The tubes were then centrifuged (2,500 r.p.m.; 10 minutes) and the upper layer carefully removed using a pasteur pipette attached to a water pump. An aliquot of the lower chloroform extract (2 x 1 ml) was transferred by automatic pipette to a 7 ml scintillation counting insert tube. The chloroform was then evaporated off at room temperature using a stream of nitrogen, the whole process taking about 10-15 minutes for completion.

The residue was dissolved in scintillation fluid (4 ml) and the ^{125}I determined by liquid scintillation counting. Blank plasma and urine samples (without added fazadinium) were similarly treated for the estimation of background extractable ^{125}I.

Determination of pancuronium in human plasma and urine

A standard calibration curve for pancuronium was established as described for fazadinium. The samples were likewise treated with 0.2M phosphate buffer (1 ml), ^{125}I-Rose Bengal (0.5 ml) and taken through the same procedure as described for fazadinium.

Ion-pair formation by other quaternary ammonium compounds

It was of interest to determine whether or not other quaternary ammonium compounds as well as fazadinium and pancuronium could interact with ^{125}I-Rose Bengal. For example, suxamethonium is generally used prior to the administration of these neuromuscular blockers and MIP is the major metabolite of fazadinium in man. Hence it is important that the assay should distinguish between these compounds and fazadinium or pancuronium.

For this purpose 1μ g. of each of the quaternary ammonium compounds listed below was added as an aqueous solution of the appropriate salt to 0.2M phosphate buffer (1 ml) and then taken through the same radiometric procedure described above. For the estimation of background extractable

^{125}I, phosphate buffer (1 ml) with no added quaternary ammonium compound was taken through the assay procedure. The difference in counts obtained with quaternary ammonium present compared to background was used as a measure of the extent of ion-pair formation with Rose Bengal.

The following compounds were tested by this procedure:-

Monoquaternary ammonium:

Tetraethylammonium, MIP, neostigmine, dibenzyldimethylammonium, cetyltrimethylammonium, tribenzylmethylammonium.

Bisquaternary ammonium:

Suxamethonium, decamethonium, paraquat, diquat, fazadinium, pancuronium, 3-desacetyl-pancuronium (NE35), 17-desacetyl-pancuronium (NB68), 3,17-didesacetyl-pancuronium (NA96), and (+) tubocurarine.

Results and discussion

Fig. 2.4 and Fig. 2.5 represent typical calibration curves for fazadinium and pancuronium in plasma and urine as measured by the ^{125}I-Rose Bengal radiometric procedure.

There is a linear relationship between extractable ^{125}I-Rose Bengal and amount of quaternary ammonium compound; in both cases, the correlation coefficient after linear regression was 0.99 over the concentration range of 0.01-1.0μg ml^{-1}.

Fig 2.4 Calibration curve of Fazadinium and Pancuronium in human plasma (0.01 - 1.0µg ml^{-1})

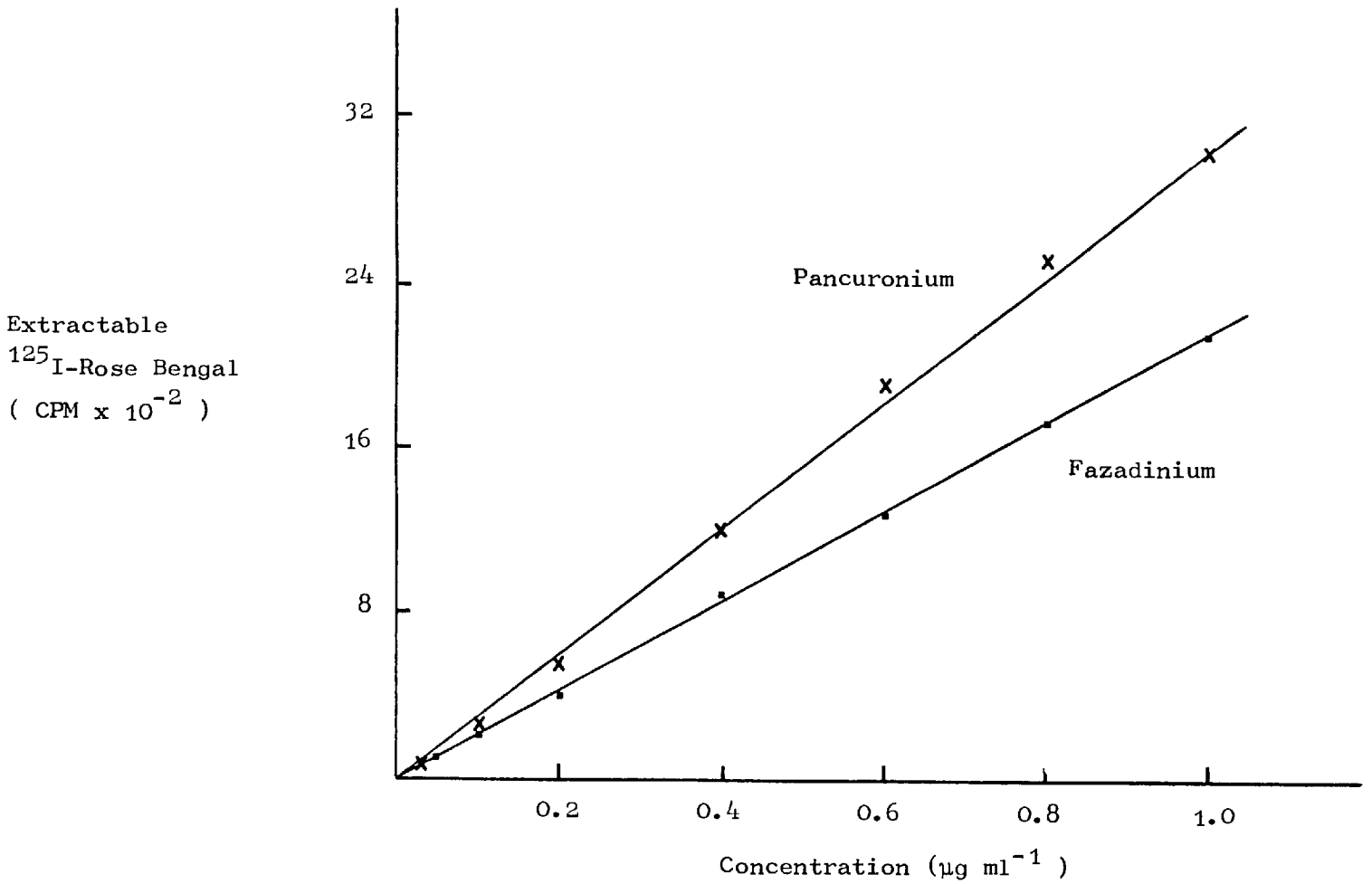
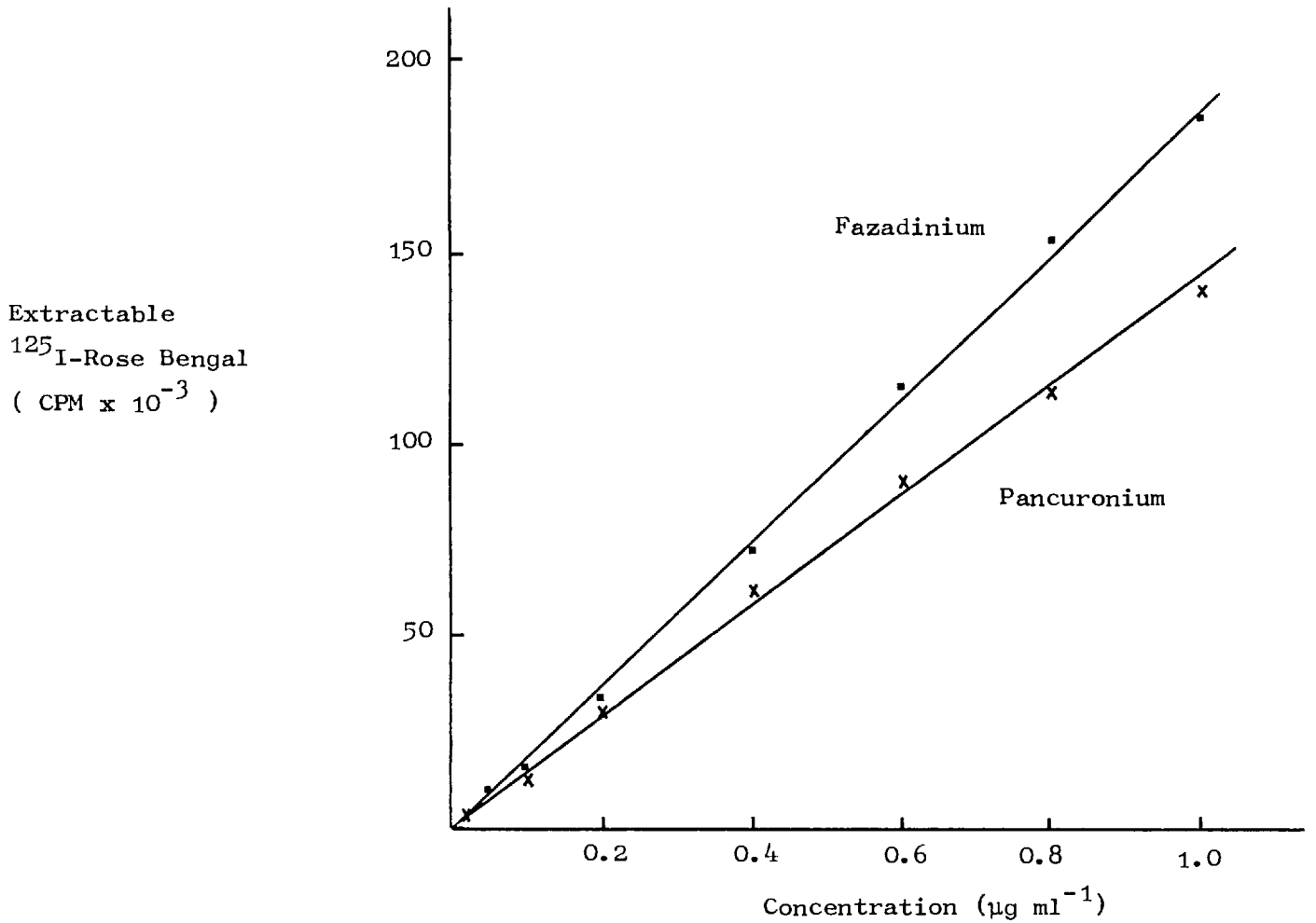


Fig 2.5 Calibration curve of Fazadinium and Pancuronium in human urine (0.01 - 1.0µg ml^{-1})



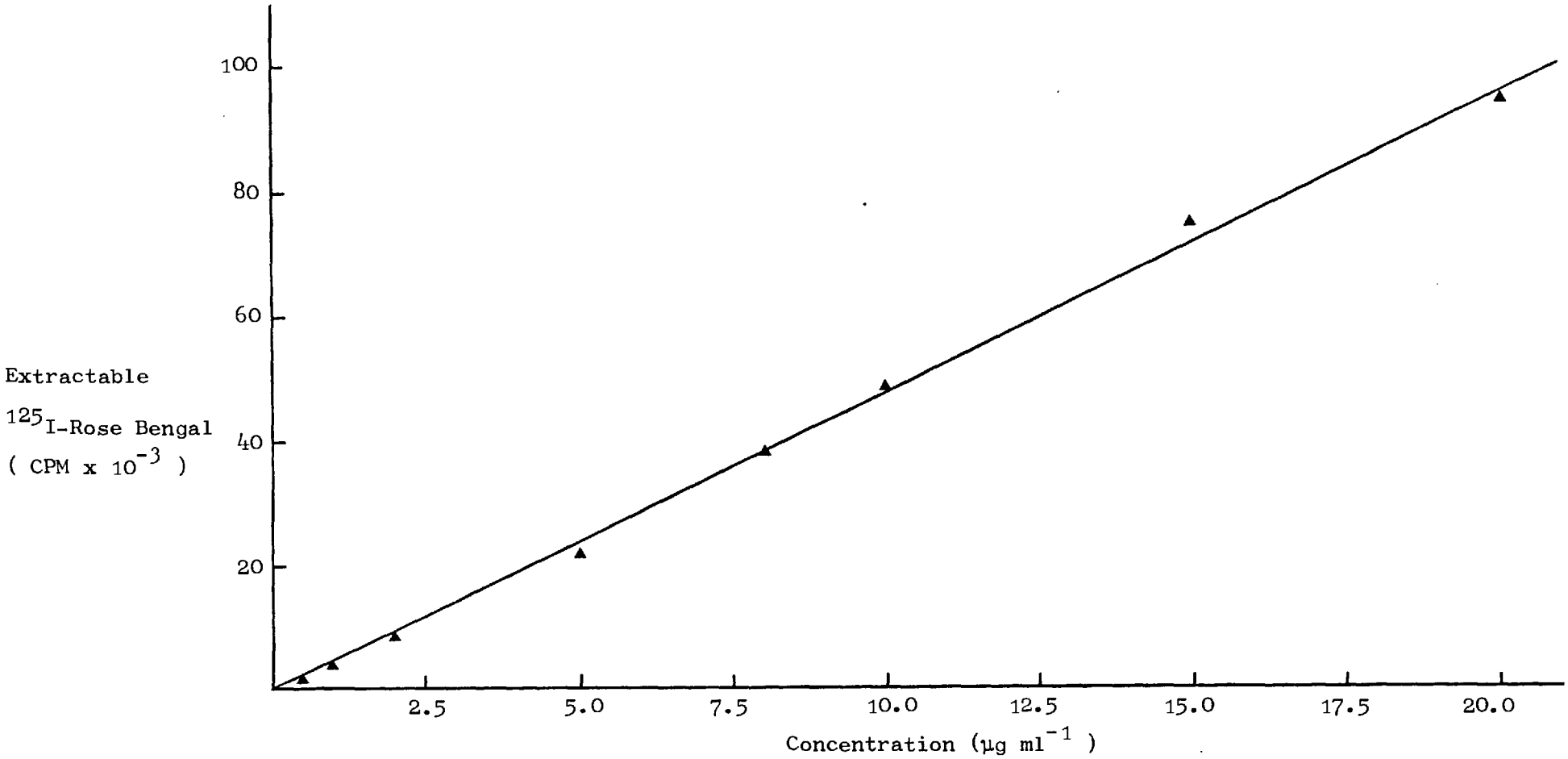
For pancuronium, less than 20 ng ml^{-1} could be detected and measured in plasma by this method. This indicates that the method is at least as sensitive as the fluorimetric method described by Kersten and others (1973).

Fazadinium is used at a dose level which is about ten times the amount of pancuronium; hence it was necessary to extend its calibration curve. A typical calibration for fazadinium in plasma over the concentration range 0.5-20 $\mu g\ ml^{-1}$ is shown in Fig. 2.6. Yet again there was a linear correlation between extractable ^{125}I-Rose Bengal and fazadinium concentration ($r = 0.99$). For fazadinium, the reproducibility of the assay over the concentration range 0.01-10 $\mu g\ ml^{-1}$ was $\pm$ 5% ($n = 5$).

Neither fazadinium nor pancuronium were appreciably bound to red blood cells since addition of known concentrations (0.01 - 50 $\mu g\ ml^{-1}$) to whole blood gave a mean recovery in plasma of 89% for fazadinium (range 85-102) and 88% for pancuronium (range 78-101), with reference to appropriate calibration curves.

Furthermore, the assay appears to measure only the unbound fraction of the drug present in plasma since after ultrafiltration of plasma (Amicon Centriflo CF 25A membrane) to which fazadinium or pancuronium had been added, the ultrafiltrate contained the same amount of drug as measured by assay of the whole plasma. Also addition of various concentrations of bovine serum albumin (up to 6g per 100 ml) to urine containing either drug, resulted in the apparent loss of the drug when

Fig 2.6 A typical calibration curve for Fazadinium in human plasma (0.5 - 20µg ml^{-1})



assayed; the disappearance of drug being related to protein concentration (see Fig 2.7)

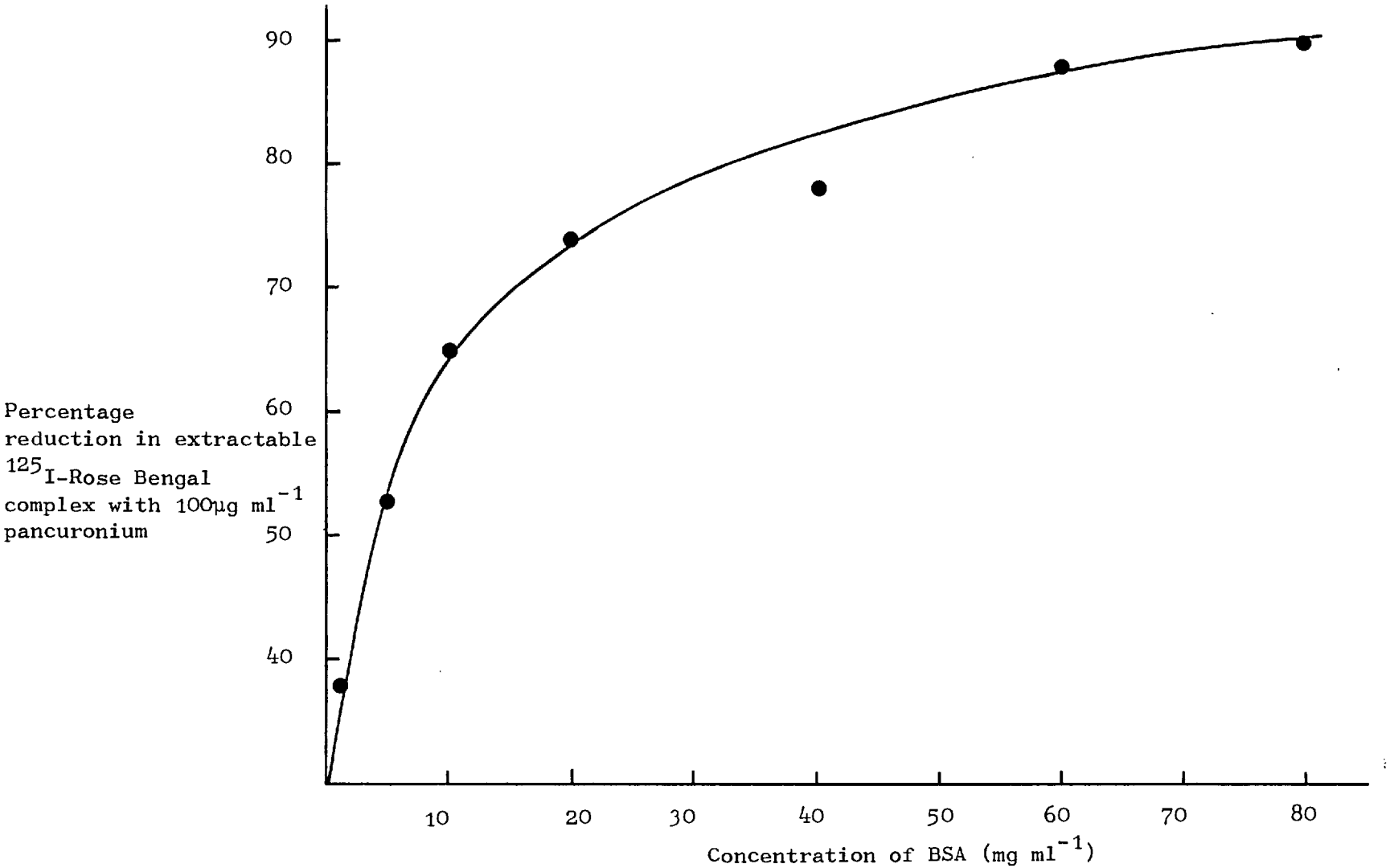
In urine, the mean recovery after addition of known concentrations of fazadinium or pancuronium (0.01-10 μg ml^{-1}) was 90% for fazadinium (range 82-132) and 94% for pancuronium (range 89-109).

It should be noted however, that although the background extractable ^{125}I-Rose Bengal is much higher in urine than in plasma, this does not affect the linear relationship between drug concentration and extractable ^{125}I-Rose Bengal over the range 0.01-10 μg ml^{-1}, after appropriate allowance for background.

Recently, Duvaldestin and others (1978) assayed fazadinium bromide using a slight modification of the fluorimetric method employed by Kersten and others (1973). However, the lowest concentration which they could measure was 0.5 μg ml^{-1} but they gave no indication as to the accuracy of this determination. The ^{125}I-Rose Bengal method described above is the only radiometric method described for the assay of fazadinium in biological fluids.

This radiolabelling method is more rapid, convenient and sensitive than others described hitherto (see table 1.15 chapter one). Perhaps, the greatest advantage of the method is that a large number of biological samples can be handled simultaneously (over 100 samples including standards can

Fig 2.7 Effect of bovine serum albumin (BSA) on the extractability of ^{125}I Rose Bengal - Pancuronium complex



be assayed within a day). In the fluorimetric assay the dye solution has to be prepared immediately prior to analysis and stored in the dark before use. The ^{125}I reagent solution can be prepared several months before use and problems associated with the instability of the fluorescent complex are avoided.

Morever, this method utilises a liquid scintillation spectrometer which is part of the standard equipment in most laboratories.

Specificity of the radiometric determination

Table 2.2 is a list of quaternary ammonium compounds (in order of increasing molecular weight) that have been examined in this assay.

It can be seen that the ability to form chloroform-soluble ion-pair complexes with Rose Bengal is not restricted to fazadinium and pancuronium since a number of other mono- and bis-onium compounds also do this.

With the simple monoquaternary compounds such as tetraethylammonium, neostigmine and MIP, there is no detectable formation of a chloroform-soluble complex with ^{125}I-Rose Bengal. However, with monoquaternaries of higher molecular weight, viz, dibenzyldimethyl-, tribenzylmethyl- and cetyltrimethyl- ammonium compounds, there occurs considerable complexation with Rose Bengal.

Table 2.2 Formation by some quaternary ammonium compounds of chloroform-soluble ion-pair complexes with ^{125}I Rose Bengal

Compound	Mol.wt. of cation	Chloroform-extractable ^{125}I (c.p.m.) formed from interaction of the compounds (1μg) with ^{125}I-Rose Bengal
Mono-quaternary ammonium		
Tetraethylammonium	126	0
3-Methyl-2-phenylimidazole (1,2α)pyridinium (MIP)	210	24
Neostigmine	223	0
Dibenzyldimethylammonium	226	7,720
Cetyltrimethylammonium	285	29,180
Tribenzylmethylammonium	302	6,900
Bis-quaternary ammonium		
Diquat	184	3,750
Paraquat	186	9,020
Decamethonium	258	0
Suxamethonium	290	0
Fazadinium	444	78,300
3-Desacetyl-pancuronium	471	16,122
17-Desacetyl-pancuronium	471	18,705
3,17-Desacetyl-pancuronium	488	19,690
Pancuronium	572	16,098
(+)-Tubocurarine	625	14,800

In the case of the bisquaternary ammonium compounds, all those investigated with the exception of suxamethonium and decamethonium formed extractable Rose Bengal complexes under the conditions used. This method could not distinguish between Pancuronium and its deacetylated analogues. As with the monoquaternaries, the most extensive extraction occurred with the higher molecular weight compounds such as fazadinium, pancuronium and (+)-tubocurarine, suggesting that this may be a factor, but not the sole one, influencing the extent of formation of chloroform-soluble complexes with Rose Bengal.

The results also agree closely with those of Kersten and others (1973) who found no extractable Rose Bengal complexes formed with acetylcholine, suxamethonium, N-methylnicotinamide, neostigmine and edrophonium.

Kersten and others (1973) concluded that the Rose Bengal complex only occurs with mono- and bis-quaternary ammonium compounds with molecular weights (cation) exceeding 200 and containing mostly ring structures. Tanaka and others (1974) using bromophenol blue (tetrabromophenolsulfopthalein) as the extracting dye for pancuronium, suggested that ion-pair formation with quaternary ammonium compounds also depends upon their lipophilic character. This obtains with Rose Bengal where cetyltrimethylammonium is extracted to a greater extent (four fold) than either dibenzyldimethylammonium or tribenzylmethylammonium (see table 2.2).

The reaction to form an ion pair in aqueous media appears to be a property only of quaternary ammonium compounds and not of tertiary amines. Auerbach (1943) using bromophenol blue tested fifty tertiary amines with negative results. Later, Mitchell and Clarke (1952) confirmed Auerbach's results and also obtained negative results with a number of other tertiary nitrogen compounds. Similarly, the Rose Bengal radiometric method gave negative results with tertiary amines such as nicotinamide, papaverine, tribenzylamine, ergotamine, the tertiary amine analogue of pancuronium (NB 62), droperidol and Fentanyl.

Finally, the radiometric method described for fazadinium is sufficiently specific and its sensitivity should enable the measurement of plasma concentrations when the effect of the drug has almost disappeared; this feature being most useful when studying the pharmacodynamics of fazadinium.

Summary

1) A rapid, sensitive and convenient radiometric method utilising ^{125}I-Rose Bengal for the assay of fazadinium in biological fluids is described.

2) There is a linear relationship between extractable ^{125}I-Rose Bengal and fazadinium concentration over the range 0.01-10μg ml^{-1} in plasma or urine.

3) Fazadinium is not appreciably bound to red blood cells and the assay only measures the free drug in plasma.

4) Drugs that are commonly used during surgery and contain either quaternary ammonium or tertiary nitrogen functions do not interfere with the assay of fazadinium.

5) The specificity and potential applicability of the assay to the determination of other quaternary ammonium compounds is discussed with respect to molecular size and lipophilic character.

CHAPTER THREE

Disposition of fazadinium bromide in Man and the Dog

Contents

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List of Tables

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Introduction

The physiological changes associated with renal failure often have pronounced effects on the disposition and pharmacological response to neuromuscular blocking agents.

All the muscle relaxants with the exception of suxamethonium are extensively excreted unchanged in the urine (see Table 3.1) and elimination by this route has been assumed to be a prime determinant of their duration of action. For instance, gallamine is eliminated mainly via the kidneys and prolonged, sometimes fatal postoperative neuromuscular blockade has frequently been reported in patients with impaired renal function (Churchill-Davidson and others, 1967; McLaughlin and others, 1972). Renal excretion of these compounds is also important in dogs. For example, Feldman and others (1969) recovered 84% of unchanged gallamine in dog urine after 24h. Furthermore, when the renal pedicles were ligated, there was no change in the biliary excretion (<1%), but a sustained blood gallamine concentration (10% of dose) was maintained for 24h producing prolonged paralysis.

Although renal excretion is the primary pathway of elimination for (+)-tubocurarine and pancuronium, both these drugs appear to have an alternative biliary route of excretion. In the absence of renal function, the biliary elimination of (+)-tubocurarine is increased four-fold in the dog (Cohen and others, 1967). Whether the same compensatory mechanism occurs with pancuronium is unknown. However, blockade from pancuronium lasts two to three times longer in anephric cats compared to those with normal renal function (Adler and Pilon, 1972). The use of (+)-tubocurarine and pancuronium in patients with impaired renal function consequently requires extreme caution.

Table 3.1 Excretory pattern of neuromuscular blocking drugs in man

	% Excretion in 24h		
Drug	Urine	bile	Reference
gallamine	15-100	<0.1	Agoston and others (1978)
(+)-tubocurarine	38	-	Miller and others (1977)
pancuronium	43 (30h)	11 (30h)	Agoston and others (1973b)
fazadinium	70-80 (48h)	3 * (48h)	Blogg and others (1973b)

Bile samples were obtained by 'T' tube drainage (Cholecystectomy and Choledocystectomy patients)

* Denotes faecal elimination

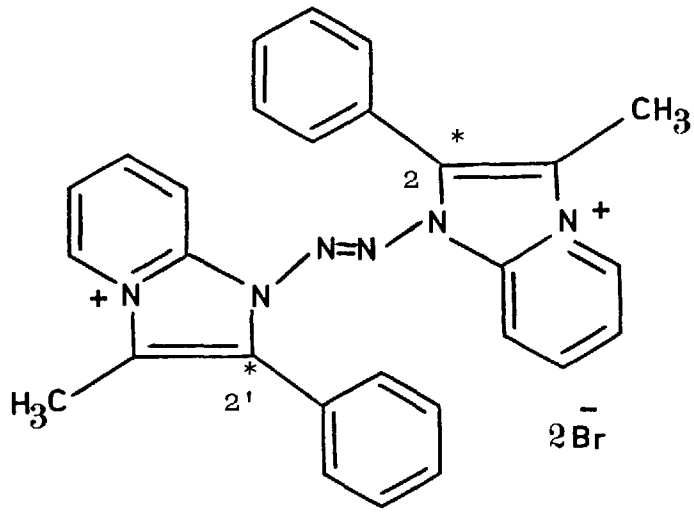
Fazadinium has undergone extensive clinical testing with respect to its neuromuscular blocking properties, but little is known about its metabolic fate in man. The only study (Blogg and others, 1973a)reports that 70-80% of a dose (0.5-0.75 mg Kg^{-1}) was excreted in the urine after 48h with only 3% in the faeces.

The study described here was designed to investigate the disposition and fate of fazadinium in man. The molecular weight of fazadinium (404) suggests that it could undergo appreciable renal and hepatic elimination but it is currently very difficult to evaluate hepatic elimination directly in man. Many workers (Cohen and others, 1965; 1967; Feldman and others, 1969; Manani and others, 1972) studied the disposition of muscle relaxants (e.g. (+)-tubocurarine, gallamine and pancuronium) by using the dog as a model species for the human situation. Accordingly, the anaesthetised greyhound was used to study the effects of either facilitating or impairing renal excretion on the disposition of fazadinium.

Materials and Methods

Radiochemicals

^{14}C-Fazadinium bromide (labelled at C-2 and 2' of the imidazole rings (see below), specific activity 1μCi mg^{-1}) and ^{3}H-fazadinium bromide (generally tritiated, specific activity 24 μCi mg^{-1}) were gifts from Allen and Hanburys Research Ltd., Ware, Herts., U.K.



The radiochemical purity of both these radioactive compounds was greater than 98% as determined by thin layer chromatography (t.l.c.) and radiochromatogram scanning, using the two solvent systems shown in table 3.2

Other compounds

3-Methyl-2-phenyl-imidazo (1-2α) pyridinium bromide (MIP) and unlabelled fazadinium bromide were gifts from Allen and Hanburys Research Ltd.

Table 3.2 Chromatographic mobilities of fazadinium and MIP in two solvent systems

Compound	R_F Values in System I	II	Detection
Fazadinium	0.16	0.15	bright yellow fluorescence in visible light
MIP	0.67	0.74	bluish fluorescence when inspected under uv (254 nm)

solvent systems:

I, Acetone : 0.2N HCl , 1:1 by vol

II, Propan-1-ol : 20% NH_4Cl, 7:3 by vol. (upper phase)

Aluminium backed Kieselgel $60F_{254}$ thin layer plates were used (0.25 mm thickness; Merck E, Damstadt, W.Germany)

Humans

Five healthy, non-fasting, male human volunteers with ages ranging from 23-28 yrs participated in the study. All had previously given their informed consent.

A dose solution was prepared by dissolving ^{3}H-fazadinium bromide (2.5 mg.) in normal saline (4 ml; 0.9% $^{w}/_{v}$). For intravenous administration, this solution was rendered sterile by passing it twice through Millex filter units (0.22 μm pore size; Millipore S.A., France).

Intravenous dosing

All five volunteers received 1 ml of the dose solution (0.63* mg; 15 μCi) by injection into the antecubital fossa of the left arm; this procedure was conducted by an anaesthetist. For the first half-hour after dosing, the subjects were kept under close clinical supervision in an anaesthetic room where ventilation equipment was available.

Oral dosing

Three volunteers also received the same dose of ^{3}H-fazadinium orally. This study was conducted about six months after the first experiment.

Sample collection

After intravenous dosing urine samples were collected hourly for 8h, thence from 8-12h, 12-24h and 24-30h. After oral dosing, 0-12h, 12-24h and 24-30h urines were collected.

Faeces were collected daily in both studies, for a period of five days.

* Dose of fazadinium administered was one-hundredth the clinical dose and no pharmacological effects were anticipated.

Animals

Adult female greyhounds (weight 25 ± 2.5 Kg) were used in the study. Anaesthesia was induced with thiopentone sodium (Pentothal^{R}; Abbott Laboratories Ltd., Queensborough, Kent, U.K., 30 mg Kg^{-1} i.v.) and maintained with intermittent doses of ketamine (Ketalar^{R}, Parke Davies & Co., Pontypool, Gwent, U.K., 10 mg Kg^{-1} i.m.) as described by Bevan and Budhu (1975).

Following induction of anaesthesia, the animals were intubated with oxygen-enriched room air to maintain PaO_2 above 100 mm Hg and $PaCO_2$ at approximately 35 mm Hg.

The rectal temperature was maintained at 37^{o}C by appropriate adjustments to the body heater on the operating table.

Surgical procedure

Catheters were inserted into both ureters,the common bile duct was cannulated above the gall bladder and cannulae were also placed in the femoral artery and vein.

In other experiments (see table 3.3), the above anaesthetic procedure was repeated except that both renal pedicles (i.e. renal artery, renal vein and ureter) were ligated (Group II) to prevent urine formation or the common bile duct was ligated above the cystic duct (Group III) in order to block biliary flow.

Table 3.3 ^{14}C Fazadinium disposition in dogs : Protocol

Three groups of dogs studied:-

Groups	physiological condition	Experimental procedure
I (n=5)	Renal function normal Biliary function normal	renal pedicles cannulated bile duct cannulated
II (n=5)	renal function impaired biliary function normal	renal pedicles ligated bile duct cannulated
III (n=3)	renal function normal biliary function impaired	renal pedicles cannulated bile duct ligated

Administration of ^{14}C-fazadinium

All the dogs received ^{14}C-fazadinium at a dose of 1 mg Kg^{-1} (2.5 mg; 2.5 μCi) which was injected as a bolus into the femoral vein.

Collection of blood, urine and bile

Blood samples (5 ml) were collected into heparinised tubes at 2,4,6,8,10,15,20,30 and 60 minutes and subsequently, every hour for a further five hours. Urine and bile were collected hourly for up to five hours.

Whenever blood samples were taken, the loss in body fluids was compensated for by administration of an equal volume of isotonic saline (0.9% $^{w}/_{v}$) or dextrose (5% $^{w}/_{v}$). Arterial blood gases were frequently checked to adjust ventilation and to maintain $PaCO_2$ within physiological limits (35–40 mm Hg).

Storage of blood samples

All samples of urine, bile and faeces were stored at -20°C without pH adjustment prior to analysis. Blood samples were treated immediately (see below).

Radiochemical techniques

Urine, bile and blood

Recovery of ^{14}C and ^{3}H radioactivity was determined by liquid scintillation spectrometry (Packard Tri-carb, model 3385).

Urine (0.5 ml) and bile (0.01–0.05 ml) were counted directly in duplicate, using a dioxan based scintillation cocktail (5 ml) consisting of naphthalene (60 g);

PPO (4g); POPOP (0.2g); methanol (100 ml); ethylene glycol (20 ml) made up to 1 litre with dioxan (Bray, 1961).

Blood samples were centrifuged (2,000 r.p.m; 5 min) immediately after collection. The ^{14}C content of the separated plasma (0.5 ml) was determined in duplicate using the dioxan based scintillant as above.

Vials were counted after a sufficient time had elapsed for cooling to prevent chemiluminescence. The efficiency of counting was determined by the channel ratio method (Bridges and others, 1967), using a previously established standard curve.

Faeces

Prior to faecal collection, stool cans were calibrated on the inside with a 1 litre mark using an indelible marker. After collection, the cans were stored at -20°C.

For the purpose of quantitation, cold water was added to cover the stools and then hot water (50-60°C) was added to make up to the 1 litre mark. (The effect of the hot water on the frozen stool was to fracture it and hence facilitate rapid homogenisation). The lid was replaced tightly and the can then shaken mechanically for 2-3 hours.

Counting of faeces

To an aliquot (20 ml) of the homogenate obtained after shaking (see above), was added 5M sodium hydroxide (10 ml), isooctanol (1 ml) to prevent

frothing, and hydrogen peroxide (100 vol. 25 ml) in a 250 ml conical flask. This was covered with aluminium foil and left to stand for 48 h.

After the bleaching procedure, the mixture was neutralised using concentrated hydrochloric acid (approx. 4 ml), boiled for about 30 seconds to evaporate any excess hydrogen peroxide and made up to 100 ml with water.

Aliquots (0.5 ml) were then counted in quadruplicate in the same manner as for plasma, urine and bile.

Nature of urinary and biliary radioactivity

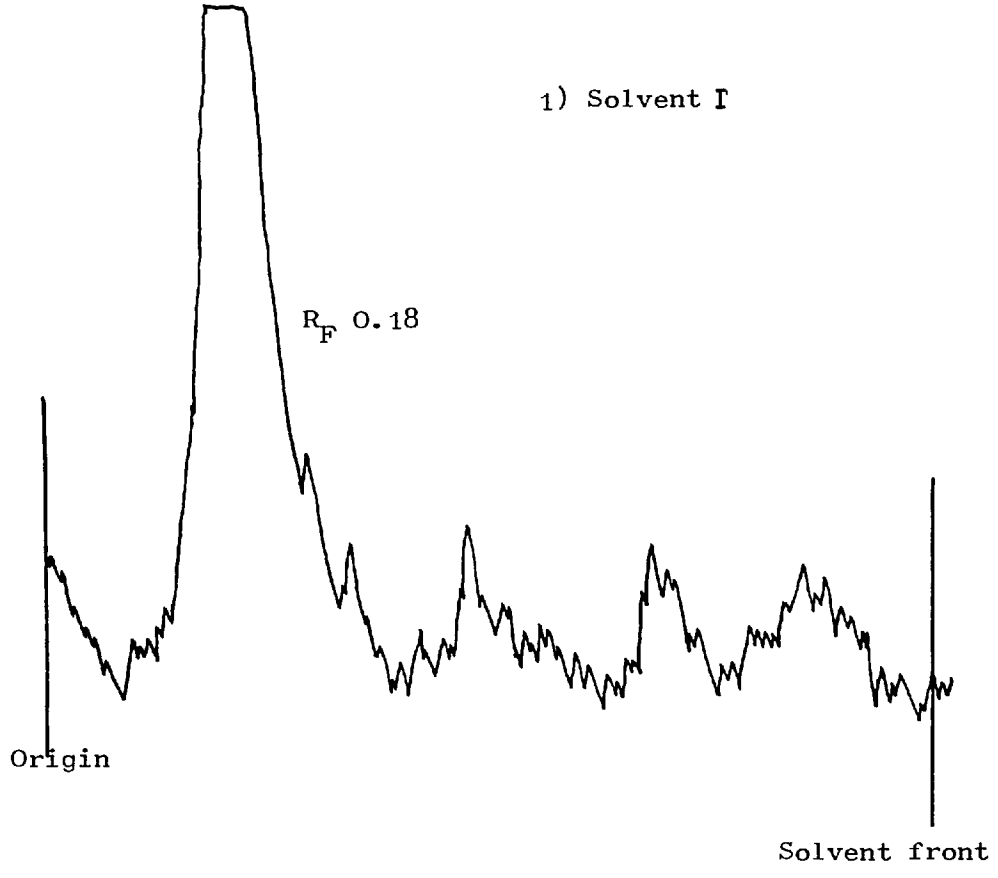
The amount of radioactivity in urine or bile as unchanged fazadinium or its metabolite (MIP) was quantitated by radiochromatography.

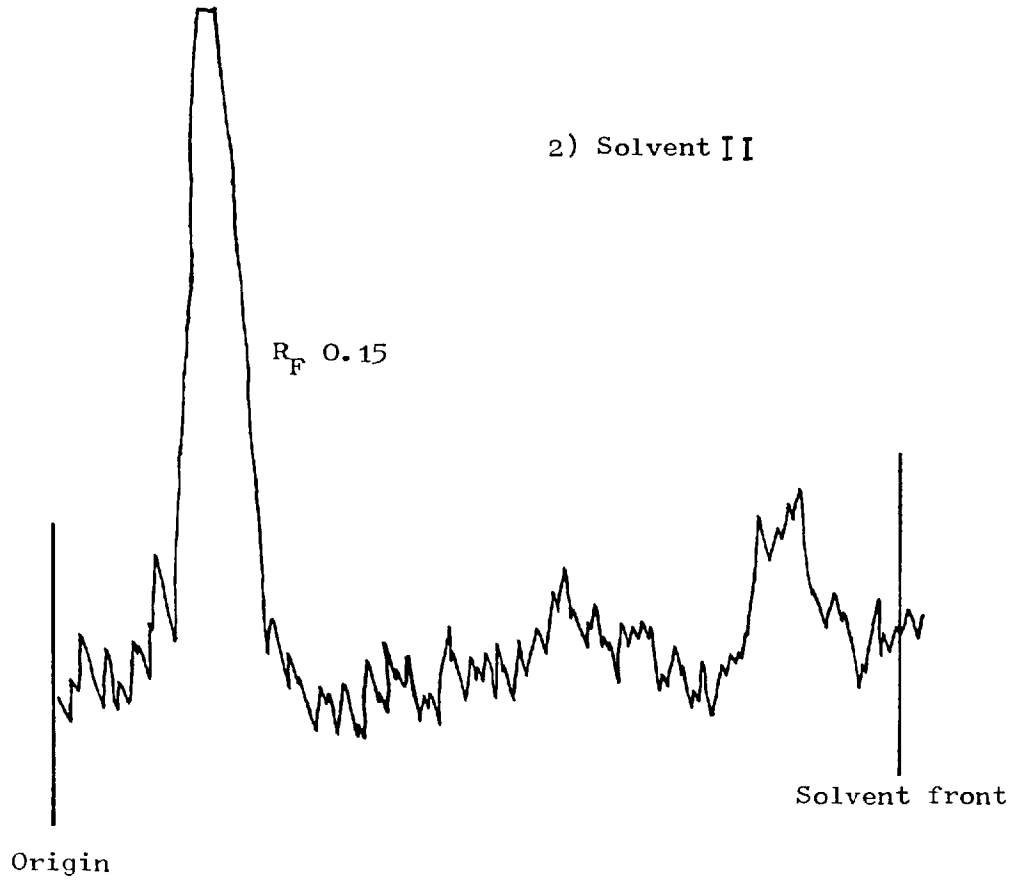
Radioactivity in urine and bile was initially concentrated using a column of Amberlite XAD-2 resin (B.D.H. Chemicals Ltd., Poole, U.K.) as described by Mule and others (1967). The methanol eluate was concentrated by rotary evaporation and examined by thin layer chromatography (see Table 3.2).

Chromatograms of the radioactive urine and bile were scanned in a Packard radiochromatogram scanner (model 7201). Fig 3.1 illustrates typical radiochromatogram scans of ^{3}H-fazadinium in the two solvent systems employed.

For quantitating the associated radioactivities, the radiochromatograms were cut laterally into small sections (1 cm wide) and placed into vials containing the dioxan scintillation cocktail.

Fig 3.1 Typical radiochromatograms of ^{3}H-fazadinium in the two solvent systems studied





Results and Discussion

Human

The dose of fazadinium bromide (0.63 mg) given to the human volunteers was well below the therapeutic range (100 times less) and no signs of neuromuscular blockade was observed. Two subjects however, complained of increased bowel motility.

Fig 3.2 illustrates the pattern of urinary excretion of ^{3}H activity after intravenous and oral administration of ^{3}H-fazadinium bromide. Until 12 h the urinary elimination was identical in both cases, but after this time, the drug was more rapidly excreted following oral administration.

The urinary (0-30 h) and faecal recoveries (0-5 day) of ^{3}H are summarised in Table 3.4. After both routes of administration, a total dose of 61%-76% of the administered radioactivity was recovered, 23% in the faeces and 38%-53% in the urine.

Assuming that the radioactivity in the faeces, after intravenous administration, is most likely to have arisen from the biliary excretion of unchanged drug or metabolites, the drug appears to be handled adequately by both routes of excretion; the ratio of the renal to hepatic elimination being 2:1. The nature of the radioactivity in the faeces or its origin was not established. Examination of the urine by thin layer chromatography showed the presence of two peaks corresponding to MIP and an unknown metabolite with chromatographic mobility between fazadinium and MIP (see Table 3.5).

Fig 3.2 Urinary excretion of ^{3}H-fazadinium in conscious human volunteers after i.v. and oral administration

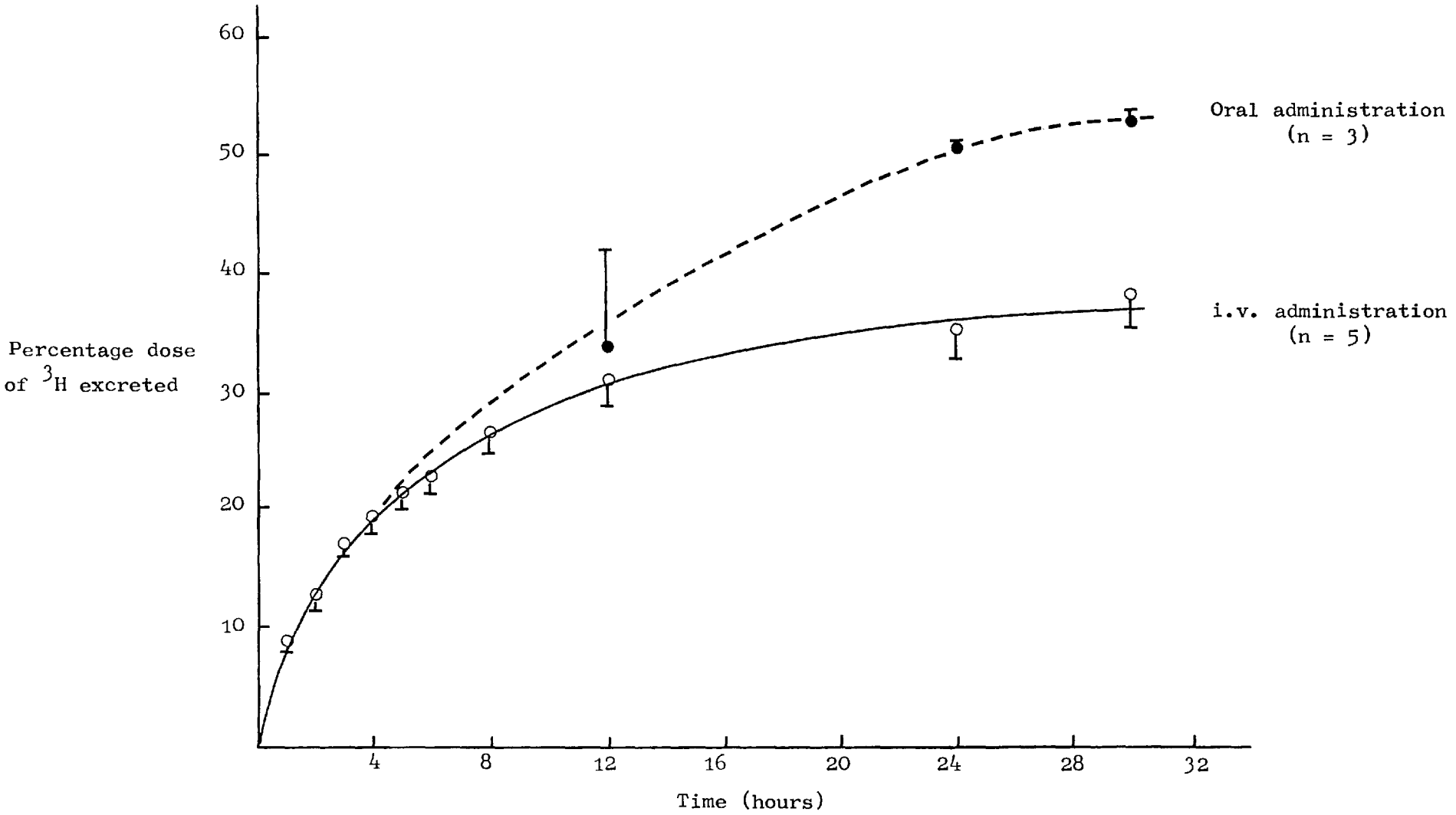


Table 3.4 Excretion of ^{3}H radioactivity by conscious human volunteers after i.v. and oral administration of ^{3}H-fazadinium bromide

Subjects	0-30h Urinary excretion	Faecal excretion (days)	Total excretion	Ratio of urinary:Faecal excretion
i.v. administration				
JCR	34.6	22.2(5)	56.8	1.6
TS	38.4	14.9(2)	53.3	2.6
SL	31.6	16.3(5)	47.9	1.9
TM	37.5	25.6(5)	63.1	1.5
Mean	*35.5	23.2	61.2	1.8
Oral administration				
JCR	52.8	24.3(4)	77.1	2.2
TS	52.9	20.1(4)	73.0	2.6
JDS	54.2	24.1(4)	78.0	2.3
Mean	*53.3	22.8	76.1	2.4

All subjects received 0.63mg (μCi) ^{3}H-fazadinium bromide

* $p < 0.001$

All others not significant

Table 3.5 Metabolites in Human Urine after ^{3}H fazadinium

Oral administration	Fazadinium	*Unknown Metabolite	MIP
JD'S	19.9	40.9	39.2
JCR	17.9	51.3	30.8
TS	32.8	59.5	7.7
i.v. administration			
JD'S	-	69.5	30.5
JCR	-	15.7	84.3
TS	20.8	30.8	48.4

* Rf = 0.48 in solvent II (see below)

Solvent II : n-propanol : 20% NH_4Cl; upper phase

These two metabolites accounted for most of the radioactivity on the plate; little or no fazadinium was present in the urines of these patients.

Significant amounts of ^{3}H-fazadinium are absorbed from the gastrointestinal tract since after oral dosage, some 52%-54% of the ^{3}H is excreted in the urine. Chromatographic analysis of the urines showed that all three compounds viz unchanged fazadinium, MIP and the unknown metabolite were present (the nature of the unknown metabolite was not further investigated.)

It is interesting to consider the present results in the light of the work of Blogg and others (1973). They found that 70-80% of an intravenous dose of ^{3}H-fazadinium (0.5-0.75 mg Kg^{-1}) was excreted in the urine of surgical patients (n=7, undergoing peripheral vascular surgery and termination hysterectomy), with only 3% in the faeces. The results in this study showed that 36% and 23% of an i.v. dose of ^{3}H-fazadinium (0.63 mg; approx. 0.01 mg Kg^{-1}) were eliminated in the urine and faeces, respectively. The results of the two studies are therefore not consistent. The only differences between them are those of dose and nature of subjects, but these are probably not sufficient to explain the large discrepancies.

Furthermore, after examination of the urines by high voltage paper electrophoresis (HVPE), Blogg and others (1973b) showed the presence of three peaks corresponding to Fazadinium, MIP and a "neutral" metabolite which did not migrate from the anode during HVPE. Chromatographic analysis of the urines in this study also indicated the presence of three peaks

which corresponded to Fazadinium, MIP and an unknown metabolite. In this respect, the two studies may be compatible.

Dog

The excretion pattern of ^{14}C-fazadinium in the three groups of dogs studied is shown on Table 3.6 and represented graphically on Fig 3.3.

In the control dogs some 66% of the dose was excreted in 5 h, 19% in the urine and 47% in the bile. Ligation of the renal pedicles did not increase biliary excretion of ^{14}C-fazadinium, and similarly there was no significant increase in urinary excretion when the common bile duct was ligated. In both groups, the total excretion of the drug was less than that of the control dogs.

Biliary excretion is the predominant route of drug elimination in the dog and obstructing this route by ligation of the common bile duct results in a significant decrease in the total excretion of fazadinium (Table 3.6).

Plasma ^{3}H radioactivity recovery profiles are shown in Fig 3.4. A typical biphasic pattern was observed for the distribution and elimination of fazadinium. The bold line is a line of best fit drawn through the data points, assuming a two compartment open model (see Appendix I). This regression line was drawn by the non-linear least squares method using a digital computer with the E04FBF programme (see Materials and Methods Chapter 4).

Table 3.6 Urinary and biliary excretion of ^{14}C in dogs after i.v. administration of ^{14}C-fazadinium bromide

	% Dose ^{14}C (range) excreted in 5h			
	Urine	Bile	Total	
I Control Group (n=4)	18.6(9-27)	47.1(36-53)	65.7(62-74)	
II Ligated renal pedicles Group (n=5)	-	52.8(45-62)	52.8(45-62)	*
III Ligated common bile duct Group (n=3)	21.7(17-32)	-	21.7(17-32)	**

* $p < 0.025$ of control

** $p < 0.005$ of control

All others not significant

Fig 3.3 Urinary and Biliary excretion of ^{14}C-fazadinium in Dogs

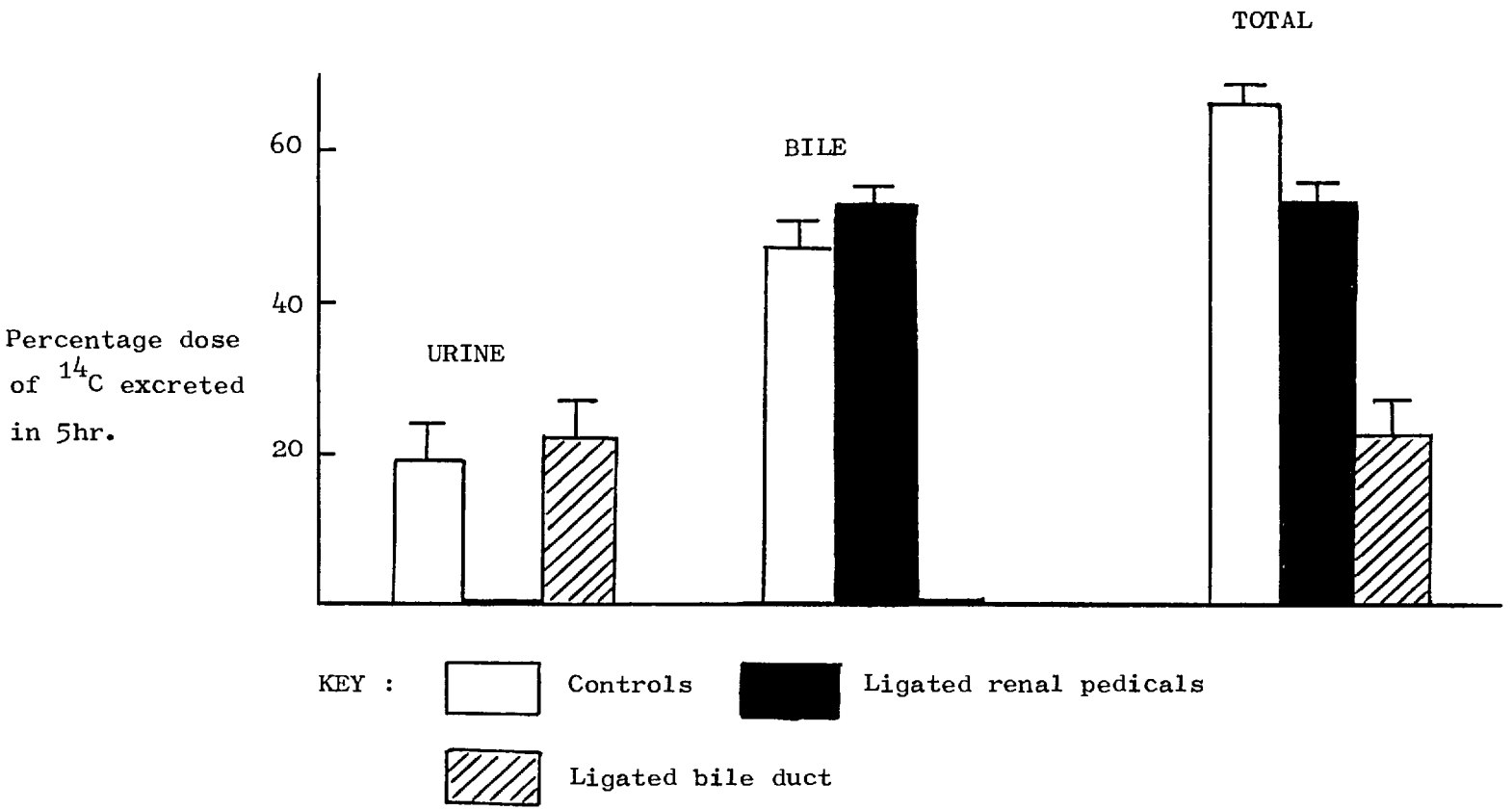
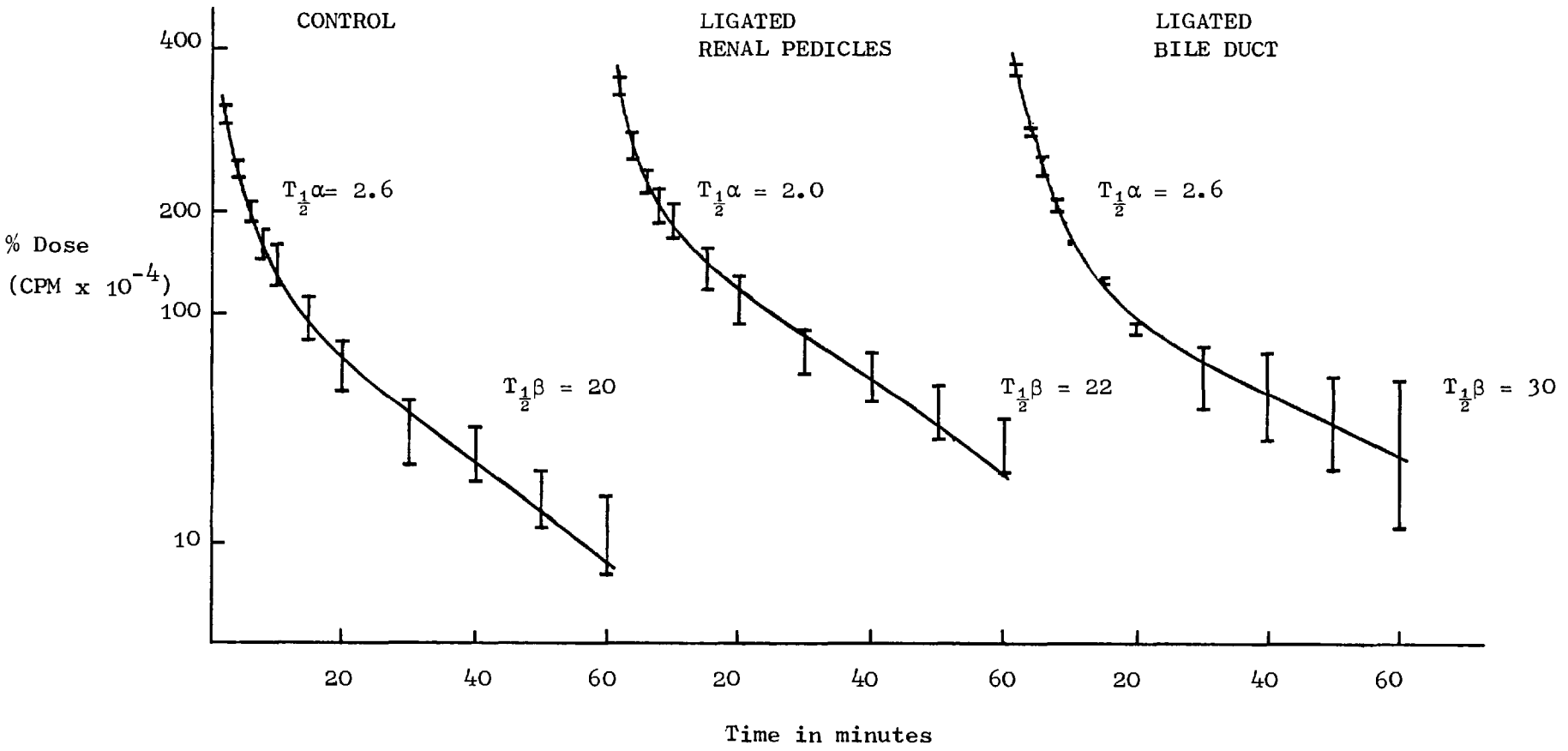


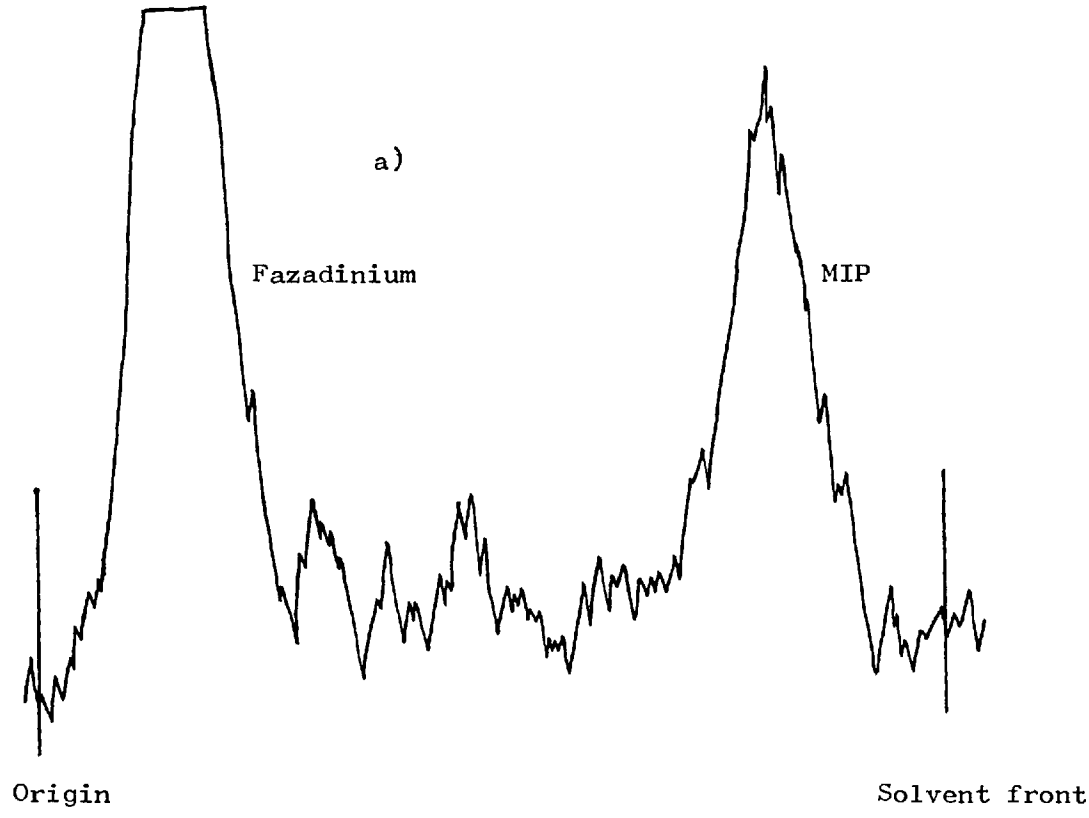
Fig 3.4 Semi-logarithmic plot of ^{14}C in plasma (± SEM)



The initial rate of decline of plasma ^{3}H is very similar in all the three groups ($T_{\frac{1}{2}}\alpha$ range 2.0-2.6 min) suggesting that the distribution of fazadinium is unaffected by the surgical procedures. There were differences in the plasma elimination half-lives ($T_{\frac{1}{2}}\beta$) which were 20 min (control group), 22 min (renal pedicle ligated group) and 30 min (bile duct ligated group). Since biliary excretion is an important route for the elimination of fazadinium, the increase in the latter group can be attributed to decreased drug clearance due to prevention of biliary excretion.

Examination of the urine and bile by t.l.c. using two solvent systems (see table 3.2) indicate the presence of only two peaks corresponding to unchanged fazadinium and MIP (see Fig 3.5). Results obtained from the three groups of dogs are summarised on Fig 3.6. The major excretion product appears to be unchanged fazadinium. In the control dogs the proportion of fazadinium to MIP was roughly in the ratio 2:1 (see Table 3.7); there being slightly more of the metabolite in the bile than in the urine. The bile of renal pedicle ligated dogs contained approximately equal amounts of these two compounds, and the urine of the ligated bile duct dogs contained a large proportion of unchanged fazadinium (ratio 6:1).

Fig 3.5 Typical radiochromatogram scans of (a) urine, and (b) bile in the dog after a bolus injection of ^{14}C-fazadinium



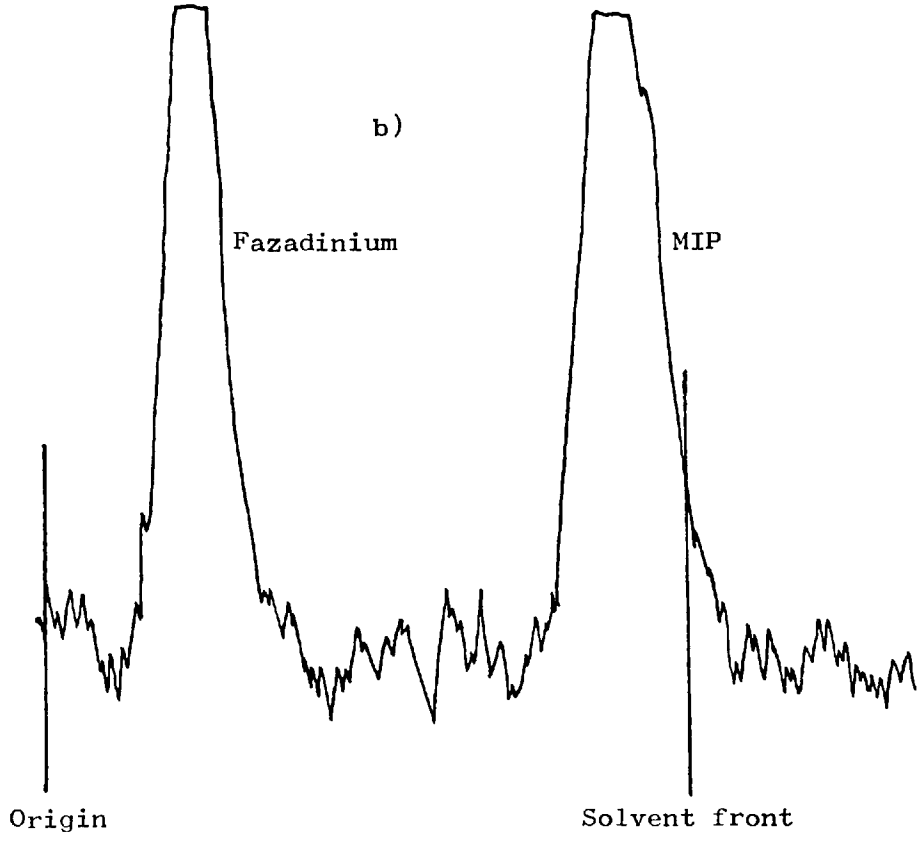


Fig 3.6 Metabolites in the urine and bile of dogs [1st hr]

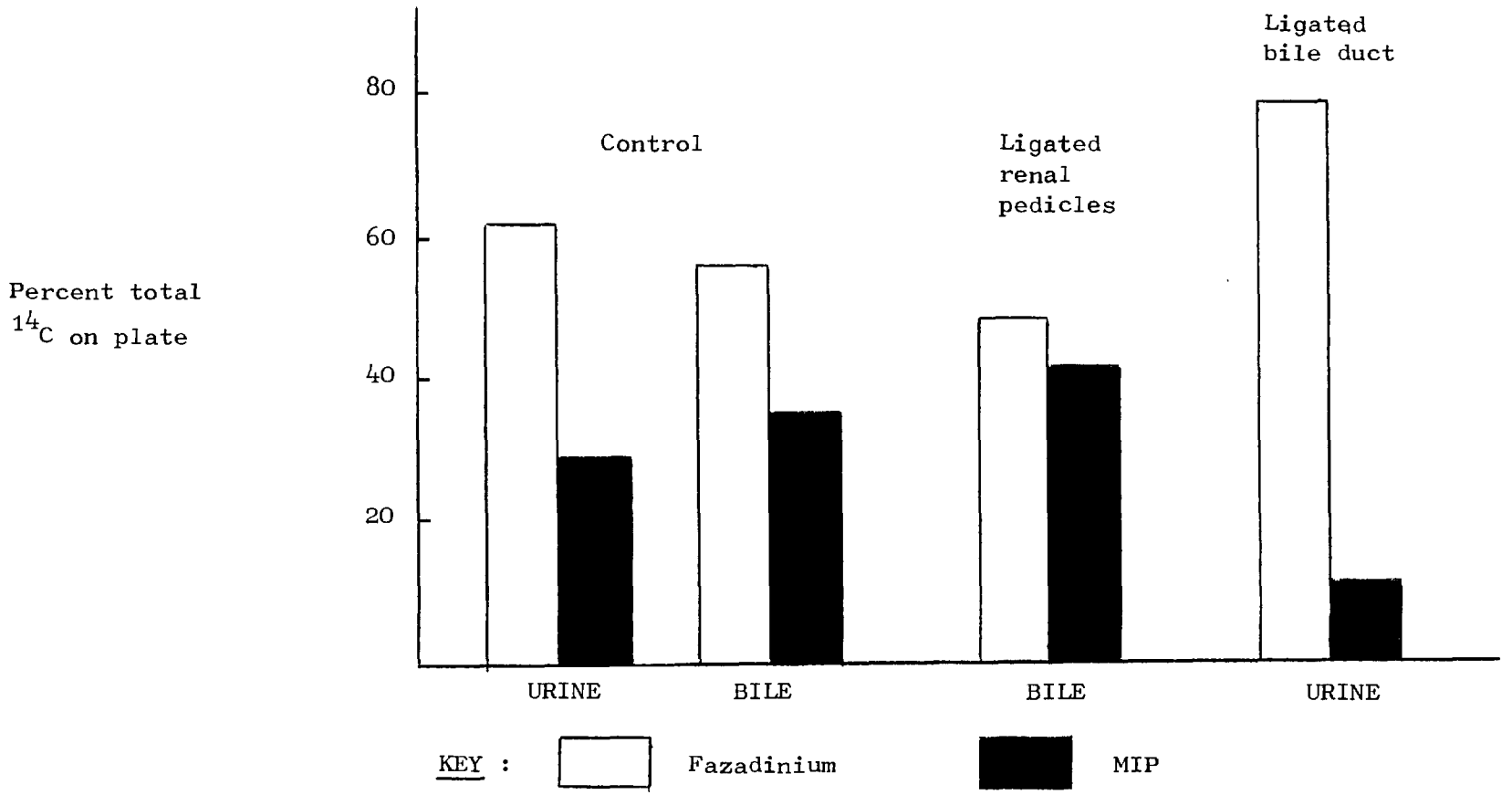


Table 3.7 Chromatographic quantitation of fazadinium and MIP in the urine and bile of dogs dosed with ^{14}C-fazadinium

	Intact		Anephric	Ligated bile duct
% Dose eliminated as:	urine	bile	bile	urine
Fazadinium	68(46-85)	62(46-81)	55(35-72)	85(71-92)
MIP	32(16-54)	38(19-54)	46(28-65)	15 (8-29)
Ratio: Fazadinium/MIP	2.1	1.6	1.2	5.7

Summary and Conclusions

(A) Results from the human study showed that both renal and hepatic routes of excretion are relatively important in the elimination of fazadinium. The faecal excretion, presumably reflecting biliary excretion, contributing to approximately one third the total elimination.

(B) Data from the dog study indicate:

(1) biliary excretion is the main pathway of elimination of fazadinium, the ratio of the hepatic to renal excretion being approximately 3:1

(2) the two pathways are not complementary since one pathway does not compensate when the other is occluded.

(3) Ligation of the bile duct results in a significant decrease in the total elimination of fazadinium, with a prolongation of the elimination half life.

(4) ligation of the renal pedicles does not appreciably alter the disposition of fazadinium as seen by the plasma levels, distribution and elimination half-lives and the excretory pattern, none of which differed significantly from controls.

The data suggest that the disposition of fazadinium in man should be relatively unaffected by impaired renal function as occurs with (+)-tubocurarine (Miller and others, 1977) and pancuronium (Mcleod and others, 1976). Hence from the drug disposition viewpoint, fazadinium could have advantages for patients with impaired renal function. This possibility is further investigated in chapter four.

CHAPTER FOUR

Clinical Pharmacokinetics of Fazadinium bromide in patients with normal renal function and those with renal failure

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Introduction

The lack of a sensitive method for the analysis of neuromuscular blocking drugs in biological fluids, has made it difficult to establish a relationship between the plasma levels of these agents and their clinical effect. Following the development of a specific radioimmunoassay method for (+)-tubocurarine (Horowitz and Spector, 1973), a highly significant correlation was demonstrated between the serum concentration of (+)-tubocurarine and the intensity of its neuromuscular blockade in man (Matteo and others, 1974).

It has since been generally assumed, that the intensity of neuromuscular blockade of competitive muscle relaxants depend on drug concentration at cholinergic receptors, and that this concentration, in turn, is closely related to the plasma levels of these compounds.

Feldman (1975) however, proposed an alternative mechanism on the basis of observations made with (+)-tubocurarine (Feldman and Tyrrell,1970). When this drug was given intravenously into an arm, isolated from the general circulation by a tourniquet, they found that neuromuscular blockade took as long as fifteen minutes to disappear, and suggested that the duration of neuromuscular block depends solely on the rate of dissociation of the relaxant-receptor complex. Later, Waud (1975) attempted to rationalise both Matteo's and Feldman's observations on the basis of the conventional views of neuromuscular physiology and pharmacology.

Direct correlations between plasma levels and neuromuscular blockade in anaesthetised patients have been recently demonstrated for pancuronium (Agoston and others, 1977) However, using a continuous infusion of pancuronium to establish a constant plasma level, during which a steady-state exists between the concentration of the drug in plasma and at the receptors, Shanks and others (1978) concluded that both plasma levels and relaxant-receptor mechanisms can function as the rate-determining factor in the termination of action of non-depolarising muscle relaxants. They suggested that when plasma levels fall rapidly as in Feldman and Tyrell (1970), the rate of dissociation of the relaxant-receptor complex is important, but when drug concentrations in plasma decline more slowly, as in Matteo and others (1974) the rate of decrease of the drug in plasma becomes predominant.

Plasma levels of drugs depend on a variety of factors such as distribution, metabolism and excretion. Identification of the rate-limiting step for the termination of action of neuromuscular blocking drugs has important implications, particularly with regard to the influence of impaired renal function. For instance, in patients with renal failure the neuromuscular blocking effect of gallamine (Lowenstein and others, 1970; Singer and others, 1971; Anand and others, 1972), (+)-tubocurarine (Logan and others, 1974; Miller and Cullen, 1976) and pancuronium (Abrams and others, 1975; Somogyi and others, 1977; Geha and others, 1976) are prolonged because elimination by the kidney plays a major role in the excretion of these drugs.

Clinical evaluation of fazadinium bromide has demonstrated a rapid onset of action, comparable to suxamethonium and having a shorter action than either (+)-tubocurarine or pancuronium. (Blogg and others,1973a; Brittain and Tyers, 1973). There has been no pharmacokinetic studies reported in the literature on the use of fazadinium in patients with impaired renal function. Previous conclusions from the present study (see chapter three) suggests that from the drug-disposition viewpoint, fazadinium could have advantages over other muscle relaxants, in patients with renal failure.

The purpose of this study was to determine whether the plasma concentration of fazadinium is in fact related to the degree of neuromuscular blockade in man, and to assess simultaneously, both neuromusculor blockade and pharmacokinetics of fazadinium in patients with normal renal function compared to those with renal failure.

Materials and Methods

Fazadinium

Fazadinium bromide (Fazadon^{R}) was purchased from Duncan, Flockhart & Co. Ltd., London E2, in 5 ml ampoules. Each ampoule contained 75 mg fazadinium made isotonic by inclusion of sodium chloride and stablised with α-thioglycerol (0.3% $^{w}/_{v}$). The ampoules were stored in the dark below 20^{o}C.

Patients

Sixteen patients who had been admitted to St. Mary's Hospital, London W2, were included in the study. All had previously given their informed consent and none of these patients showed any biochemical evidence of neuromuscular disease.

The patients were divided into three groups according to the dose and type of surgery. Groups I (2M, 2F; ages 27-73 yrs; wt 50-97 Kg) and II (2M, 3F; ages 52-74 yrs; wt 53-80 Kg) were those patients undergoing a variety of surgical procedures but free of renal and hepatic function. These patients received fazadinium intravenously, at a dose of 0.5 mg Kg^{-1} (Group I) or 1 mg Kg^{-1} (Group II), according to the anticipated duration of surgery (see Table 4.1). Group III (7M; ages 24-38 yrs; wt 47-80 Kg) were patients with renal failure about to undergo renal transplantation (see Table 4.2). The participants in this group all had haemodialysis within 48h of surgery and none of these patients showed any biochemical evidence of hepatic dysfunction. All patients in this group received fazadinium intravenously, at a dose of 1 mg $Kg.^{-1}$

Anaesthetic technique

A standard anaesthetic technique was employed for all the patients studied. Premedication was achieved by intramuscular injections of papaveretum (Omnopon^{R}; Roche Products Ltd., Herts, U.K. , 10-20 mg) and atropine sulphate injection B.P. (Antigen Ltd., Roscrea, Ireland; 0.6 mg) at approximately one hour before surgery.

Anaesthesia was induced by intravenous injections of droperidol (Droleptan^{R}; 10 mg), fentanyl (Sublimaze^{R}, 0.5-1 mg) both of which were purchased from Jansen Pharmaceuticals Ltd., Marlow, Bucks., U.K., and thiopentone (Intraval^{R}; May and Baker Ltd., Dagenham, Essex, U.K., 200 mg). Anaesthesia was maintained with nitrous oxide (70%) in oxygen and with repeated doses of fentanyl.

Table 4.1 Details of patients with normal renal function

Group I : Fazadinium 0.5 mg Kg^{-1}

Sex	Age	Wt(Kg)	Operation
M	27	97	Inguinal hernia
M	47	78	Varicose veins
F	49	50	Mastectomy
F	73	49	Mastectomy

Group II : Fazadinium 1 mg Kg^{-1}

Sex	Age	Wt(Kg)	Operation
F	52	53	Partial gastrectomy
M	61	80	Hemicolectomy
M	68	60	Laparotomy
F	69	68	Melanoma excision
F	74	70	Repair of rectal prolaps

Table 4.2 Details of surgical patients with end stage renal failure

Group III : Fazadinium 1 mg Kg^{-1}

Sex	Age	Wt(kg)	Operation
M	24	80	Renal transplantation
M	26	55	Renal transplantation
M	28	50	Renal transplantation
M	28	47	Renal transplantation
M	29	62	Renal transplantation
M	31	64	Renal transplantation
M	38	61	Renal transplantation

Endotracheal intubation was performed without the use of muscle relaxants. Respiratory ventilation was controlled using a Manley ventilator and the minute volume was adjusted to maintain end-tidal $PaCO_2$ between 4.5% and 5% using a Godart capnograph.

Measurement of neuromuscular function

Neuromuscular function was monitored by the train-of-four technique of Ali and others (1973),the ulnar nerve was stimulated supramaximally by means of 25-gauge needle electrodes placed subcutaneously at the elbow. Train-of-four square wave pulses (0.2 msec duration) were delivered at a frequency of 2Hz; each train repeated every ten seconds. Stimuli were delivered through a stimulus isolation unit (Grass S1U5) from a Grass stimulator (model S48).

The hand and forearm were immobilised in a splint and the force of contraction of the adductor pollicis was measured using a force displacement transducer (Grass FT10), the resulting isometric tension recorded on a chart recorder (Washington instruments).

Administration of fazadinium

After allowing for stabilisation of the train-of-four recording (15-20 min) all the patients under investigation received fazadinium intravenously to the left antecubital fossa by bolus injection through an indwelling cannula.

Blood sampling

Venous blood samples were collected into heparinized tubes from all patients. Initially, a blood sample (25 ml) was taken immediately prior to the administration of fazadinium and this was used as a blank for subsequent analysis, and to establish a calibration curve.

Following administration of the neuromuscular blocker, further blood samples (5 ml) were taken at regular intervals (2,4,6,8,10,15,23,30 and 60 min) and then at times to approximately 10% increments in the return of twitch tension compared to control valves.

Storage of blood samples

Immediately after all the blood samples were taken, whole blood was centrifuged and the separated plasma was removed and stored at -20^{o}C until assayed for fazadinium.

Reversal from neuromuscular blockade

At the termination of surgery all patients were given atropine (1.2 mg) and neostigmine (2.5 mg) intravenously, to reverse the effects of fazadinium, after which no further blood samples were collected.

Patients who had undergone renal transplantation (Group III) were returned to an intensive care area where respiratory function was monitored closely.

Fazadinium assay

For the estimation of fazadinium in plasma, the ^{125}I-Rose Bengal radiometric method was used. Details of this analytical procedure have been given in Chapter Two.

Computer Analysis

Plasma concentration-time data were analysed assuming a two compartment open model (Riegelman and others, 1968). With such a model, the equation relating the plasma concentration (C) to time (t) after a bolus intravenous injection is represented by the following equation:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$ (see Appendix I)

where A and B are the zero-time intercepts of the two components of the biexponential decay and α and β are the slopes of these lines.

The data were processed with a digital computer using the EO4FBF routine (Nottingham Algorithms Group, NAGF library), which obtains least squares fit to a non-linear function. The routine is similar to that applied by Powell (1968). The method is iterative and thus requires an initial estimate of the position of a solution. The initial estimates of A, α, B and β were obtained from log plasma concentration-time plots by the classical feathering method (see Appendix II).

Dimensions of the compartments, rate constants and other parameters of the model were calculated from A, α, B and β using the equations proposed by Greenblatt and Koch-Weser (1975).

Attempts were also made to fit the data to a three compartment open model using the above programme.

Results

Neuromuscular transmission

In patients with normal renal function, complete abolition of twitch height occurred after both low (0.5 mg Kg^{-1}) and high (1 mg Kg^{-1}) doses of fazadinium. Data on the recovery of response to the first stimulus in the train-of-four, expressed as a percentage of the initial pre-relaxant twitch height (I/C) is shown in Table 4.3. The time between drug administration and each point of recovery (5% to 50%) was significantly less ($p < 0.01$) after the smaller dose of fazadinium (see Fig. 4.1). Once started, the rate of recovery was more rapid in the smaller dose group, for instance, the mean recovery time between points from 10% to 50% were 18 min. and 47 min. after the low and high doses respectively.

Complete abolition of twitch height was also observed in patients with renal failure after 1 mg Kg^{-1} fazadinium. Table 4.4 shows the I/C recovery values obtained in these patients compared to values obtained after the same dose of fazadinium in patients with normal renal function (control group). Both groups showed a similar pattern of recovery (Fig 4.2) and there was no significant difference at any of the recovery points (5% - 50%), however, values obtained from the renal failure group were more widely scattered. For example, the times to 10% recovery ranged from 65-141 min in the control group, whilst in the renal failure group they varied from

Table 4.3 Spontaneous recovery of single twitch (I/C) following i.v. administration of fazadinium at two dose levels in patients with normal renal function

Groups	n	Recovery time in minutes					
		5%	10%	20%	30%	40%	50%
I ($0.5mg\ Kg^{-1}$)	4	24.3 ±3.2	26.6 ±3.3	31.2 ±3.0	35.8 ±1.8	39.8 ±2.4	44.3 ±1.8
II ($1\ mg\ Kg^{-1}$)	5	75.4 ±14.1	90.1 ±13.6	111.1 ±16.5	128.8 ±26.4	130.8 ±26.4	137.2 ±26.2

Values are mean ± S.E.

Recovery times were significantly longer in Group II patients ($2p < 0.02$)

Fig 4.1 Mean recovery (min ± SEM) in response of first stimulus of the train-of-four as a percentage of initial twitch height after 0.5 mg Kg^{-1} and 1 mg Kg^{-1} fazadinium in patients with normal renal function

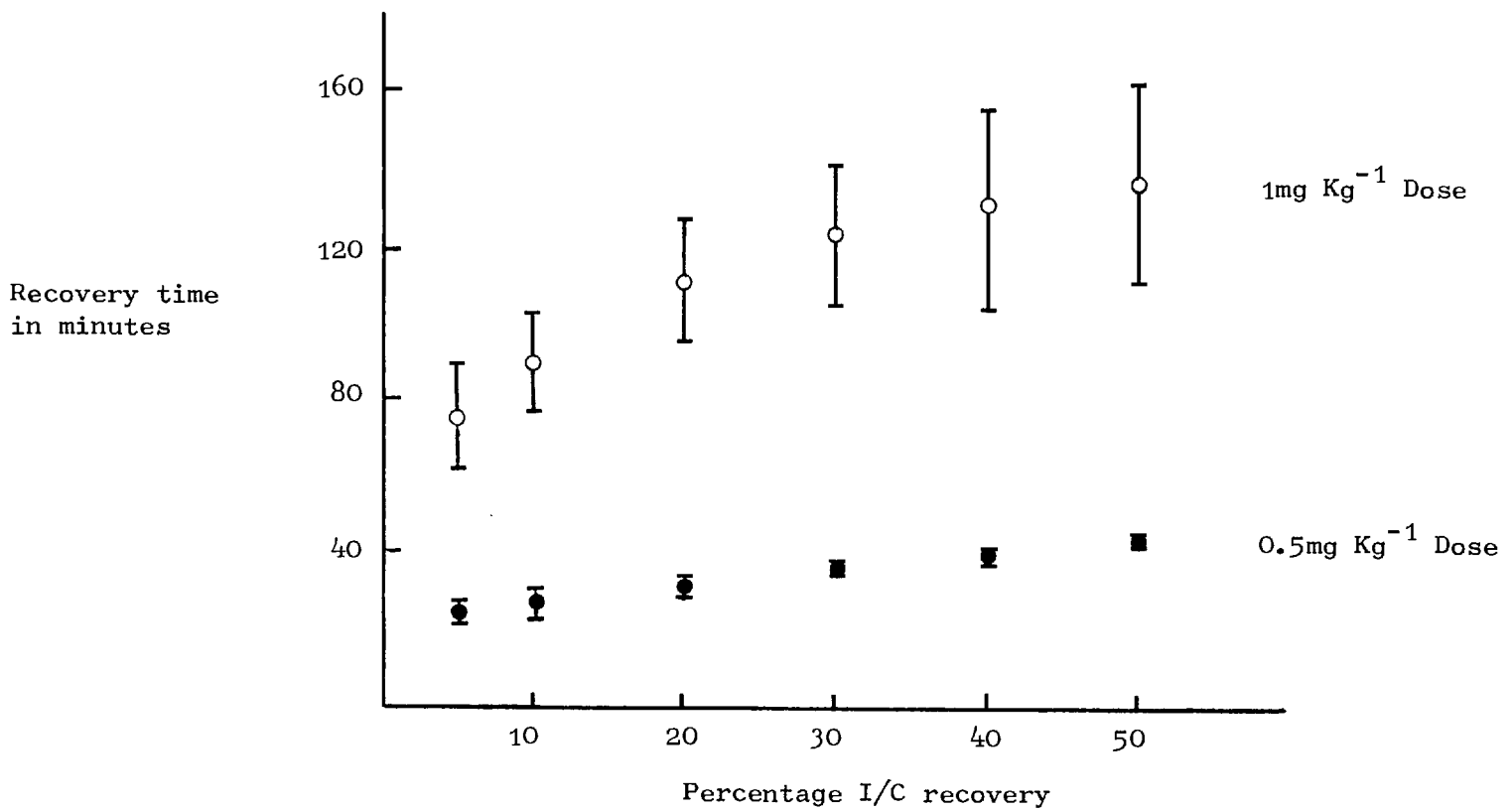
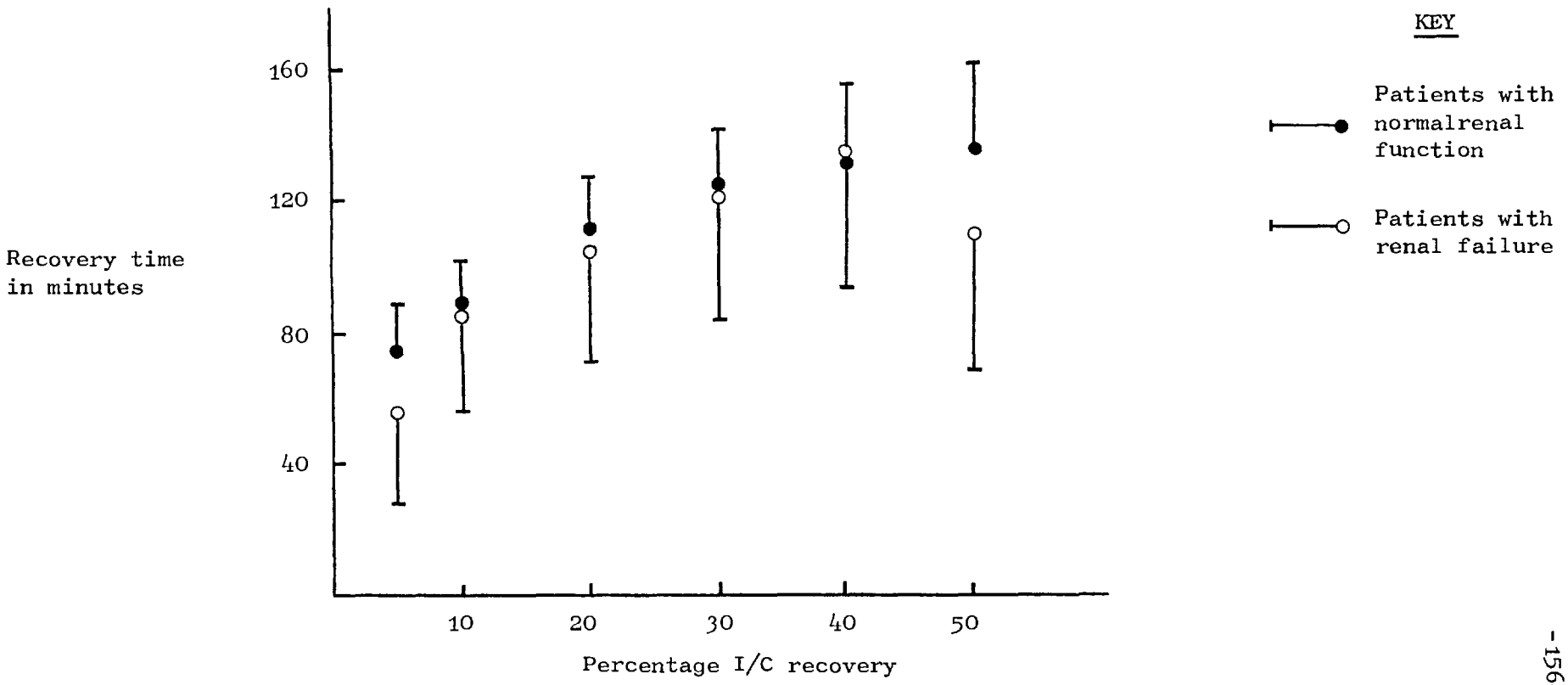


Table 4.4 Spontaneous recovery of a single twitch (I/C) following i.v. administration of fazadinium (1 mg Kg^{-1}) in patients with and without renal failure

Groups	n	Recovery time in minutes					
		5%	10%	20%	30%	40%	50%
Patients with normal renal function (Group II)	5	75.4 ±14.1	90.1 ±13.6	111.0 ±16.5	123.8 ±18.9	130.8 ±26.4	137.2 ±26.2
Patients with renal failure (Group III)	7	57.3 ±29.4	86.3 ±29.9	105.1 ±34.8	121.6 ±38.3	134.8 ±42.4	118.3 ±50.8

Values are mean ± S.E.

Fig 4.2 Mean recovery (min ± SEM) in response to the first stimulus of the train-of-four as a percentage of initial twitch height after 1 mg Kg^{-1} fazadinium in patients with and without renal failure



20 - 137 min. Moreover, the longest time to 10% recovery, 141 min occurred in the control group and the shortest time, 20 min, in the renal failure group.

Recovery of the train-of-four (T_4), the height of the fourth evoked twitch expressed as a percentage of the first twitch in the train, indicated a similar pattern as I/C recovery in all the three groups of patients. Assessment of neuromuscular function by this method also demonstrated complete reversal of neuromuscular blockade after neostigmine and atropine. In no cases was there any evidence of residual paralysis (curarisation) of neuromuscular block by fazadinium or prolongation of effect after surgery. Furthermore, at the end of operations, all the patients were able to breathe adequately without ventilatory support in the recovery room.

Pharmacokinetics

The disappearance of fazadinium in plasma exhibited a biexponential decay pattern, and this was analysed assuming a two compartment open model. A representative plasma concentration-time curve for fazadinium in man is shown in Fig 4.3. The plasma concentration of fazadinium in this patient declined rapidly ($T\frac{1}{2}\alpha$ = 2.4 min) over a period of above 10 min due to distribution of the drug, followed by a slower elimination phase ($T\frac{1}{2}\beta$ = 70 min).

Plasma concentration-time data were also analysed assuming the three compartment open model; the goodness of fit as indicated by the sum of the squared residuals, after computer analysis is shown in Table 4.5

Fig 4.3 A typical log-plasma concentration-time curve of fazadinium (1mg Kg^{-1}) in a patient with normal renal function

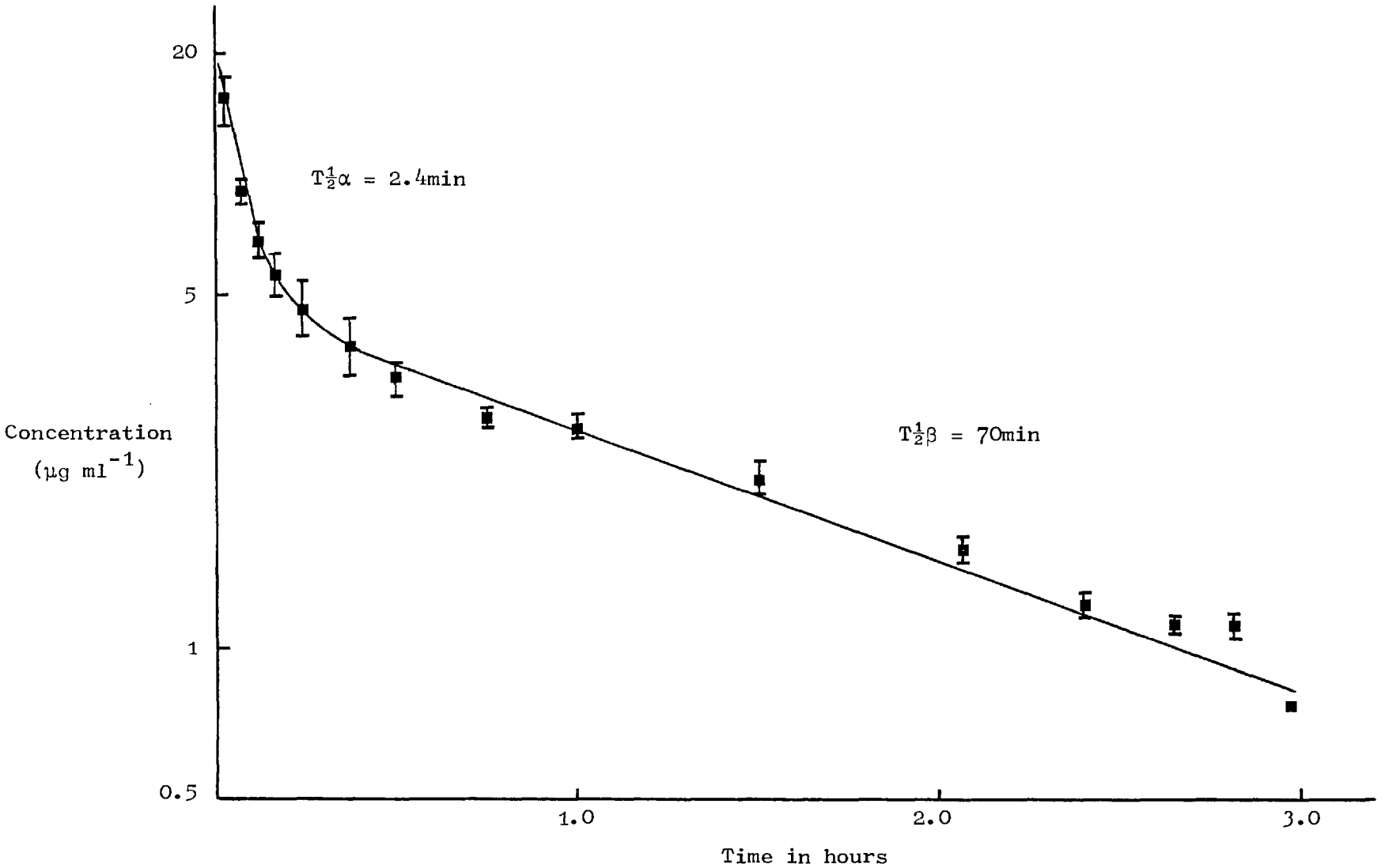


Table 4.5 Comparison of two and three compartment open models to explain the pharmacokinetics of fazadinium in man

Subjects *	No of data points	Sum of squared residuals	
		2 compartment model	3 compartment model
Group I (0.5 mg kg^{-1})	16	0.52	0.21
	15	1.05	0.64
	14	0.69	0.84
	11	0.71	No fit
	16	0.97	0.41
Group II (1 mg Kg^{-1})	14	1.04	No fit
	10	0.97	No fit
	12	0.95	0.41
	12	0.14	No fit

* All subjects had normal renal and hepatic function

In four patients there was a slight improvement of the goodness of fit using the three compartment model, but in one case the two compartment model was better, and in all others, the data could not be fitted satisfactorily to the three compartment model. Hence the simplest model consistent with the plasma data to describe the pharmacokinetic behaviour of fazadinium in man, is the two compartment open model. Pharmacokinetic parameters derived for fazadinium in all the three groups of patients using this model are shown in Table 4.6.

In patients with normal renal function, the mean peak plasma concentration (Co) at time t=0, was 34 $\mu g\ ml^{-1}$ and 95 $\mu g\ ml^{-1}$ after administration of fazadinium at dose levels of 0.5 mg Kg^{-1} and 1 mg Kg^{-1} respectively. The area under the plasma concentration-time curve (AUC) were significantly different ($p < 0.05$), as expected after the two dose levels, but all other pharmacokinetic parameters remained unaltered by the change of dose.

The pharmacokinetic parameters of fazadinium in patients with renal failure were also not significantly different from controls. In these patients the mean values for the elimination half-life ($T\frac{1}{2}\beta$) was longer, the apparent volume of distribution (Vd) was larger, the elimination rate constant (Kel) was lower, and the plasma clearance (Cp) was more rapid than the control group, however, the values did not achieve statistical significance. Thus it appears from these results, that the pharmacokinetic behaviour of fazadinium in man is unaltered by renal failure.

Table 4.6 Pharmacokinetics of fazadinium in patients with and without renal failure (mean ± S.E.)

	Patients with normal renal function		Patients with renal failure
Parameters	0.5 mg Kg^{-1}	1 mg Kg^{-1}	1 mg Kg^{-1}
A ($\mu g\ ml^{-1}$)	31.1 ± 8.9	92.0 ± 65.0	33.2 ± 13.7
α (hr^{-1})	52.8 ± 18	30.6 ± 6.6	23.2 ± 3.7
$T_{\frac{1}{2}}\alpha$ (min)	1.2 ± 0.5	1.6 ± 0.3	2.3 ± 0.5
B ($\mu g\ ml^{-1}$)	3.3 ± 0.9	5.3 ± 1.7	4.4 ± 3.6
β (hr^{-1})	1.7 ± 0.4	0.9 ± 0.1	0.7 ± 0.1
$T_{\frac{1}{2}}\beta$ (min)	30.2 ± 7.2	50.1 ± 6.4	80.2 ±29.5
V_1 ($L\ Kg^{-1}$)	0.024 ± 0.012	0.041 ± 0.014	0.066 ± 0.023
V_2 ($L\ Kg^{-1}$)	0.096 ± 0.031	0.143 ± 0.052	0.215 ± 0.063
Vd ($L\ Kg^{-1}$)	0.157 ± 0.057	0.225 ± 0.076	0.341 ± 0.100
K_{12}(h^{-1})	35.2 ± 12.8	17.3 ± 2.1	14.7 ± 2.4
K_{21}(h^{-1})	6.75± 4.25	4.25± 0.75	3.78 ± 0.66
Kel (h^{-1})	12.7 ± 3.3	9.8 ± 5.3	5.4 ± 1.5
C_p ($ml\ min^{-1}$)	221 ± 18	185 ± 49	220 ± 65
AUC ($\mu g\ ml^{-1}\ min^{-1}$)	155 ± 22	470 ± 113	466 ± 112

Plasma levels

Mean plasma fazadinium concentrations in the three groups of patients studied during 60 min post-administration of the relaxant is shown in Table 4.7 and respective log concentration-time profiles are illustrated in Fig. 4.4. Although in general, the plasma levels after 0.5mg kg^{-1} dose were lower than after 1 mg Kg^{-1}, they did not achieve statistical significance except at 8 min post-injection ($p < 0.05$). The plasma levels of patients with renal failure were similar to those patients with normal renal function.

Dose-response relationship

Following the construction of log plasma concentration-time plots for individual patients, plasma fazadinium concentrations at 10% increments in the return of twitch tension were obtained by interpolation.

Data for patients with normal renal function are shown in Table 4.8 Fazadinium concentrations after the low and high doses were similar, and in both instances, a steep dose-response relationship for fazadinium was observed (Fig. 4.5). Combining the values obtained for both groups of patients with normal renal function, the mean plasma concentration at 10% and 50% recovery (i.e. 90% and 50% paralysis) were 1.39 $\mu g\ ml^{-1}$ and 0.87 $\mu g\ ml^{-1}$ respectively, thus indicating the steepness of the dose-response curve.

Mean fazadinium concentrations from both control groups (I and II) were compared with corresponding values obtained from patients with renal failure (Table 4.9). The relationship between plasma

Table 4.7 Plasma concentrations ($\mu g\ ml^{-1}$) during various time intervals after i.v. administration of fazadinium in the three groups of patients studied

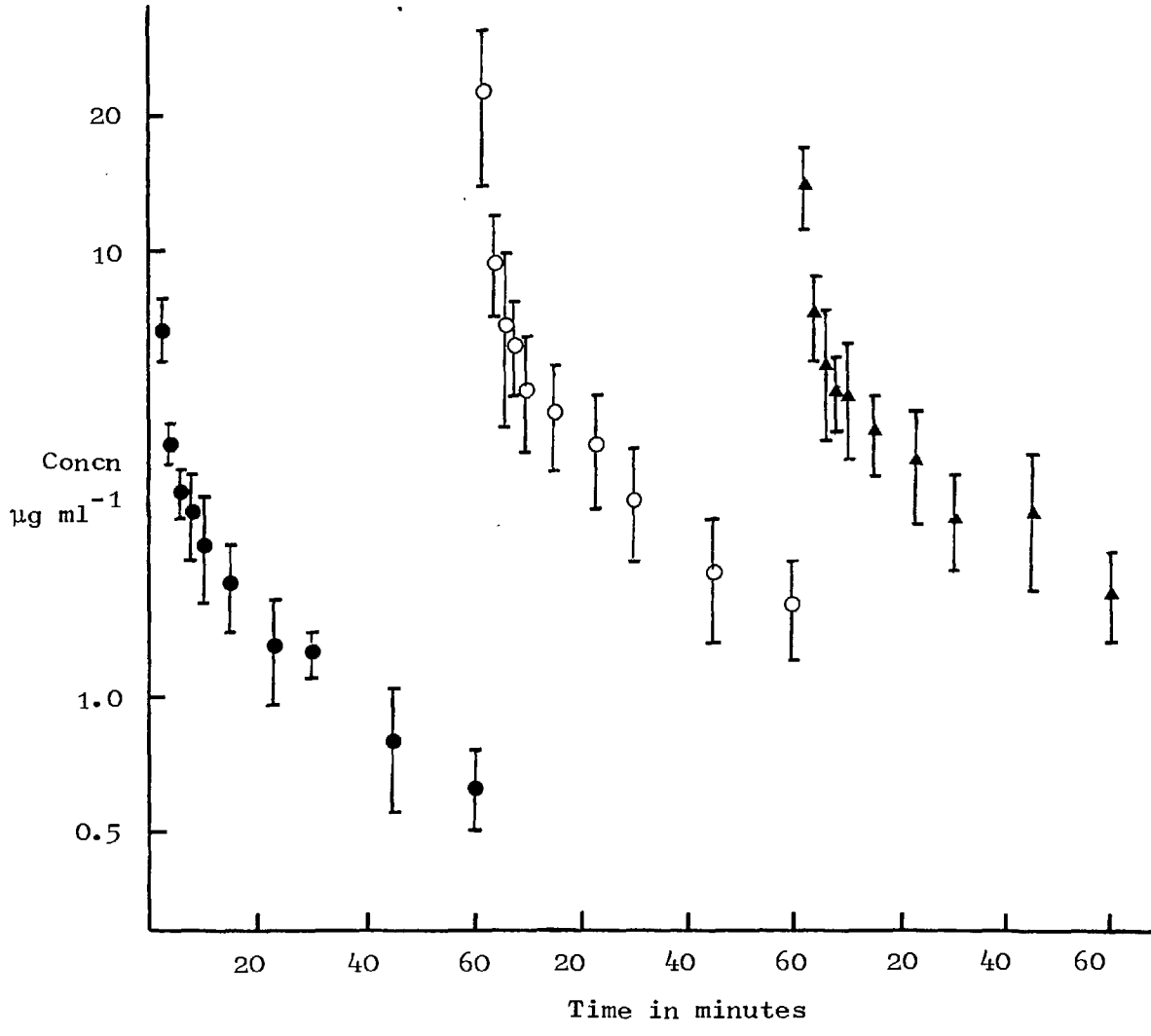
Groups	Time in minutes									
	2	4	6	8	10	15	23	30	45	60
Patients with normal renal functions										
I ($0.5\ mg\ Kg^{-1}$) n=4	6.67 ±1.1	3.70 ±0.41	2.92 ±0.44	2.57* ±0.63	2.24 ±0.59	1.76 ±0.41	1.27 ±0.35	1.25 ±0.15	1.80 ±0.25	1.63 ±0.13
II ($1\ mg\ Kg^{-1}$) n=5	22.79 ±8.71	9.46 ±2.52	6.86 ±2.88	6.21* ±1.51	4.91 ±1.43	4.36 ±1.21	3.67 ±1.08	2.81 ±0.81	1.90 ±0.57	1.61 ±0.42
Patients with renal failure										
III ($1\ mg\ Kg^{-1}$) n=7	13.98 ±3.33	7.15 ±1.62	5.49 ±1.75	4.82 ±0.91	4.82 ±1.45	3.87 ±0.89	3.39 ±1.03	2.48 ±0.62	2.64 ±0.94	1.72 ±0.38

* $p < 0.05$ (groups II against I)

Values are mean ± S.E.

All others not significant

Fig. 4.4 Semilog plasma concentration-time curves of fazadinium in the three groups of patients studied (values are mean + S.E.)



KEY

Patients with normal renal function (0.5mg Kg^{-1})

Patients with normal renal function (1.0mg Kg^{-1})

Patients with renal failure (1.0mg Kg^{-1})

Table 4.8 Plasma fazadinium concentration ($\mu g\ ml^{-1}$) during recovery of twitch tension(I/C) in patients with normal renal function

Group	n	% Recovery of twitch tension						
		5	10	20	30	40	50	60
I ($0.5\ mg\ Kg^{-1}$)	4	1.49 ±0.39	1.48 ±0.32	1.28 ±0.28	1.13 ±0.41	1.04 ±0.23	0.94 ±0.22	0.86 ±0.21
II ($1.0\ mg\ Kg^{-1}$)	5	1.60 ±0.41	1.30 ±0.32	1.09 ±0.17	0.93 ±0.02	0.78 ±0.02	0.79 ±0.03	0.76 ±0.03

Values are mean ± S.E.

Fig 4.5 Dose-response relationship in patients with normal renal function after 0.5 mg Kg^{-1} and 1 mg Kg^{-1} fazadinium

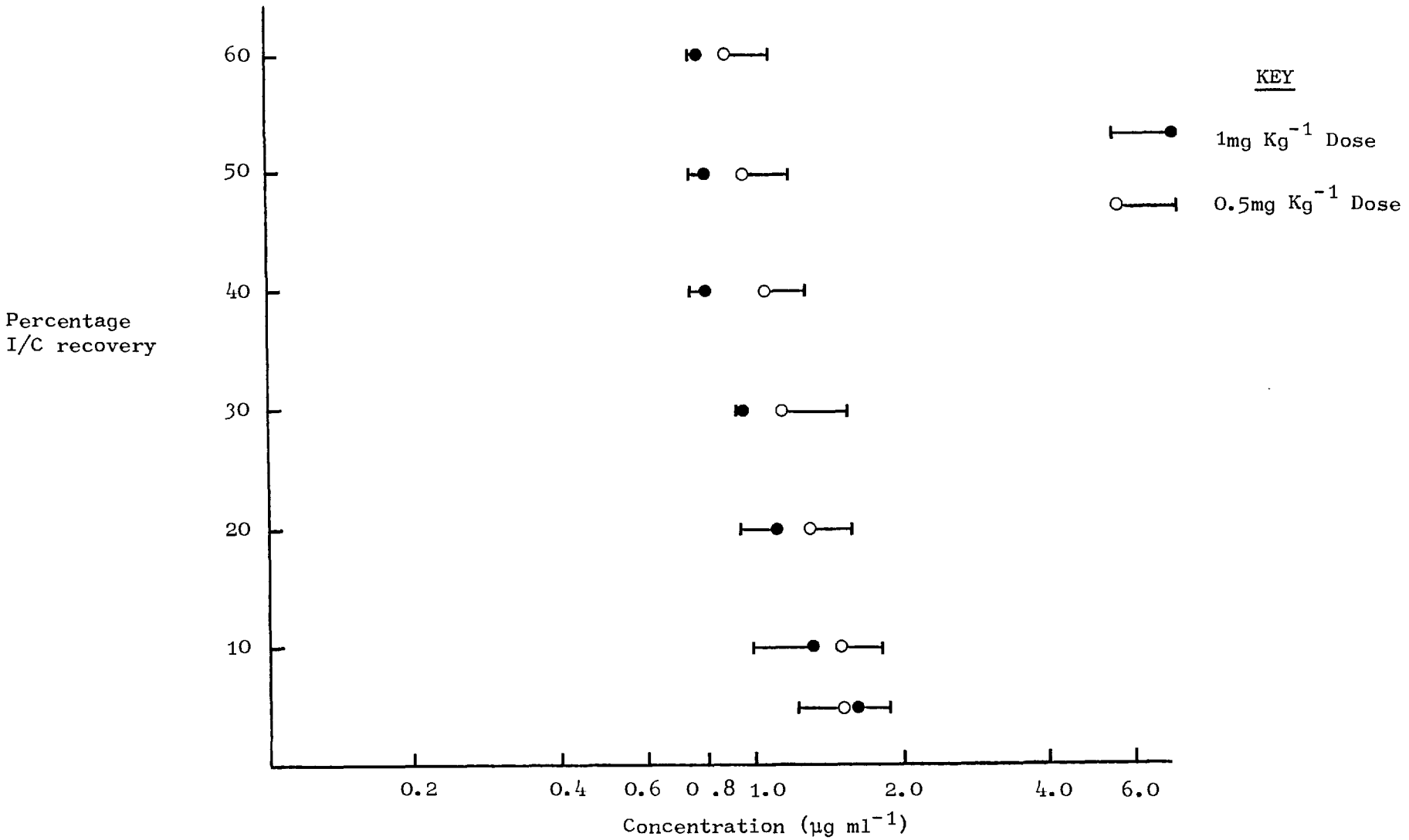


Table 4.9 Plasma fazadinium concentration ($\mu g\ ml^{-1}$) during recovery of twitch tension (I/C) in patients with and without renal failure

Group	n	% Recovery of twitch tension						
		5	10	20	30	40	50	60
Patients with normal renal function (Groups I & II)	9	1.56 ±0.28	1.38 ±0.21	1.17 ±0.14	1.03 ±0.11	0.89 ±0.11	0.87 ±0.11	0.81 ±0.09
Patients with renal failure (Group III)	7	2.13 ±0.77	1.69 ±0.5	1.39 ±0.39	1.16 ±0.27	1.04 ±0.27	1.18 ±0.12	1.10 ±0.12

Values are mean ± S.E.

fazadinium levels and depression of twitch height in patients with and without renal failure is illustrated in Fig. 4.6. Although the mean plasma fazadinium concentration was higher at each 10% increment in the renal failure group, the valuesdid not achieve statistical significance. Consequently, dose-response relationships in patients with and without renal failure were essentially the same.

Discussion of results

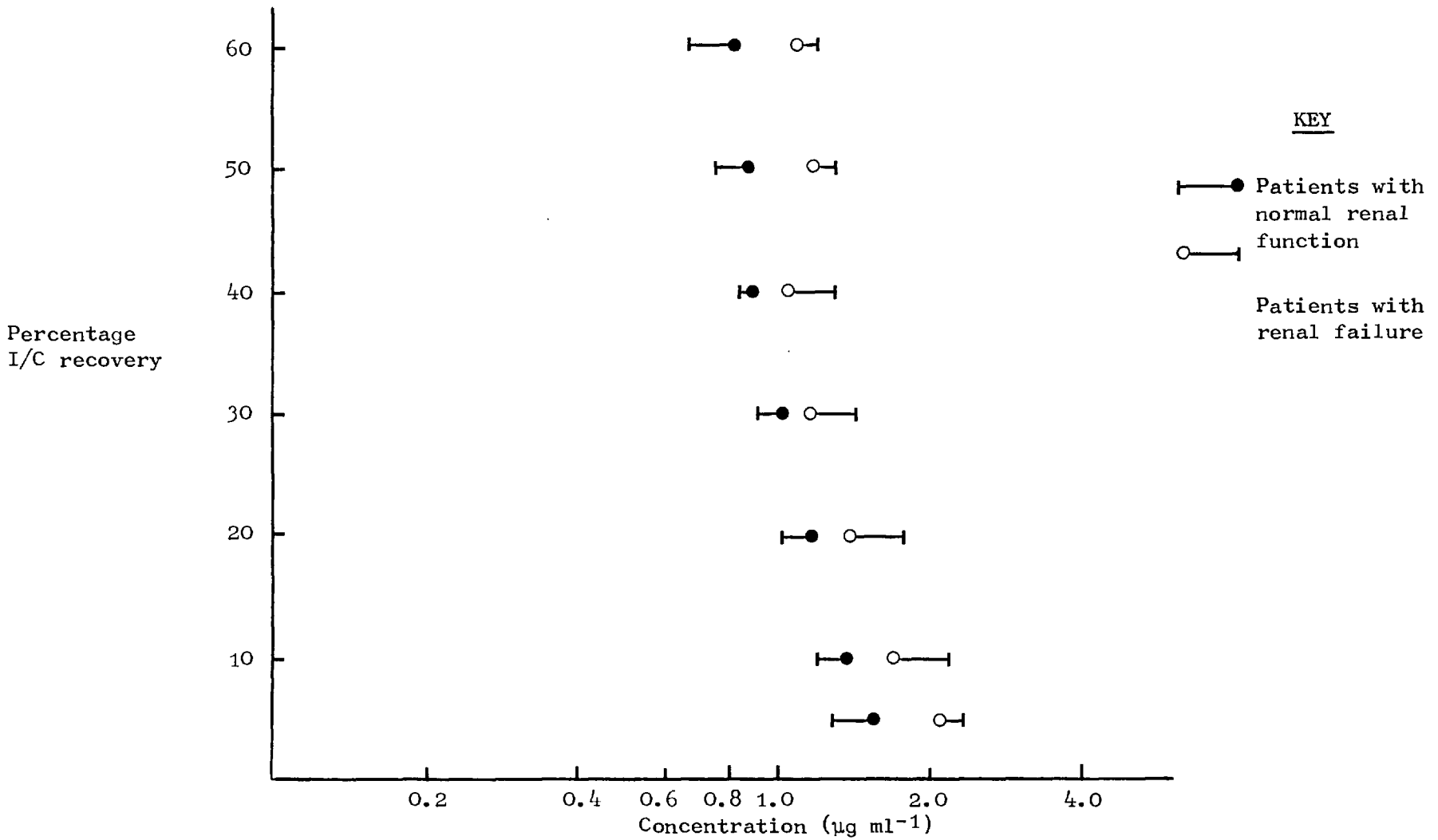
The findings show the relationship between the clinical effect and the pharmacokinetics of fazadinium, when administered as a single bolus, to patients with and without renal failure.

In this study, the use of inhalation agents (e.g. halothane, enflurane) and other muscle relaxants (e.g. suxamethonium) were avoided by employing neuroleptanaesthesia. Many reports (Gissen and others, 1966; Miller and others, 1971; Ali and Saverese, 1976) have demonstrated a prolongation of neuromuscular blockadedue to alteration of both the pharmacokinetic and pharmacodynamic properties of muscle relaxants, by inhalation agents used in anaesthetic practice.

Duration of action of Fazadinium

The mean duration of action of fazadinium to 10% recovery, in patients with normal renal function, was 27 min and 90 min after 0.5 mg Kg^{-1} and 1 mg Kg^{-1} does respectively. These values were similar to previous reports (Simpson and others, 1972; Blogg and others, 1973a;

Fig 4.6 Plasma fazadinium concentration during recovery of neuromuscular blockade in patients with and without renal failure



Camu and D'Hollander, 1978) despite considerable intra-patient variation and the use of different anaesthetic techniques. Duration of neuromuscular block depends on the time for the concentration of the muscle relaxant in the extracellular fluid to diminish to a particular value (Horowitz and Spector, 1973). The variation in drug concentration with time and consequently, the degree of neuromuscular blockade is influenced by the processes of drug distribution, metabolism and excretion.

In patients with renal failure, the reduced renal elimination of pancuronium (Miller and others, 1973), (+)-tubocurarine (Miller and others, 1977) and gallamine (Singer and others, 1971) results in the prolongation of neuromuscular blockade. In the case of fazadinium, Camu and D'Hollander (1978) observed shorter recovery times at early stages of recovery (5%) from neuromuscular block, and slightly longer recovery times for later stages of recovery (75%, 95%) in patients with renal failure compared with those with normal renal function. These differences were however, not statistically significant even when increasing doses of fazadinium (0.25 mg Kg^{-1}, 0.5 mg Kg^{-1} and 1 mg Kg^{-1}) were used. In the present study with fazadinium, there was no change in the mean duration of action of neuromuscular blockade, and the subsequent recovery from block was as rapid in the renal failure patients as in the control patients (those patients with normal renal function).

Kinetics of Fazadinium

With regard to the disposition of neuromuscular blocking drugs, there has been some controversy in the literature over whether the pharmacokinetics of muscle relaxants are better described by a three compartment rather than a two compartment open model (see Table 4.10). Thus, Agoston and others (1973a) claimed that the plasma level-time curve of pancuronium is triphasic, but Mcleod and others (1976) found that the two compartment open model was sufficient to explain their data. Somogyi and others (1976) fitted similar data on pancuronium to both two and three compartment models and showed that the three compartment model has no advantages over the two compartment model with this drug. They concluded that the two compartment open model is the simplest model consistent with their observed data. For (+)-tubocurarine, Gibaldi and others (1972b) proposed a three compartment open model and many workers (Wingard and Cook, 1976; Miller and others, 1977) have satisfactorily explained their data with this model. In the case of gallamine, Duvaldestin and others (1977) utilised the two compartment model, but Agoston and others (1978) suggest a triphasic pattern for this drug, although the only dimensions given are the half-lives of the three phases. In this study, the pharmacokinetics of fazadinium was best described by the two compartment open model, as assessed by the ability of the model to fit the observed data. Recently, Duvaldestin and others (1978) also successfully utilised this model to explain the pharmacokinetics of fazadinium.

Table 4.10 Pharmacokinetic models to describe the disposition of neuromuscular blocking drugs

Drug	studies employing the 2 compartment open model	studies employing the 3 compartment open model
Pancuronium	McLeod and others (1976) Somogyi and others (1976)	Agoston and others (1973b)
(+)-Tubocurarine	-	Gibaldi and others (1972a) Wingard and Cook (1976) Miller and others (1977)
Gallamine	Duvaldestin and others (1977)	Agoston and others (1978)
Fazadinium	Present study Duvaldestin and others (1978)	-

In the present study, the pharmacokinetics of fazadinium in patients with normal renal function, were consistent with the rapid plasma decay pattern observed. Plasma clearances (Cp) were 221 ml min^{-1} after 0.5 mg Kg^{-1} dose and 185 ml min^{-1} after 1 mg Kg^{-1} dose, whereas Duvaldestin and others (1978) reported a value of 132 ml min^{-1} after 1.5 mg Kg^{-1} dose. There were similar differences in the elimination half-lives ($T\frac{1}{2}\beta$) which in this study, were 30.2 min and 50.1 min after the low and high doses of fazadinium, respectively. Nevertheless, both studies demonstrated the rapid plasma elimination of this drug compared to other non-depolarising muscle relaxants (Table 4.11)

Using the two compartment model for (+)-tubocuarine, Gibaldi and others (1972a)and later Wingard and Cook (1976) both predicted a prolonged elimination half-life for patients with renal failure compared to controls and this was confirmed by the pharmacokinetic studies of Miller and others (1977). Substantial changes in other pharmacokinetic parameters, have also been reported to occur for pancuronium (Mcleod and others, 1976; Somogyi and others, 1976) in patients with renal failure compared to controls (see Table 4.12). The main alterations were in the elimination half-life ($T\frac{1}{2}\beta$), elimination rate constant (Kel), plasma clearance (Cp) and the apparent volume of distribution, although Somogyi and others (1976) found no significant difference in the last parameter.

In the present study with fazadinium there were no significant changes in any of the pharmacokinetic parameters in patients with renal failure or those with normal renal function. Furthermore, the plasma concentration-time curves

Table 4.11 Terminal elimination half-life ($T_{\frac{1}{2}}\beta$) of some competitive neuromuscular blocking drugs

Drug	Dose (mg Kg^{-1})	$T_{\frac{1}{2}}\beta$ (min)	Reference
Fazadinium	0.5	30.2	Present study
	1.0	50.1	
	1.5	76.4	Duvaldestin and others (1978)
Pancuronium	0.13	110	Buzello (1975)
	0.09	132	Somogyi and others (1976)
	0.06	102	Mcleod and others (1976)
(+)-Tubocurarine	0.3	150	Horowitz and Spector (1973)
	0.3	231	Gibaldi and others (1972a)
	0.3	152	Wingard and Cook (1976)
	0.5		Miller and others (1977)
Gallamine	2.5	128	Dulvaldestin and others (1977)
	2.5	141	Agoston and others (1978)

Table 4.12 Comparison of some pharmacokinetic parameters after pancuronium in patients with and without renal failure

Parameters	Patients with normal renal function	Patients with renal failure	t-test
$T\frac{1}{2}\beta$ (min)	102	492	< 0.005
	103	257	< 0.01
Kel (h^{-1})	0.809	0.136	< 0.005
	1.2	0.5	< 0.01
Cp (ml min^{-1})	74	20	< 0.005
	123	53	< 0.001
Vd (L Kg^{-1})	0.148	0.236	< 0.05
	0.338	0.330	Not significant

(taken from Mcleod and others, 1976; and Somogyi and others, 1976)

were essentially identical in these two populations, indicating that the plasma elimination of fazadinium occurred at the same rate in the two groups of patients studied.

Plasma levels and effect

Plasma levels in patients with normal renal function were generally lower after the smaller dose (0.5 mg Kg^{-1}) of fazadinium and the dose-response curves in the two instances were similar but the time to recovery from clinical effect was more rapid after the smaller dose. These results are consistent with the concept that it is the concentration of drug at the receptor-site which in turn, depends on the plasma concentration, that determines the extent of neuromuscular blockade of fazadinium. Estimation of plasma concentration and measurement of clinical effect illustrates a steep dose-response curve, with 90% block at a mean concentration of 1.39 $\mu g\ ml^{-1}$ and 50% block at 0.87 $\mu g\ ml^{-1}$. Plasma concentrations at similar degrees of neuromuscular block can also be derived from studies involving pancuronium (Somogyi and others, 1976; Agoston and others, 1977) and (+)-tubocurarine (Matteo and others, 1974; Ham and others, 1979) which demonstrates the potency of fazadinium to be one-half that of (+)-tubocurarine and one-eight that of pancuronium (see Table 4.13) . Similar studies relating plasma relaxant concentration to depression of neuromuscular response using (+)-tubocurarine (Miller and others, 1977) and pancuronium (Somogyi and others, 1976) fail to show any change in the sensitivity of the neuromuscular junction to the relaxant in patients with renal failure. The present study with fazadinium also supports this conclusion.

Table 4.13 Plasma concentrations ($\mu g\ ml^{-1}$) of some muscle relaxants at 90% and 50% block

Drug	% Block		Reference
	90%	50%	
Fazadinium	1.42	0.88	Present Study
Pancuronium	0.18	0.11	Somogyi and others (1976) Agoston and others (1977)
(+)-Tubocurarine	0.67	0.45	Matteo and others (1974) Ham and others (1979)

Summary

1) Fazadinium at doses of 0.5 mg Kg^{-1} and 1 mg Kg^{-1} produced complete abolition of pre-relaxant twitch height, and recovery from neuromuscular blockade occurred significantly faster after the low dose.

2) The disappearance of fazadinium in plasma exhibited a bi-exponential decay pattern. Pharmacokinetic analysis demonstrated high plasma clearances of 221 and 185 ml min^{-1}, and short terminal half lines, 30 and 50 min, for the two doses of fazadinium given.

3) A relationship between plasma concentration and depression of neuromuscular blockade during spontaneous recovery after fazadinium was established. Mean plasma concentrations at 10% and 50% recovery were 1.39 $\mu g\ ml^{-1}$ and 0.88 $\mu g\ ml^{-1}$ respectively, thus demonstrating the potency of fazadinium to be approximately one-half that of (+)-tubocurarine and one-eight that of pancuronium.

4) In renal failure patients undergoing kidney transplantation, fazadinium also produced complete abolition of pre-relaxant twitch height and recovery of neuromuscular blockade did not differ significantly from controls.

5) No significant differences were found in the pharmacokinetic or pharmacodynamic properties of fazadinium in patients with or without renal failure.

CHAPTER FIVE

N-Methylation and Quaternisation of Pyridine in vivo

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Introduction

Biological methylation is an important metabolic route for many compounds of both endogenous and exogenous origin(Axelrod, 1971). Various types of oxygen, nitrogen and thiol functions are known to undergo this reaction including phenols, catechols, primary, secondary and tertiary amines (see Table 5.1). One type of methylation that has been little explored despite the fact that its occurrence has long been known is that of the methylation of tertiary nitrogen functions in azaheterocycles.

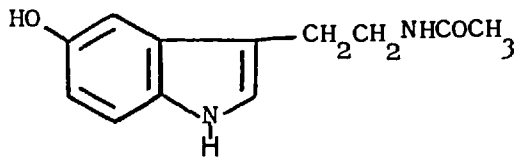
N-Methylation of tertiary amines

His in 1887 first observed the occurrence of N-methylation when he isolated N-methylpyridinium hydroxide from the urine of dogs dosed with pyridine acetate, and this was confirmed by other workers (Cohn, 1894; Abderhalden and Brahm, 1909 ; Tomita, 1921). Subsequently, it was also shown that other species, including pig, goat, frog, hen, and man but apparently not the rabbit and rat, were also able to N-methylate pyridine (see Table 5.2).

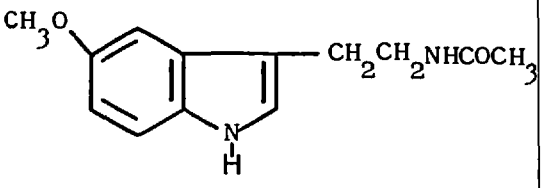
Later it was shown that N-methylation and quaternisation occurred with other pyridine derivatives such as nicotinic acid (Ackerman, 1912), ethionamide (Biedor and Brunel, 1971), and chloromethiazole (Herbertz and others, 1973) as well as other azaheterocycles such as quinoline and isoquinoline (Tamura, 1924; Kamei, 1927, Takahashi, 1927). For the chemical structures of these compounds see Table 5.3

Table 5.1 Biological methylation of oxygen, sulphur and nitrogen functions

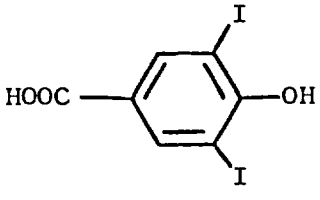
O-Methylation



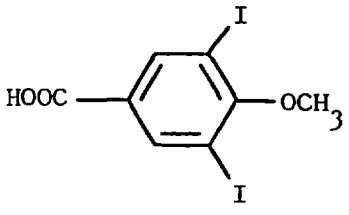
N-Acetylserotonin



Melatonin

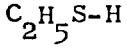


4-Hydroxy-3,5-diodo-benzoic acid

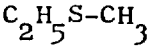


3,5-Diodo-4-methoxybenzoic acid

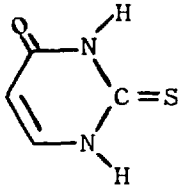
S-Methylation



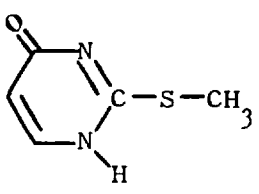
Mercaptoethanol



S-Methylmercaptoethanol



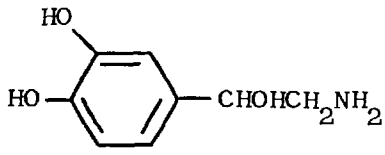
Thiouracil



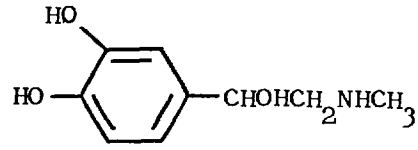
S-Methylthiouracil

Table 5.1 Biological methylation of oxygen, sulphur and nitrogen functions (cont'd)

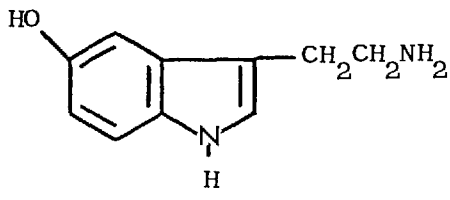
(i) Primary amines



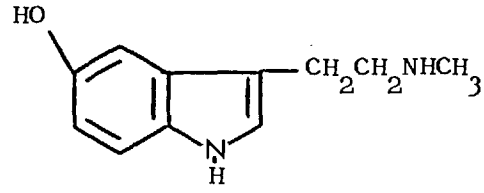
Noradrenaline



Adrenaline

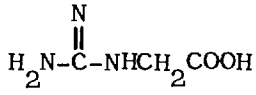


Serotonin

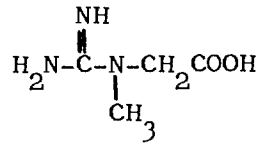


N-Methylserotonin

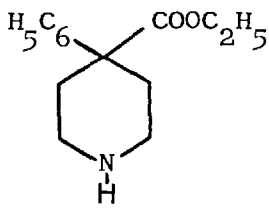
(ii) Secondary amines



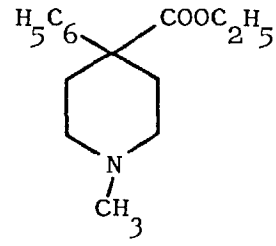
Guanidoacetic acid



Creatine



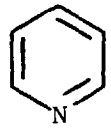
Norpethidine



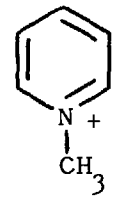
Pethidine

Table 5.1 Biological methylation of oxygen, sulphur and nitrogen functions (cont'd)

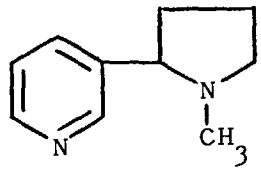
(iii) Azaheterocycles



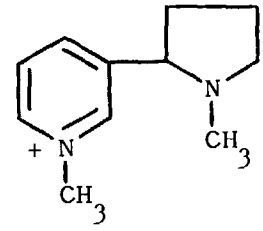
Pyridine



N-Methylpyridinium



Nicotine

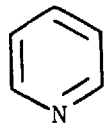


N-Methylnicotine

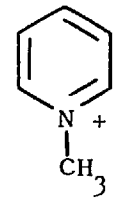
Table 5.2 N-Methylation of pyridine in various species

Species	N-Methylation expressed as % dose administered	Workers
Dog	4.0	His (1887) Cohn (1894) Abderhalden & others (1909)
	2.5 (p.o.) 6.1 (s.c.)	Okuda (1959b)
Eck Fistula dogs	2.38	Kamei (1927)
Pig		Totani & others (1910
Goat		Totani & others (1910
Frog		Tomita (1921) Mageda & others (1914
Rabbit	0.0	Abderhalden & others (1909)
	0.75	Tomita (1921)
	0.85 (p.o.) 1.05 (s.c.)	Okuda (1959a)
Rat	0.0	Baxter & Mason (1947)
Hen		Hoshiai (1909)
Man		Kutscher & others (1907)

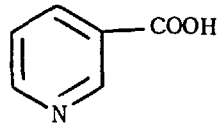
Table 5.3 Some examples of quaternisation of azaheterocycles by N-methylation



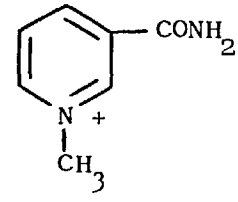
Pyridine



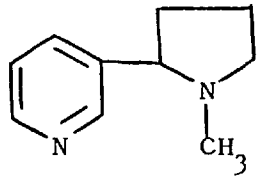
N-Methylpyridinium



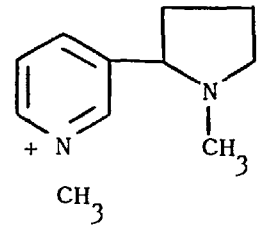
Nicotinic acid



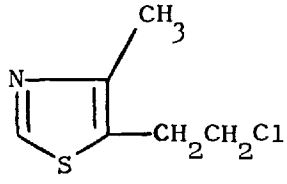
N-Methylnicotinamide



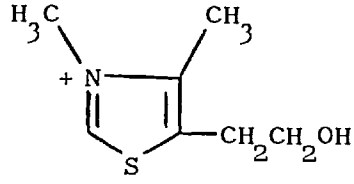
Nicotine



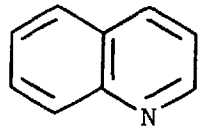
N-Methylnicotine



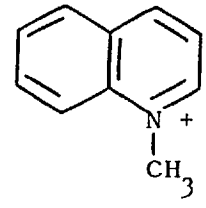
Chloromethiazole



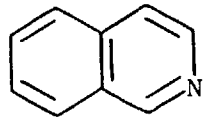
N-Methylthiazole derivatives in free and conjugated forms



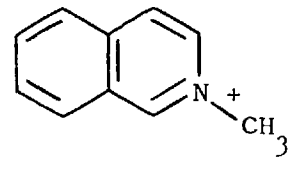
Quinoline



N-Methylquinolinium



Isoquinoline



N-Methylisoquinoline

Enzymology of methylation

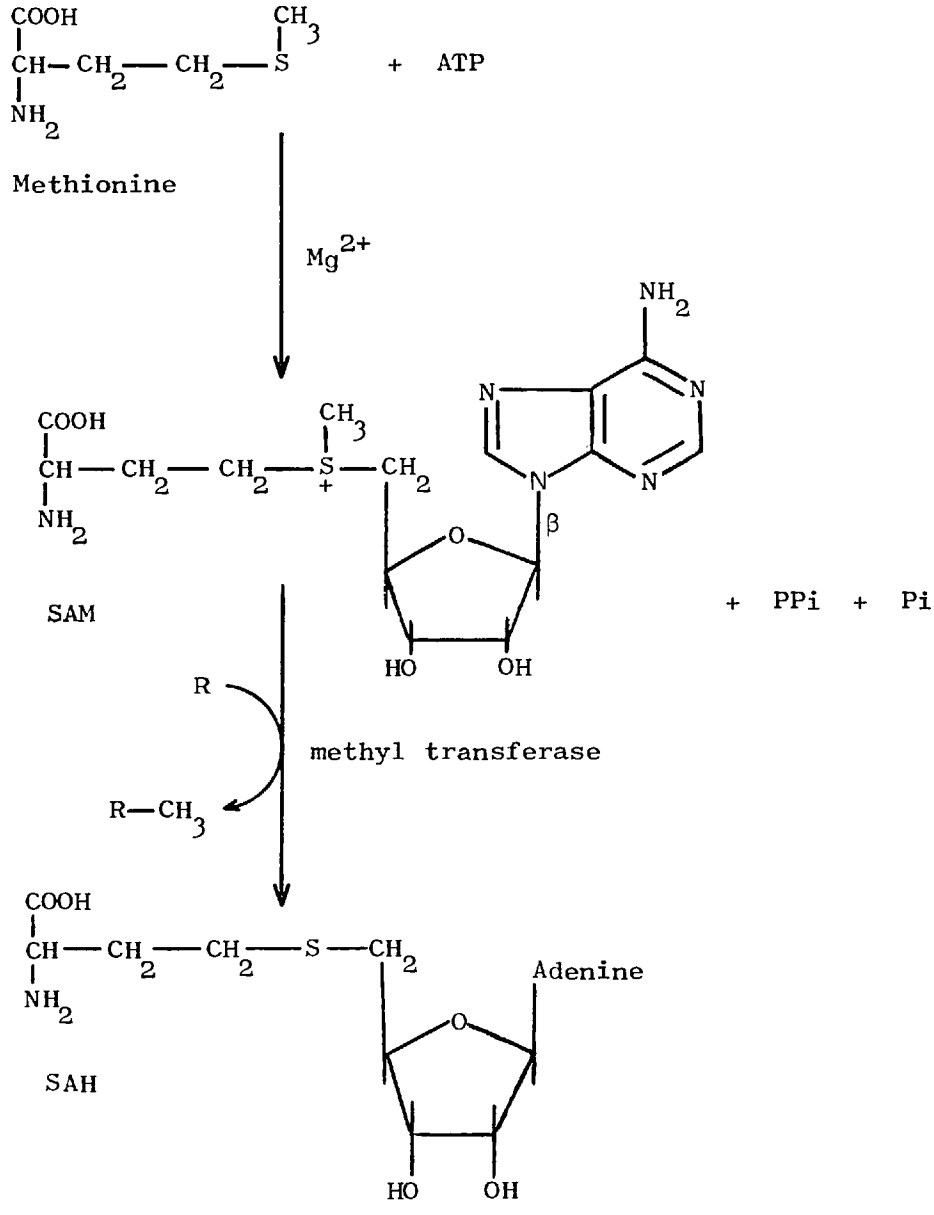
The enzymology of methylation has been studied largely with endogenous substrates e.g. the biogenic amines. It is now known that the main source of methyl groups for the methylation of various compounds *in vivo* is methionine. (Fig 5.1) Methylation however, requires an active form of methionine which is formed enzymatically from methionine and adenosine triphosphate (ATP). This substance is S-adenosylmethionine (SAM) and plays an important role as the intermediary nucleotide in many methylation processes. (Cantoni, 1953).

Specificity in methylation depends on the nature of the transferase, and several are known to exist which catalyse O-, S- and N-methylations. The methyl-transferases differ from each other in terms of: cofactor requirements, they demonstrate substrate and species specificity, variations in tissue distribution and also differ in sensitivity to different inhibitory drugs. Furthermore, some are known to exist in multiple molecular forms in different species, and in a few cases, within tissues of the same species (Axelrod and Vessel, 1970).

Importance of the quaternisation process

The conversion of tertiary nitrogen heterocycles to quaternary ammonium functions could be of great pharmacological and toxicological interest for two main reasons. Firstly, this conversion is accompanied by a marked change in physicochemical properties such as charge and solubility which could affect disposition.

Fig. 5.1 Activation of methionine for biological methylations



KEY

ATP : Adenosine triphosphate

SAM : S-adenosyl methionine

PPi : pyrophosphate

Pi : inorganic phosphate

SAH : S-adenosyl homocysteine

R : Acceptor

Secondly, quaternary ammonium compounds are frequently biologically active and they could differ in this respect from their parent tertiary amines. Atropine-like and neuromuscular blocking activity is not infrequently associated with structures containing one or more quaternary ammonium centres.

This study is concerned with the in vivo N-methylation of the simple azaheterocycle, pyridine. It was undertaken to ascertaining certain features of this metabolic reaction, namely its occurrence in respect of species, the influence of dose and route of administration as well as the provision of supplementary methionine on this metabolic process.

Materials and Methods

Compounds and Radiochemicals

Pyridine

[2,6 - ^{14}C] Pyridine (250 μCi; specific activity 27 mCi mM^{-1}) was purchased from the Radiochemical Centre, Amersham, U.K. The radiochemical purity and chemical purity of this compound were >97% and >96% respectively.

A stock solution was prepared by diluting the ^{14}C-pyridine with unlabelled pyridine hydrochloride (Fisons, Loughborough, Leics., U.K.) in 0.01N HCl to give a concentration of 25 mg ml^{-1} and 25 μCi ml^{-1}. This solution was stored at -20°C. For dosing, aliquots were appropriately diluted with 0.01N HCl and additional carrier added where necessary.

N-Methylpyridinium Iodide

N-Methylpyridinium iodide (m.p. 116-118°C uncorr.) was prepared by the method of Prescott (1896), in which pyridine was mixed with methyl iodide.

N-Methyl - [2,6 - ^{14}C] - Pyridinium iodide

[2,6 - ^{14}C] Pyridine (25μCi; 88mg) dissolved in ethanol (3 ml) was refluxed for one hour with methyl iodide (3 ml). After this time, excess methyl iodide was removed by distillation in vacuo. The straw-coloured crystalline residue was washed with cold ethanol and ether, to give white needle crystals, m.p. 116-118°C (literature value 117°C, Prescott (1896)) with a yield of 40% (90 mg).

The compound was shown to have a radiochemical purity of > 98% by paper chromatography (Whatman No. 3 mm) followed by radiochromatogra scanning (Packard model 7201).

Both labelled and unlabelled N-methylpyridinium iodide were kept in a vacuum dessicator as the material was found to be hygroscopic.

Animals

Seven species of animals were used, as follows:
rat (Wistar albino, female, body wt. 240 ± 30 g); guinea pig (Dunkin - Hartley albino, male and female, body wt. 535 ± 70g); mice (Tuck strain, female,body wt. 140 ± 10g); gerbil (female, body wt. 63 ± 4g); rabbit (New Zealand White, male and female, body wt. 4.0 ± 0.5 Kg) and cat (mongrel, female, body wt. 2.5 Kg).

Administration of ^{14}C-Pyridine

^{14}C-Pyridine was administered as an aqueous solution of its hydrochloride salt. All the animals received the drug intraperitoneally. Rats and guinea pigs were also given the drug by stomach garage. Dose schedules for the administration of pyridine to the various species is shown in Table 5.4.

Administration of N-Methyl- [2,6-^{14}C] - pyridinium iodide

Rats and guinea pigs were dosed with N-methylpyridinium (8 mg Kg^{-1}; 10 μCi Kg^{-1}), given as an aqueous solution of its iodide by intraperitoneal injection.

Table 5.4 Dose schedules for the administration of [2,6-^{14}C] pyridine to the various animal species studied

Animals	Route of administration	Dose of pyridine
Rat Mouse Guinea pig Hamster Gerbil Rabbit Cat	i.p.	7 mg Kg^{-1}
Rat Guinea pig	i.p.	1 mg Kg^{-1} 7 mg Kg^{-1} 500 mg Kg^{-1}
Rat Guinea pig	p.o.	7 mg Kg^{-1}
Rat	p.o.	7 mg Kg^{-1} 68 mg Kg^{-1} 357 mg Kg^{-1}

[2,6-^{14}C] Pyridine (0.3-7.6μCi) was administered as its hydrochloride in aqueous solution

Collection of urine samples

For the duration of the experiment, rats, guinea pigs,mice, hamsters and gerbils were housed in metabolism cages which allowed separate collection of urine and faeces ("Metabowls"; Jencons Scientific Ltd., Hemel Hempstead, U.K.). The animals were housed singly except for mice which were kept in groups of five.

Rabbits and cats were housed separately in cages with wire-mesh floors and mounted on stainless steel funnels such that urine and faeces could be collected separately.

In all cases 0-24h and 24-48h urines were collected. Cages and funnels were thoroughly washed with distilled water and the washings retained separately. All animals were allowed free access to their normal diet and water for the duration of the experiment.

Feeding rats with DL-methionine

Rats were injected intraperitoneally (1g Kg^{-1}) with an aqueous solution of DL-methionine 24h before dosing with ^{14}C-pyridine (7 mg Kg^{-1}, i.p). During the course of the experiment a 1% supplement (by weight of diet) was also added to the basic diet.

Human volunteers

Two male human subjects participated in the study after having given their informed consent. Each volunteer took [2,6-^{14}C] - pyridine hydrochloride (5 mg; 5 μCi) orally in orange juice. 0-24h and 24-48h urines were collected.

Storage of urine samples

All urine samples were stored at -20^{o}C without pH adjustment prior to analysis.

Radiochemical techniques

Determination of radioactivity

^{14}C radioactivity in urine was determined by liquid scintillation spectrometry (Packard, Tri-Carb model 3385) Aliquots (0.1 to 0.5 ml) were counted directly in duplicate using a Triton-toluene based scintillation cocktail (see Materials and Methods chapter Two). Quench correction was performed by the external standard ratio method (Higashimura and others, 1962).

Reverse isotope dilution procedure

The reference compound, N-methylpyridinium iodide (0.5-1g weighed accurately) was added to a urine sample containing a known amount of radioactivity (10^5 - 5x 10^5 d.p.m.). Enough distilled water was added to dissolve the solid completely, and the solution then lyophylized. The solid residue was treated with warm alcohol (approx. 20 ml) and the mixture filtered, the filtrate then reduced in bulk by rotary evaporation and diethylether (approx. 5 ml) added to precipitate N-methylpyridinium iodide. The latter was filtered and recrystallised to constant specific activity and melting point.

Chromatography

Urine samples (10-50µl) derived from animals dosed with ^{14}C-pyridine were chromatographed in the following two solvents using Whatman No. 3 mm paper:-

I : n-propanol / 0.88 NH_3; 7:3 by volume

II : n-butanol / acetic acid / water; 4:1:2 by volume.

The developed chromatograms were then scanned for ^{14}C radioactivity in a Packard radiochromatogram scanner (model 7201) and the N-methylpyridinium peak located by using a sample of radiolabelled N-methylpyridinium iodide as a standard for reference. The R_F values were 0.41 (solvent I) and 0.51 (solvent II).

Chemical detection of N-methylpyridinium

The location of N-methylpyridinium on paper chromatograms were also ascertained using a chemical detection reaction. For this purpose, the dry, developed chromatogram was placed for 60 min. in a glass tank containing a beaker with equal volumes of methyl ethyl ketone and 0.88 s.g. ammonia. Following this, the plate was examined under ultra-violet (uv) light (245 nm) where N-methylpyridinium is seen as a bluish-white fluorescing spot. This is a characteristic reaction for quaternary ammonium compounds (Reddi and Kodicek, 1953).

Results

Identification of N-methylpyridinium in urine after dosing with ^{14}C-pyridine

The quaternisation of pyridine by metabolic N-methylation *in vivo* to form N-methylpyridinium, was confirmed by chromatographic and reverse isotope dilution analysis of urine samples obtained from animals which had been dosed with ^{14}C-pyridine.

For instance, paper chromatography of urine samples from the rat and guinea pig, using solvent system I, showed on radiochromatogram scanning a ^{14}C peak at R_F 0.40 which corresponded to that produced by the authentic sample of N-methylpyridinium iodide, R_F 0.41 (see Fig 5.2). The areas on the chromatograms relating to this peak also developed a bluish white fluorescence when subject to methyl ethyl ketone : NH_3

The presence of N-methylpyridinium was further substantiated by the reverse isotope dilution procedure. This technique was used for the quantitative assessment of N-methylpyridinium in urine samples obtained from the various other species under investigation.

Species variation in the urinary excretion of N-methylpyridinium

Table 5.5 shows the excretion of ^{14}C and N-methylpyridinium by various species following administration of ^{14}C-pyridine. The excretion of ^{14}C was satisfactory for all the species examined (43-78% in 24h) and no attempt was made to account for the ^{14}C fully, as the emphasis was to identify possible species variation

Fig 5.2 Typical Radiochromatogram scans of Urine in the Guniea Pig and Rat after ^{14}C pyridine

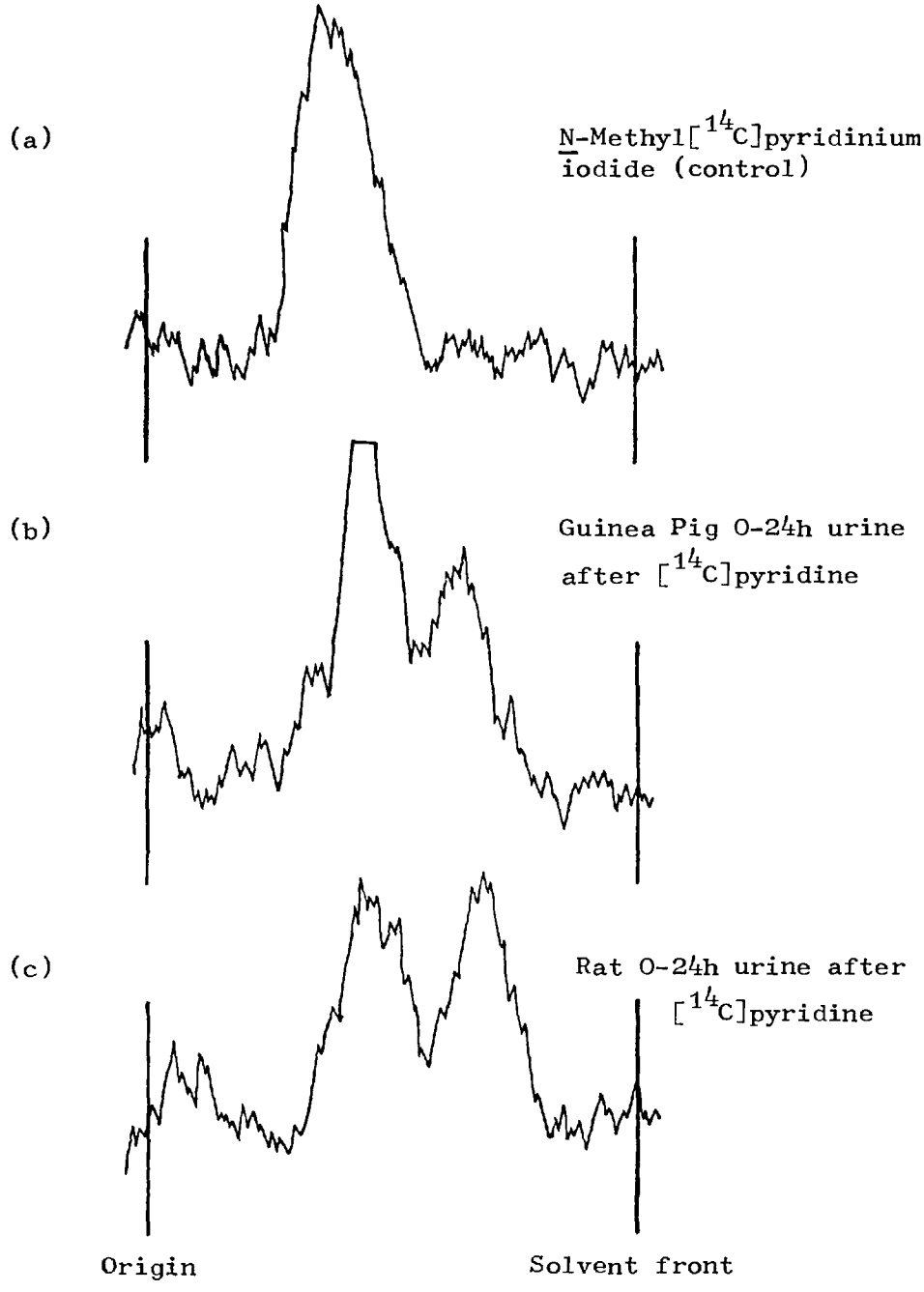


Table 5.5 Species Variations in the N-methylation of [^{14}C] Pyridine

[^{14}C] Pyridine (7mg Kg^{-1};10μCi approx) was given as an aqueous solution of the hydrochloride salt by intraperitoneal injection to the various species. In the case of the two human volunteers the compound (3.4mg; 4μCi) was given orally also as a solution of the hydrochloride. Results are means with ranges in parentheses. Where only 2 animals were used individual values are given.

		% dose excreted in 24h urine:	
Species	No. and Sex	Total ^{14}C	As N-methylpyridinium
Rat	3 ♀	48 (46-50)	5.0 (3.9-6.8)
Mouse	5 ♀	66	12
Guinea pig	2 ♀ , 1 ♂	66 (43-78)	30 (25-35)
Hamster	3 ♀	67 (63-70)	26 (8.0-42)
Gerbil	2 ♀	54 , 50	33 , 20
Rabbit	1 ♀ , 1 ♂	77** , 36**	15 , 26
Cat	3 ♀	75* (67-88)	40 (37-44)
Man	2 ♂	65 , 68	5.5 , 12

* 48h collection; ** 72h collection

in the extent of the formation and excretion of the N-methylated metabolite of pyridine.

The cat and rabbit did not urinate in 24h and hence the 48h and 72h samples respectively, were analysed for N-methylpyridinium by the usual method (see Table 5.5).

N-methylpyridinium was identified as a urinary metabolite of pyridine in all the species examined but there were major differences in the extent of its occurrence. Thus, it was eliminated in large amounts in the rabbit, hamster, gerbil, guinea pig and cat (mean 21-40% of dose), whereas the rat, mouse and man excreted relatively little N-methylpyridinium (mean 5-12% of dose).

Fate of N-Methyl-[2,6-^{14}C]-pyridinium iodide in the rat and guinea pig

The 24h urinary elimination of ^{14}C radioactivity in the rat and guinea pig after dosing with N-methyl-^{14}C-pyridinium iodide was 53% and 85% respectively. A further 2-3% of the dose was excreted in the subsequent 24-48h period (see Table 5.6).

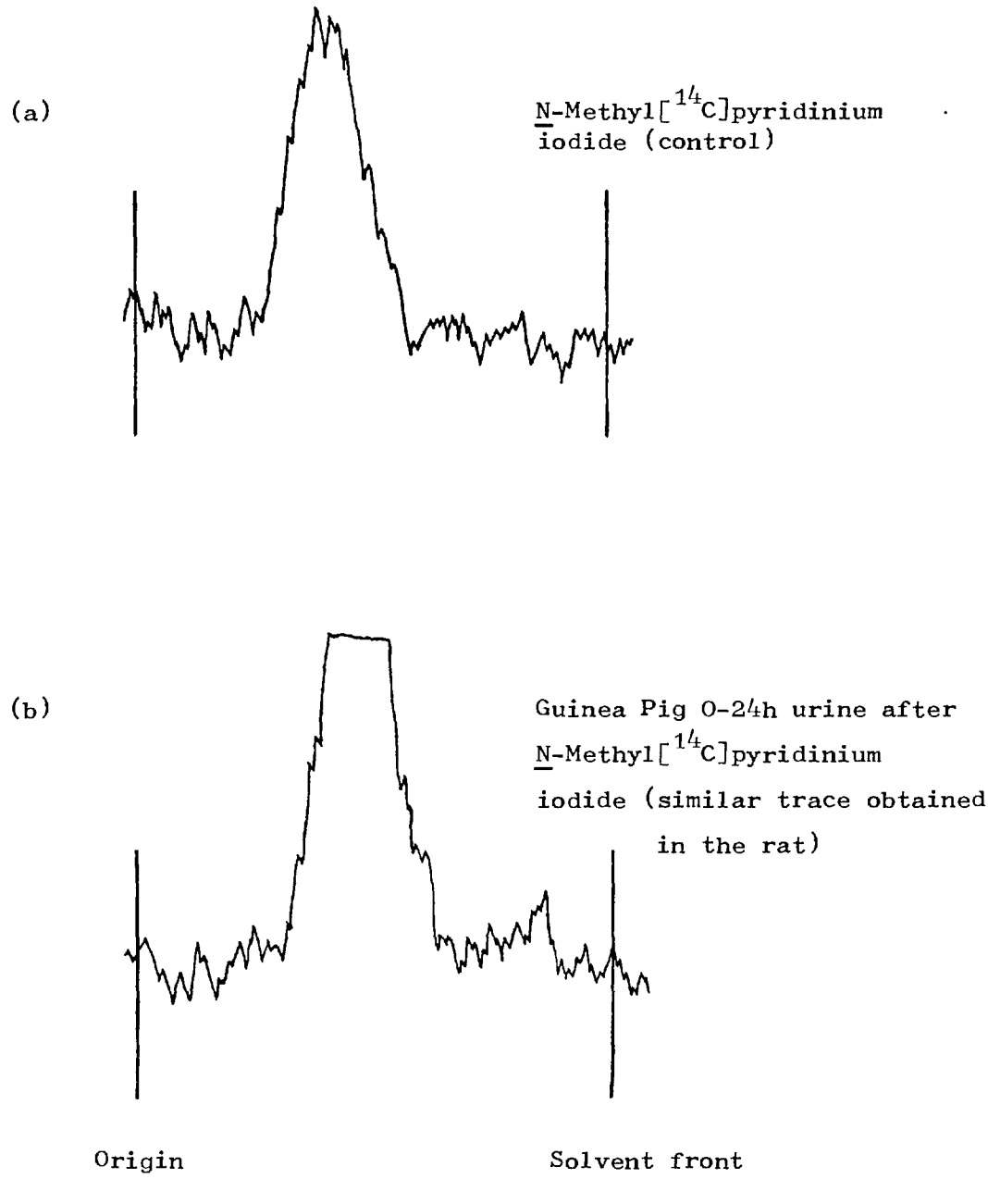
Chromatographic analysis (Fig 5.3) and reverse isotope dilution procedures both confirmed that >95% of the ^{14}C activity was present as N-methylpyridinium, hence indicating that N-methylpyridinium is largely metabolically stable.

Table 5.6 Fate of N-Methyl [^{14}C] pyridinium in the Rat and Guinea Pig

Species	No. and Sex	% ^{14}C excreted in urine in 0 - 24h	% ^{14}C excreted in urine in 24 - 48h	% dose excreted in 0 - 24h as N-methylpyridinium
Rat	3 ♀	53 (40-62)	2.2 (1.2-3.7)	51 (38-61)
Guinea pig	3 ♀	85 (79-94)	2.9 (2.0-4.7)	81 (75-89)

Animals were injected intraperitoneally with N-methyl [2,6^{14}-C] pyridinium (8 mg Kg^{-1}; 10μCi Kg^{-1}) given as an aqueous solution of the iodide. The urine was collected and analysed for ^{14}C and N-methylpyridinium as described in the text.

Fig 5.3 Typical radiochromatogram scans of urine in the Guinea Pig and Rat after N-Methyl [^{14}C] pyridinium iodide



Factors affecting the quaternisation of pyridine in the rat and guinea pig

The rat and guinea pig were found to be poor and good methylators of pyridine, respectively. Hence from the comparative point of view these two species were chosen for further evaluation.

Influence of route of administration and dose

Table 5.7 shows the urinary elimination of ^{14}C and N-methylpyridinium in the rat and guinea pig following the administration of ^{14}C-pyridine orally and intraperitoneally at various dose levels.

The urinary excretion of both ^{14}C and N-methylpyridinium was clearly dose dependent for both species when labelled pyridine was injected intraperitoneally. Thus, in the rat, the urinary excretion of ^{14}C accounted for 70% and 48% dose within 24h of dosing at 1 mg Kg^{-1} and 7 mg Kg^{-1} respectively. At a higher dose (500 mg Kg^{-1}) however, the recovery dropped to 5.8% dose. Similarly, with the guinea pig there occurred a sharp drop in the overall ^{14}C and N-methylpyridinium elimination at the highest dose level (500 mg Kg^{-1}) compared with the two lower doses.

N-methylation also occurred when pyridine (7 mg Kg^{-1}) was given by the oral route; the values obtained for ^{14}C elimination and N-methylpyridinium formation in the rat and guinea pig were similar to those found after the intraperitoneal route. In the case of the rat, the overall urinary excretion of ^{14}C and

Table 5.7 Influence of Route of Administration and Dose on the N-methylation of ^{14}C-Pyridine in the Rat and Guinea Pig

	RAT		GUINEA PIG	
	% dose excreted in 24h		% dose excreted in 24h	
Dose (mg Kg^{-1})	Total ^{14}C	as N-Methylpyridinium	Total ^{14}C	as N-Methylpyridinium
Intraperitoneal				
1	70 (65-76)	9.8 (8.9-11)	79 (78-81)	36 (32-43)
7	48 (47-51)	5.0 (3.9-6.8)	66 (43-78)	31 (25-35)
500	5.8 (3.0-10)	0.8 (0.4-1.2)	11 (8.5-13)	1.9 (1.7-2.3)
Oral				
7	58 (32-75)	3.1 (1.8-4.5)	76 (74-77)	31 (26-35)
68	13 (11-14)	1.2 (0.9-1.7)	-	-
357	20 (8.4-26)	2.4 (1.2-3.1)	-	-

Rats and guinea pigs were appropriately dosed with ^{14}C-pyridine (10-20μCi Kg^{-1}).

The urine was collected for 24h and analysed for ^{14}C and N-methylpyridinium.

Results are means for three animals with ranges in parentheses.

N-methylpyridinium also declined with increase of dose during oral administration, as was found after intraperitoneal injection.

Influence of supplementary DL-methionine

In the rat, the formation of N-methylpyridinium after administration of pyridine was low compared to other species such as the guinea pig and cat (see Table 5.5). One possible explanation for the low N-methylation in this species could be due to a relative deficiency of a source of methyl groups for the purpose of metabolic quaternisation in vivo.

Table 5.8 shows the influence of treatment with DL-methionine on the N-methylation of pyridinium in the rat. The results show that the excretion of N-methylpyridinium and total ^{14}C radioactivity is similar in the controls rats compared with those animals that had been pretreated with the DL-methionine supplementary diet.

Table 5.8 Influence of Supplementary DL-Methionine Administration on the N-methylation of ^{14}C-Pyridine in the Rat

Female rats (body wt. 200g) were injected intraperitoneally (1g Kg^{-1}) with an aqueous solution of DL-methionine 24h before dosing with pyridine and a 1% supplement was included in the diet. ^{14}C-Pyridine (7mg Kg^{-1}) was injected intraperitoneally as an aqueous solution of its hydrochloride. Results are mean values for 3 animals with ranges in parentheses

	% dose ^{14}C excreted in urine in		
Treatment	0 - 24h	24 - 48h	% dose in 0-24h as N-methylpyridinium
None	48 (47-51)	3.7 (2.4-5.1)	5.0 (3.9-6.8)
DL-Methionine	45 (30-58)	6.1 (5.1-6.9)	4.2 (3.8-5.0)

Discussion

Pyridine has been found to undergo N-methylation and hence quaternisation in vivo thereby confirming the findings of earlier workers. It has been confirmed to occur in the rat and also been shown for the first time to occur in the mouse, guinea pig, gerbil, cat and man. Earlier studies (Abderhalden and others, 1909) indicated that the rabbit may be deficient with respect to the N-methylation of pyridine but results reported in this study do not support this view. Okuda (1959b)also found that the rabbit was able to N-methylate pyridine, but to a small extent (1%).

A marked species difference occurred in respect of the N-methylation process and two distinct groups could be identified. The cat, rabbit, guinea pig, gerbil and hamster are relatively good N-methylators (20-40% of dose) while the rat, mouse and man are comparatively poor (5-12% of dose) at this reaction.

The question arises as to how far the measurement of urinary levels of N - methylpyridinium adequately reflect the extent of N-methylation of pyridine in vivo. It is possible that N-methylpyridinium itself is further metabolised once formed and therefore its measurement will not be a fully adequate parameter of N-methylation, and hence the quaternisation of pyridine. However chromatographic and reverse isotope dilution analysis both showed that N-methylpyridinium is largely metabolically stable, and its measurement could therefore be considered as a valid index for the N-methylation process.

The quaternisation of pyridine in the rat and guinea pig was found to be independent of the route of administration (oral or intraperitoneal) but not of the dose. Thus, pyridine is N-methylated to about the same extent by both species irrespective of whether it is given orally or by intraperitoneal injection. In both species, the amount of N-methylpyridinium excreted fell with increase in dose and this was clearly evident at the maximum dose (500 mg Kg^{-1}). The metabolic reaction therefore appears to be saturable but however, there is still a species difference in the capacity for this reaction since values in the rat remain lower than corresponding amounts in the guinea pig.

No difference in the excretion of N-methylpyridinium was observed in rats, whether or not they were pretreated with a supplementary diet of DL-methionine before challenge with ^{14}C-pyridine. The metabolic deficiency in the rat could possibly be attributed to enzymic defects for the transfer of methyl groups to pyridine.

In this study, the quaternisation of pyridine could be seen as an "intoxication reaction" resulting in the formation of a more toxic conjugation product. Thus the LD_{50} (subcutaneous) of N-methylpyridinium chloride in rats is 0.28g Kg^{-1} compared to 1.0g Kg^{-1} for pyridine (Brazda and Coulson, 1946). In the mice the LD_{50} (intraperitoneal) for the quaternary ammonium compound is 0.22g Kg^{-1} compared to 1.2g Kg^{-1} for pyridine (Baxter and Mason, 1947). By contrast N-methylation decreases the toxicity of nicotinamide and coramine,

and has little or no apparent influence on the toxicity of nicotinic acid (Brazda and Coulson, 1946).

The possibility that some heterocycles can undergo N-methylation *in vivo* and perhaps extensively so in some species could be important for some aspects of their biological properties.

Summary

N-Methylation of pyridine

1. Pyridine undergoes N-methylation *in vivo* to give the quaternary ammonium compound, N-Methylpyridinium (NMP).
2. NMP is metabolically stable and hence excreted unchanged.
3. Species differences occur in the N-methylation process: high (20-40%) in Gerbil, Guinea pig, Hamster, Rabbit and Cat and low (5-12%) in Rat, Mouse and man.
4. In Rats and Guinea pigs, the metabolic process is:
   (a) independent of route of administration (oral or i.p.)
   (b) saturable at high dose levels
   (c) unaffected by methionine supplement (Rat)
5. Quaternisation can lead to both toxic and non-toxic products.

CHAPTER SIX

General Disucussions and Conclusions

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For the purpose of discussion it will be convenient to deal with the findings presented in this study under three main headings : (A) the assay and its applications, (B) clinical pharmacokinetics, and (C) methylation.

A) The assay and its applications

The ^{125}I-Rose Bengal radiometric method described for the determination of fazadinium and pancuronium in biological fluids is rapid, convenient and sufficiently sensitive for pharmacokinetic evaluation of these drugs.

In the case of fazadinium, the assay was satisfactory in determining patient plasma levels for as long as three hours post-administration of this relaxant; the lowest measured concentration being $0.30\ \mu g\ ml^{-1}$, a value well within the limits of sensitivity. During this time also, the pharmacological effect of fazadinium was monitored which enabled the correlation between plasma levels and recovery of neuromuscular function to be established from the start of muscle relaxation until the effects had almost disappeared. This was of great advantage for the determination of the pharmacodynamics of fazadinium in patients with normal and impaired renal function.

Although no precise definition of the nature of the complex formed between fazadinium and Rose Bengal was made, studies with both mono- and bisquaternary ammonium compounds demonstrated that both molecular weight and lipophilic character are important

requisites for ion-pair formation. Cohen (1963) reported that the halogens in the Rose Bengal molecule also formed an intimate part of the ion-pair complex with (+)-tubocurarine.

Dye extraction methods have been available for many years (Auerbach, 1943) but relatively few investigations have been conducted with regard to the relationship between extractability and structure of quaternary ammonium compounds. Using bromophenol blue, Mitchell (1952) reported that only compounds of the general structure which includes at least one chain of four carbons or longer, or a benzyl group, can be determined by the dye extraction method. More recently, Schill (1965) concluded that extractability increases with increasing number of carbon atoms.

In order to determine the stoichiometric relation in the formation of these ion-pair complexes Ballard (1954) found the molar ratio between gallamine and bromothymol blue (BTB) was 1:3, one molecule of dye being associated with each quaternary nitrogen atom. In complete contrast, Tanaka & others (1974) reported the molar ratio of quaternary ammonium compound to bromophenol blue was 1:1 and 2:1 for pancuronium and cetyltrimethylammonium respectively, thus indicating that one dye molecule forms an ion-pair with two quaternary nitrogens.

It is interesting to note that whereas tertiary amines could not be extracted under the conditions used, their quaternary ammonium analogues readily formed chloroform-extractable ion-pair complexes with Rose Bengal thus providing a method for

distinguishing the latter. Such a property could also be utilised for the determination of tertiary amines by preliminary N-methylation using methyl iodide; the quaternary ammonium compounds so formed could be extracted and quantitated by the radiometric procedure.

The technique therefore shows wide applicability in the study of quaternary ammonium compounds which as a group have diverse uses in medicine, as muscle relaxants, antiseptics, bactericides herbicides, and in the textile, paper and leather industries. Furthermore, the method could be converted into a rapid screening procedure potentially capable of automation for such compounds, but considerable amount of work remains to be done before the theoretical basis of the method can be fully established.

B) Clinical pharmacokinetics

The effects in man, of fazadinium bromide, the latest non-depolarising muscle relaxant introduced into clinical practice has been described.

Pharmacokinetics is not an esoteric abstract exercise as long as its limitations are acknowledged. Pharmacokinetic studies are important in providing dose regimes to varying clinical circumstances and could be of value in dealing with the problem of prolonged activity of a drug. The ideal muscle relaxant can therefore only be achieved if the drug is based on sound pharmacokinetic and clinical principles.

In this study, there were no cases of prolonged paralysis with fazadinium; this was in accordance with the pharmacokinetic data obtained in patients with normal and impaired renal function. Clinical studies with fazadinium presented in this thesis have contributed towards providing a rationale for its safe use in patients with impaired renal function. For instance, disposition studies in the dog and man indicated that biliary excretion plays an important role in the elimination of fazadinium. Upon renal pedicle ligation, its disposition was not altered in the dog but after ligation of the bile duct, an increase in the plasma elimination half-life was apparent together with a marked reduction in the total elimination. Furthermore, by alternatively occluding either routes of elimination, the two pathways were not found to be complementary.

It is tempting to suppose that renal and biliary pathways for the excretion of fazadinium (m.w. 444) might be complementary. There is rather a narrow range of compounds (m.w. 350-450) for which these two pathways are complementary (Hirom and others, 1976) however, these studies were performed in the rat and due to the large species difference, extrapolation to the dog and man is not possible.

The present theories of the pharmacokinetics of recovery from neuromuscular blockade are as follows: the "competitive" theory suggests that the concentration of drug at the receptor site controls the rate of recovery, whilst the "rate" theory postulates that the drug-receptor interaction is the primary factor in the termination of the action of muscle relaxants.

In this study complete blockade with fazadinium appeared to remain until the plasma concentration was reduced to 1.42μg ml^{-1} and 50% recovery was attained at 0.88μg ml^{-1}. This steep dose-response relationship supports, but in no way proves the "competitive" theory nor does it disprove the "rate" theory.

In the past, the choice of muscle relaxant for use in patients with renal failure has been difficult. Gallamine was avoided because as much as 85% is normally excreted unchanged by the kidney (Feldman and others, 1969), and there seems to be no alternative route of excretion. In this respect, Miller and others (1977) suggested that (+)-tubocurarine may be preferred because it is less dependant upon urinary excretion for elimination. Others have suggested that pancuronium may be safer because it has a flatter dose-response curve than (+)-tubocurarine and therefore lends itself to a greater margin of error (Stovner and Lund, 1970).

The ideal muscle relaxant would thus be one for which the biliary excretion would fully compensate for the impaired renal elimination or as is the case with fazadinium, whose biliary excretion is important enough so that its disposition would be unaffected by renal failure. Furthermore, measurement of neuromuscular function showed that renal failure does not appear to potentiate the depth of neuromuscular blockade nor alter the duration of action of the block induced by fazadinium.

(C) Methylation

Methylation is a synthetic or conjugation reaction, one of many such reactions known to occur both in man and animal species which include glucuronide formation, glycine conjugation, mercapturic acid synthesis, ethereal sulphate synthesis, acetylation, and thiocyanate formation from cyanide. These conjugations or phase II reactions are an integral part of the fate of a foreign compound in the body. However, species defects in respect of these reactions are also known, and these are summarised in Table 6.1

In this thesis, evidence for the existence of metabolic N-methylation and hence quaternisation, of ^{14}C-pyridine in vivo in man and several animal species has been presented. A distinct species variation was observed, methylation being extensive (20-40% of dose) in the cat, guinea pig, gerbil, rabbit, and hamster, but relatively low (5-12%) in the rat, mouse and man.

It is interesting to speculate that other azaheterocycles and tertiary amines may undergo this reaction since the quaternisation reaction has also been reported for nicotinic acid, nicotine, quinoline, isoquinoline and more recently for ethionamide and chloromethiazole. Even choline which is found most abundantly in all living tissues is formed by a quaternisation reaction, the N-methylation of dimethylaminoethanol (Williams, 1959).

Table 6.1 Species defects in conjugation reactions

Species	Defective reaction
Cat, lion, lynx civet, genet	Glucuronidation
Pig	Sulphation
Indian Fruit bat	Hippuric acid formation
Dog	N-acetylation of aromatic amines and hydrazines
Non-primate mammals	i) N^1-glucuronidation of sulphadimethoxine ii) Glutamine conjugation
Guinea pig	N-acetylation of S-substituted cysteines (arising from glutathione conjugation)

(taken from Caldwell, 1979)

Quaternisation of tertiary amines leads to an alteration of the physicochemical properties (charge and solubility) of the parent compound thus enabling its active secretion via the kidneys and hence its rapid elimination from the body. The reaction can also produce toxic products (e.g. pyridine) or non-toxic products (e.g. nicotinamide); the toxicity presumably being more pronounced in those species deficient in this reaction such as the rat, due to the scavenging of essential methyl groups (Baxter and Mason, 1947). This process also appears to have an effect on the potency of neuromuscular blockers. For instance trimethyltubocurarine is ten times more potent than (+)-tubocurarine and this increase in potency can partially be ascribed to quaternisation of the tertiary nitrogen which in (+)-tubocurarine is protonated at physiological pH values.

It must be emphasised that the quaternisation reaction is not restricted to N-methylation since other conjugation reactions also produce quaternised products,such as N-glucuronidation which has recently been reported for cypropeptadine, tripelannamine and clozapine (Israili and others, 1977).

In the light of the present work, the quaternisation of commonly used drugs containing one or more tertiary nitrogen functions were examined after i.p. administration to guinea pigs, a species being relatively good at N-methylating pyridine. The ^{125}I-Rose Bengal radiometric method was employed for screening the quaternisation products in 0-24h urine samples after administration of the various compounds. Since the assay is specific for high molecular weight compounds possessing some

lipophilic character, only compounds with such properties were used.

Table 6.2 summaries the results obtained . Background extractable ^{125}I-Rose Bengal was obtained for a number of tertiary compounds tested and extensively so (>10 times background) for mepyramine and chloroquine.

In conclusion, quaternisation by N-methylation promises to be an important pathway of drug metabolism and possibly offers an explanation of the toxicity of some commonly used N-heterocyclic drugs employed in medicine. Further research is required to evaluate this possibility.

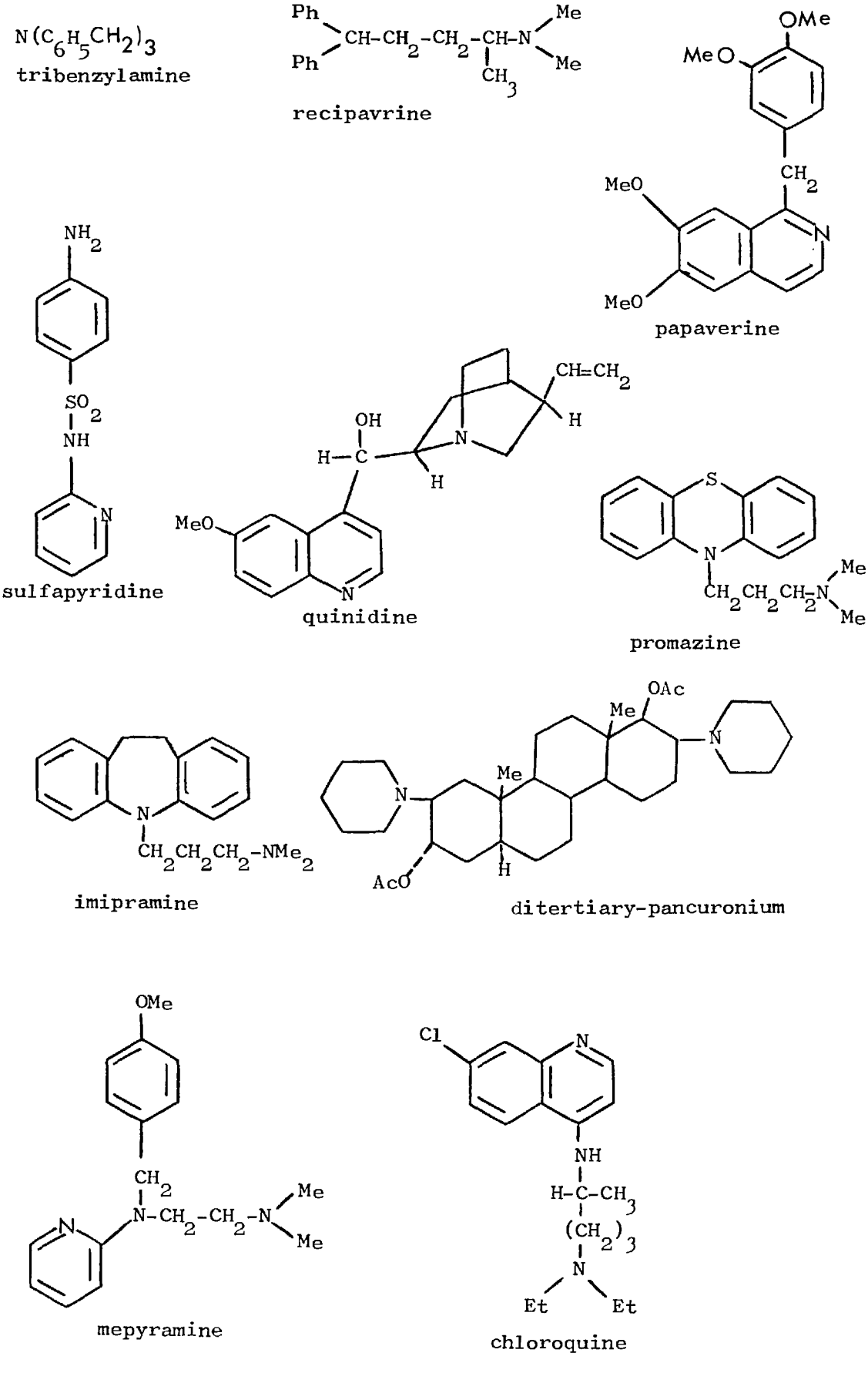
Table 6.2 Extractability in guinea pig 0-24h urine of chloroform-soluble ion-pair complexes with ^{125}I Rose Bengal after intraperitoneal injection of various drugs containing tertiary nitrogen functions

Drug	Dose ($mg\ kg^{-1}$)	Background extractable ^{125}I ^{125}I Rose Bengal
Tribenzylamine	10	-
Recipavrine	10	-
Papaverine	50	-
Sulfapyridine	50	+
Promazine	50	+
Quinidine	50	+
Imipramine	50	++
Ditertiary-Pancuronium	10	++
Mepyramine	50	+++
Chloroquine	50	+++

Key - No extractability
+ Intermediate extractability
++ Good extractability (2 times background)
+++ Extensive extractability (10 times background)

(for chemical structures see Fig 6.1)

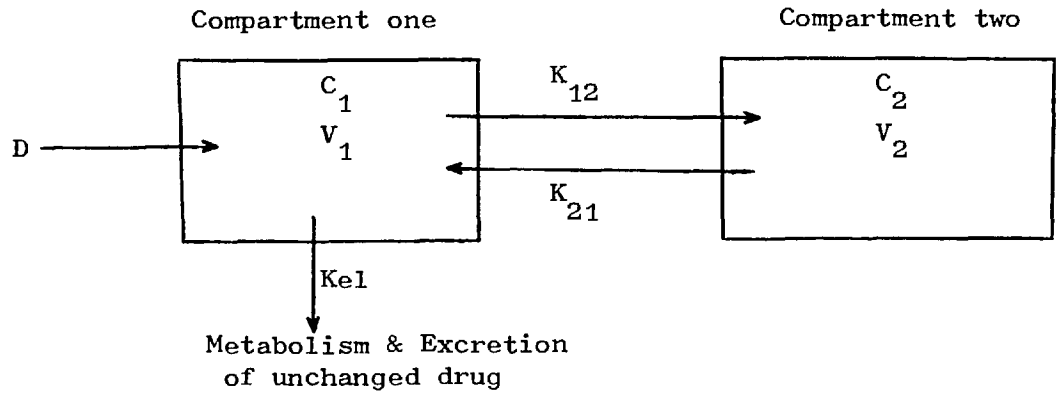
Fig. 6.1 Chemical structures of some commonly used drugs containing one or more tertiary nitrogen functions



APPENDIX

Appendix 1: "The two compartment open Model"

Fig A



D = Dose

C_1, C_2 = Concentration of drug in compartment one and compartment two, respectively at time t.

K_{12}, K_{21} = First order rate constants. K_{12} characterises the movement of drug from compartment one to compartment two, and K_{21} vice versa

V_1, V_2 = Compartmental volumes

Note: The two compartments and their derived volumes do not necessarily correspond to specific anatomical entities - they are theoretical spaces postulated to account for the experimental data.

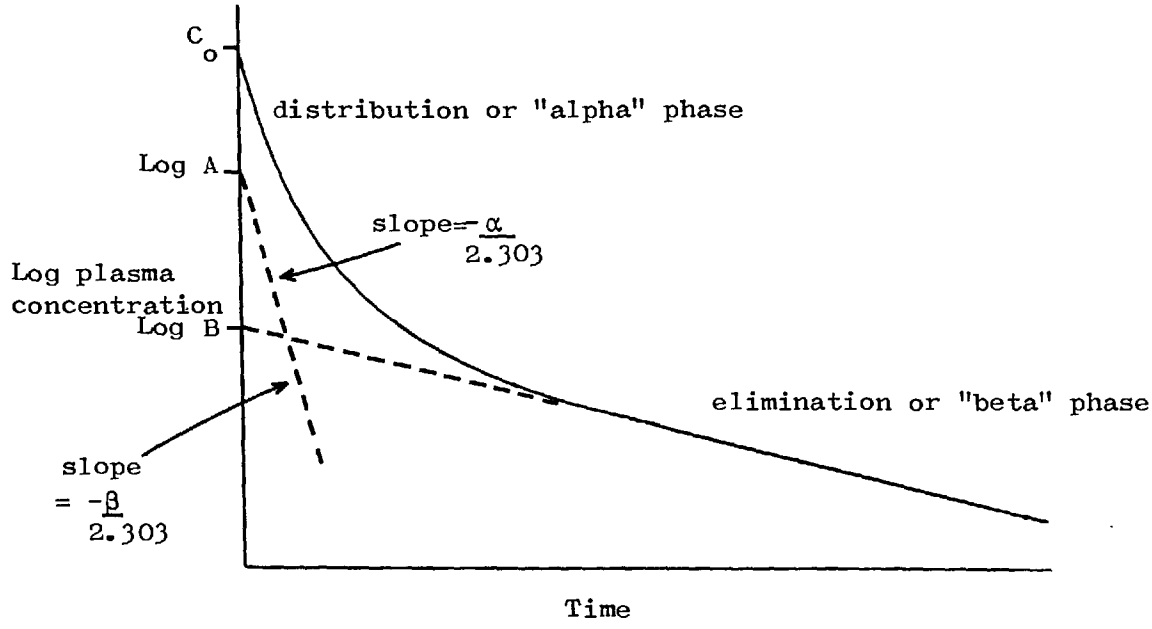
The equation describing the time course of a drug in plasma for intravenous administration is given by:

$$C_1 = A e^{-\alpha t} + B e^{-\beta t}$$

(for mathematical derivation see Gibaldi and Perrier, 1975).

A, α, B, and β are "hybrid" coefficients since their values are determined by both distribution and elimination processes (see Fig. B)

Fig B



The solid biexponential curve is the sum of two single exponents (dotted lines) A declining at rate α, and B declining at rate β Mathematical characterisation (see Appendix II) provides these hybrid coefficients which are then used to calculate the various pharmacokinetic parameters by application of a sequence of formulae (Greenblatt and Koch-Weser, 1975)

$Co = A + B$ — concentration of drug at time $t = O$

$AUC = \frac{A}{\alpha} + \frac{B}{\beta}$ — Area under the plasma concentration - time curve for $t = O$ to $t =$ infinity

$Kel = \frac{A + B}{Auc}$

$K_{21} = \frac{\alpha\beta}{Kel}$

$K_{12} = \frac{AB}{(A+B)^2} \times \frac{(\beta-\alpha)^2}{K_{21}}$

$V_1 = \frac{D}{Co}$ This volume is likely to represent those areas into which the drug can distribute rapidly such as plasma and parts of highly perfused organs.

Vd = Total apparent volume of distribution, and can be calculated by a number of methods :-

(1) "Steady-state" method

$Vd(SS) = V_1 \ (1 + \frac{K_{12}}{K_{21}})$ assumes no net transfer of drug between central and peripheral compartments; maintenance of steady-state requires a constant i.v. infusion of the drug

Since $V_2 = Vd - V_1$

$V_2 = V_1 \times \frac{K_{12}}{K_{21}}$

(2) "Area" method

$Vd(area) = \frac{D}{\beta \ (Auc)}$ provides the most useful estimate of this parameter

$Cp = V_1 \times Kel$ plasma clearance - the volume of the central compartment from which the drug is entirely eliminated (or cleared) in a specified unit of time.

Note: Changes in Cp may occur by alteration of either V_1 or Kel or both. For example in hypoproteinaemia, a drug may shift from compartment one to compartment two due to decreased protein binding resulting in a reduced clearance value.

$T1_2^{\alpha} = \frac{0.693}{\alpha}$: distribution half-life

$T1_2^{\beta} = \frac{0.693}{\beta}$: elimination half-life

(see text for units)

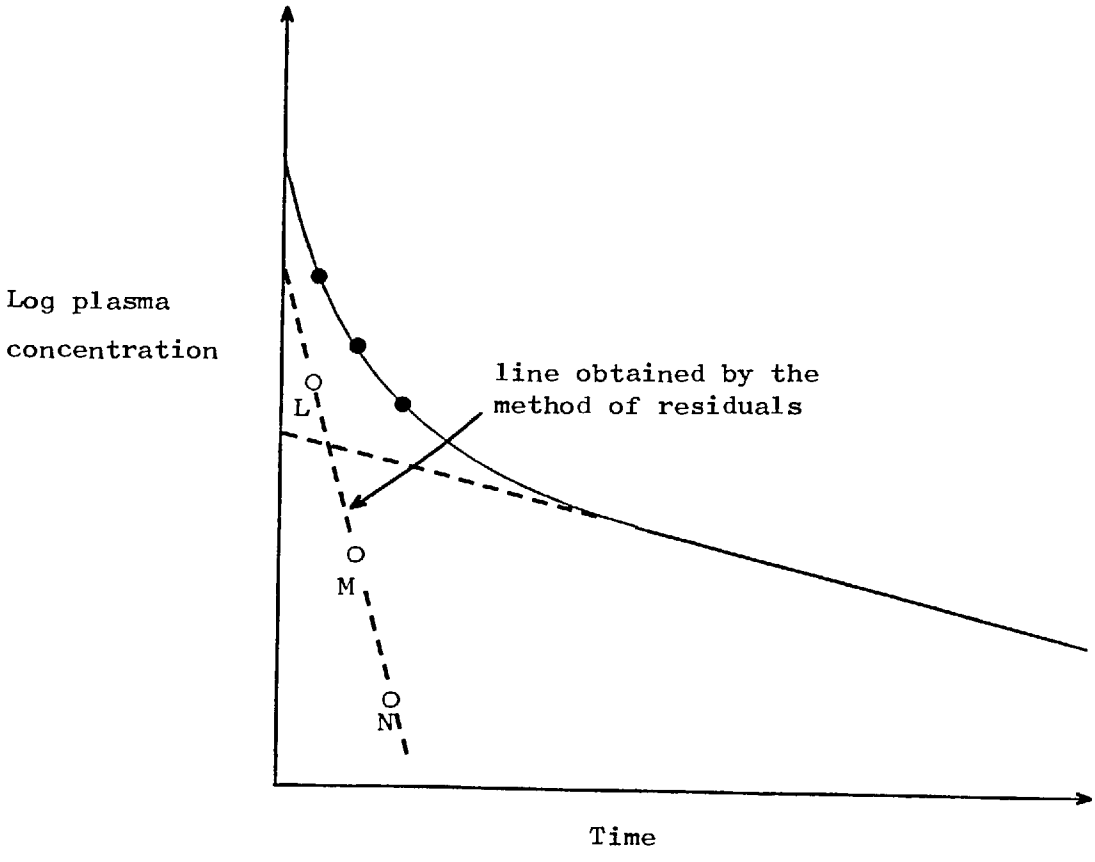
Appendix II: "Method of Residuals"

The method of residuals is frequently employed for resolving a curve into its various exponents. The terms "feathering" and "peeling" are also used in reference to this technique.

The two compartment open model can be described by the following biexponential equation

$$C_1 = Ae^{-\alpha t} + Be^{-\beta t} \quad \ldots\ldots (1)$$

A plot of the logarithm of drug plasma concentration versus time will yield a curve as depicted below:-



Note: Residuals L,M,N are obtained from antilog plasma concentrations and plotted on a Log scale.

The constant α is by definition larger than β, and therefore, at some time the term $Ae^{-\alpha t}$ will approach zero while $Be^{-\beta t}$ will still have a finite value. Equation (1) will then become

$$C_1 = Be^{-\beta t}$$

and

$$\log C = \log B - \frac{\beta t}{2.303}$$

This equation which describes the terminal linear phase has a slope of $-\beta/2.303$ with ordinal intercept of log B.

By substracting the concentration-time values on the interpolated line from the corresponding true plasma concentration-time values, a series of residual concentration-time values are obtained (e.g. points L, M and N) hence the residual concentration,

$$C_r = Ae^{-\alpha t}$$

and $$\log C_r = \log A - \frac{\alpha t}{2.303}$$

which yields a straight line of slope $-\alpha/2.303$ and an ordinal intercept of log A.

It is possible however to introduce considerable degrees of error by mistakes at this stage and these values are normally used as initial estimates for determination of the most accurate equation by utilising digital computer programs.

REFERENCES

Abbreviations are taken from the

World List of Scientific Periodicals

Fourth Edition (1900-1960)

References

Abderhalden, E. & Brahm, C. (1909) Hoppe-Seyler's Z. physiol.Chem., 62, 133.

Abrams, R.F. & Hornbein, T.F. (1975) Anesthesiology, 42, 362

Ackerman, D. (1912)Z. Biol., 59, 17

Adam, W.R. & Dawborn, J.K. (1970) Aust. Ann. Med., 19, 250

Adler, L & Pilon, R.D. (1972) Abstracts of Scientific Papers, American Society of Anesthesiologists Meeting p. 169-170

Agoston, S., Kersten, U.W. & Meijer, D.K.F. (1973a). Acta.anaesth. scand. 17, 129

Agoston, S., Vermeer, G.A., Kersten, U.W. & Meijer, D.K.F. (1973b) Acta. anaesth. scand., 17, 267

Agoston, S., Crul, J.F., Kersten, U.W. & Scaf, A.H.J. (1977) Anesthesiology, 47, 509

Agoston, S., Vermeer, G.A., Kersten, U.W. & Scaf, A.H.J. (1978) Br. J. Anaesth. 50, 347

Agoston, S., Feldman, S.A. & Miller, R.D. (1979) Anesthesiology, 51, 119

Aladjemoff, L., Dikstein, S. & Shafir, E. (1958) J. Pharmac. exp. Ther., 123, 43

Ali, H.H., Utting, J.E. & Gray, C. (1970) Br. J. Anaesth., 42, 967

Ali, H.H., Utting, J.E. & Gray, C. (1971a) Br. J. Anaesth., 43, 473

Ali, H.H., Utting, J.E. & Gray, C. (1971b) Br. J. Anaesth., 43, 478

Ali, H.H. & Savarese, J.J. (1976) Anesthesiology, 45, 216

Anand, J.S., Mehta, R.K., Munschi, C.A. & Mulla, D.H. (1972) Can. Anaesth. Soc. J., 19, 651

Anderson, N. (1962) Br. J. Anaesth., 34, 363

Anton, A.H. & Corey, W.T. (1971) Acta. pharmac . tox. 29, Suppl. III, 134

Auerbach, M.E. (1943) Ind. Engng. Chem. analyt. Edn. 15, 492

Auerbach, M.E. (1944) Ind. Engng. Chem. analyt. Edn. 16, 739

Axelrod, J. & Vessel, E.S. (1970) Molec. Pharmac. 6, 78

Axelrod, J., (1971) In : Concepts in Biochemical Pharmacology, 2, 609 Springer, Verlag, Heidelberg

Baird, W.L.M. & Reid, A.M. (1967) Br. J. Anaesth. 39, 775

Balasubramaniam, K., Mawer, G.E., Pohl, J. & Simons, P. (1972) Br. J. Pharmac., 45, 360

Ballard, C.W., Isaacs, J. & Scott, P.G.W. (1954) J.Pharm.Pharmac., 6, 971

Baraka, A. (1964) Br. J. Anaesth., 36, 272

Baxter, J.H. & Mason, M.F. (1947) J. Pharmac. exp. Ther., 91, 350

Belafasky, M.A. & Klawans, H.L. (1974) Anesthesiology, 40, 295

Bell, J.A. & Martin, L.E. (1979) Br. J. Pharmac., 66, 433

Bendixen, I.I.I.I., Surtees, A.D., Oyama, T. & Bunker, J.P. (1959) Anesthesiology, 20, 121

Bennett, A.E., McIntyre, A.R. & Bennett, A.L. (1940) J. Am. med. Ass., 114, 1791

Bennett, W.M., Singer, I. & Loggins, C.H. (1970) J. Am. med. Ass., 214, 196

Bernard, C. (1851) C.r. Seanc. Soc. Biol., 2, 195

Bernard, C. & Bunker, J.P., (1959) Anesthesiology, 29, 121

Berry, F.R. (1966) Br. J. Anaesth., 38, 829

Bevan, D.R. & Budhu, R. (1975) Br. J. Anaesth., 47, 634

Bianchetti, G., Graziani, G. & Brancaccio, D. (1976) Clin. Pharmacokinet., 1, 373

Biedor, A. & Brunnel, P. (1971) Annls. pharm. fr., 29, 461

Blogg, C.E., Savege, T.M., Simpson, J.C., Ross, L.A. & Simpson, B.R. (1973a) Proc. Roy. Soc. Med., 66, 1023

Blogg, C.E., Simpson, B.R., Martin, L.E. & Bell, J.A. (1973b) Br. J. Anaesth., 45, 1233

Bloom, P.M. & Nelp, W.P. (1966) J. Med.Sci., 251, 133

Boehm, R. (1886) Chemische Studien uber das Curare. Liepzig.

Bloger, L., Brittain, R.T., Jack, R.T., Jackson, M.R., Martin L.E., Mills, J., Poynter, D. & Tyers, M.B. (1972) Nature, 238, 354

Bono, F. & Mapelli, A. (1960) Minerva anest., 26, 29

Bovet, D., Bovett-Nitti, F. & Marini-Bettolo, G.B. (Eds) (1959) Curare and Curare-Like Agents, Elsevier Publishing Co., Amsterdam

Bray, G.A. (1961)Analyt.Biochem., 1, 279

Brazda, F.G. & Coulson, R.A. (1946) Proc. Soc. exp. Biol.Med., 62, 19

Bridgeman, J.F., Rosen, S.M. & Thorp, J.M. (1972) Lancet, 2, 506

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

Brittain, R.T. & Tyers, M.B. (1973) Br. J. Anaesth., 45, 837

Brodie, B.C. (1811) Phil.Trans. R. Soc. 101, 194

Brodie, B.C. (1812) Phil .Trans. R. Soc. 102, 194

Brodie, B.B. & Mitchell, J.R. (1973) In : Biological effects of drugs in relation to their plasma concentrations. (Ed. D.S. Davies and B.N.C. Pritchard) The Macmillan Press Ltd.

Brooks, D.K. & Feldman, S.A. (1962) Anaesthesia, 17, 161

Bryn, T.K. (1964) Curare, its History and Usage London : Pitman Medical Publishing Co.

Buzello, W. (1974) Anaesthesist , 23, 443

Buzello, W. (1975) Anaesthesist, 24, 13

Caldwell, J. (1979) Life Sci. 24, 571

Camu, F.& D'Hollander, A.A. (1978) Acta. anaesth. scand. 22, 221

Cantoni, G.L. (1953) J. biol. Chem., 204, 403

Chan, K.Williams, N.E., Baty,J.D. & Calvey,T.N. (1976) J. Chromat, 120, 349

Churchill-Davidson, H.C. (1954) Br. med. J., I, 74

Churchill-Davidson, H.C. (1965) Anesthesiology, 26, 132

Churchill-Davidson, H.C., Way, W.L. & deJong, R.H. (1967) Anesthesiology, 28, 540

Churchill-Davidson, H.C. (Ed.)(1978), : A practice of anaesthesia 4th Edition. Lloyd-Luke (Medical books) Ltd., London

Clausen, O.G. (1955) Medd. Norsk. Farm. Sels., 17, 124

Cochin, J. & Woods, L.A. (1951) J. Pharmac. exp. Ther., 101, 7

Cohen, E.N., Paulson, W.J. & Elert, B. (1957) Anesthesiology, 18, 300

Cohen, E.N. (1963) J. Lab. clin. Med., 61, 338

Cohen, E.N., Corbascia, A. & Fleischli, G. (1965) J. Pharmacol. exp. Ther., 147, 120

Cohen, E.N., Bremer, H.W. & Smith, D. (1967) Anesthesiology, 28, 309

Cohn, R. (1894) Hoppe-Seyler's Z. physiol. Chem., 18, 112

Coleman, A.J., Ripley, S.M., Sliom, O.M. & Knowles, S.L. (1966) In : Curare, p. 98-105. Basel : Schwabe & Co.

D'Anglera, P.M. (1516) In : De Orbo Novo translated by Augustus Macnutt New York. G.P. Putman's Sons, (1912) 1, 75

Dettli, L. & Spring, P. (1973) Proceedings of the 5th international congress on Pharmacology, 3, 165

Dost, F.H. (1953) Der Blutspiegel : Kinetik der Knozentration - sablaufe in der Kreislauffussigkeit. Leipzig : Thieme

Dowdy, E.G., and Fabian, L.W. (1963) Anesth. Analg. (Paris), 42, 501

Drayer, D.E. (1976) Clin. Pharmacokin., 1, 426

Drayer, D.E., Lowenthal, D.T., Woolsley, R.L., Nies, A.S. Schwartz, A. & Reidenberg, M.M. (1977) Clin. Pharmac. Ther., 22, 63

Dundee, J.W. & Richards, R.K. (1954) Anesthesiology, 15, 333

Duvaldestin, P., Henzil, D., Gilles, M.R., Roze, C. & Desmonts, J.M. (1977) Anesth. Analg. (Paris), 34, 235

Duvaldestin, P., Henzil, D., Demetriou, M. & Desmonts, J.M. (1978) Br. J. Anaesth., 50, 773

Eakins, K.E. & Katz, R.L. (1966) Br. J. Pharmac. Chemother., 26, 205

Elarmann, E.G., & Wright, E.S. (1948) Am. J. Pharmac., 120, 146

Elion, G.B., Yu, T., Gutman, A.B. & Hitchings, G.H. (1968) Am. J. Med., 45, 69

Englert,E., Brown, H., Willardson, D.G., Stanley, W. & Simons, E.L. (1958) J. clin. Endocr. Metab., 18, 36

Everett, A.J., Lowe, L.A. & Wilkinson, S. (1970) J. chem. Soc. D, 1020-1021

Fabre, J., de Freudenreich, J., Dunkert, A., Pitton, J.S., Rudhardt, M. & Virieux, C. (1967) Helv. med. Acta., 33, 307

Fabre, J. & Balant, L. (1976) Clin. Pharmacokin., 1, 99

Fairley, H.B. (1950) Br. Med. J., 2, 986

Farrell, P.C., Grib, N.L., Fry-Dell, (1972) Trans. Am. Soc. Artif. Intern. Organs, 18, 268

Feldman, S.A. (1963) Br. J. Anaesth., 35, 546

Feldman, S.A., & Levy, G. (1963) Br. J. Anaesth., 35, 804

Feldman, S.A., Cohen, E.N. & Golling, R.C. (1969) Anesthesiology, 30, 593

Feldman, S.A., & Tyrrell, M.F. (1970) Proc. Roy. Soc. Med. 63, 692

Feldman, S.A. (1973) In : Muscle Relaxants. London : Pitman Medical Publishing Co.

Feldman, S.A. (1975) Anesthesiology, 42, 644

Fleischli, G. & Cohen, E.N. (1963) Br. J. Anaesth., 35, 804

Flowtow, E. (1942) Pharm. Zeutralhalle Dtl., 83, 181

Foldes, F.F. (1960) Clin. Pharmac. Ther., 1, 345

Ford, G.C., Grigson, S.J.W., Haskins, N.J. Palmer, R.F., Prout, M. & Vos, C.W. (1967), Biomed. Mass. Spectrom, 4, 94

Gamstropp, I. & Vinnars, F. (1961) Acta.physiol.Scand., 53, 160

Geha, D.G., Blitt, C.D. & Moon, B.J. (1976) Anest. Analg.curr.Res.55,343

Ghoneim, M.M., Krammer, S.E., Barrow, R., Pandya, H. & Routh, J.I. (1973) Anesthesiology, 39, 410

Gibaldi, M., Levy, G. & Hayton, W.L. (1972a) Anesthesiology, 36, 213

Gibaldi, M., Levy, G. & Hayton, W.L. (1972b) Br. J. Anaesth., 44, 163

Gibaldi, M. & Perrier, D. (1975). Pharmacokinetics. Vol. 1 of Drugs and the Pharmaceutical Sciences. New York, Marcel Dekker.

Gill, R. (1940). In : White water and Black Magic. New York

Gissen, A.J., Karis, J.H. & Nastuk, W.L. (1966) J. Am.med.Ass.179, 770

Glick, D. (1941) J. biol. Chem., 137, 357

Goodman, L.S. & Gilman, A. (1975) The Pharmacological basis of Therapeutics, Macmillan Publishing Co. Inc. 5th Edition

Gottilieb, T.B. (1953) Dansk Tidsskr. Farm., 27, 199

Gray, T.C. (1954) Ann. R. Coll. Surg. 15, 402

Greenblatt, D.J. & Koch-Weser, J. (1975) N. Engl. J. Med., 293, 702

Griffith, H.R. & Johnson, G.E. (1942) Anesthesiology, 3, 418

Grisen, A.J. (1973) Anesthesiology, 39, 567

Gugler, R. & Azarnoff, D.L. (1976) Clin. Pharmacokin., 1, 25

Guyton, A.C. (1976) Textbook of medical physiology (5th Edition W.D. Saunders Company, Philadelphia, London, Toronto

Ham, J., Miller, R.D., Sheiner, L.B. & Matteo, R.S. (1979) Anesthesiology, 50, 528

Hanin, I. & Jenden, D.J. (1966) Experientia, 22, 537

Hanin, I. & Jenden, D.J. (1969) Biochem. Pharmac., 18, 837

Henegham, C.P.H., Findley, I.L. Gillbe, C.E. & Feldman, S.A. (1978) Br. J. Anaesth., 50, 1105

Herbertz, G., Metz, T., Reinauer, H. & Staib, N. (1973) Biochem. Pharmac ., 22, 1541

Higashimura, T., Yamada, O., Nohara, N. & Shidei, T. (1962) Int. J. appl. Radiat. Isotopes., 13, 308

Hirom, P.C., Millburn, P. & R.L. Smith (1976) Xenobiotica, 6, 55

His, W. (1887) Arch. exp. Path. Pharmak., 22, 253

Hoffman, A.W. (1851a) Justus Liebigs Annln. Chem, 78, 253

Hoffman, A.W. (1851b) Justus Liebigs Annln. Chem., 78, 253

Horowitz, P.E. & Spector, S. (1973) J. Pharmac. exp. Ther., 185, 94

Hosein, E.A., Proul, X.P. & Ara, R. (1962) Biochem. J., 83, 341

Hosein, E.A. & Koh, T.Y. (1965) Can.J.physiol. Pharmac., 43, 637

Hosein, E.A. & Orzeck, A. (1966) Nature, (Lond.), 210, 731

Hoshiai, Z. (1909) Hoppe-Seyler's, Z. physiol. Chem., 62, 118

Hug, C.C. (1978) Anesth. Analg. curr. Res., 57, 704

Hughes, R. (1970) Br. J. Anaesth., 42, 658

Hughes, R. (1973) Ph.D. Thesis University of London

Hughes, R.D., Millburn, P. & Williams, R.T. (1973) Biochem, J. 136, 979

Humboldt, A.v (1805) Voyage aux regions equinoxiales du nouveau continent, 5 , ii

Israili, Z.H., Dayton, P.G. & Kiechel, J.R. (1977) Drug Metab.Dispos., 5, 411

Jenkins, R. (1961) Br. J. Anaesth., 33, 314

Johansen, S.H., Jorgensen, M. & Molbach, S. (1964) J.Appl.Physiol., 19, 990

Joske, R.A., Ebeling, P. & Stainstreet, R.H. (1954) Med.J.Aust., 1, 856

Kalow, W. (1953) J. Pharmac. exp. Ther., 109, 74

Kalow, W. (1954) J. Pharmac. exp. Ther., 16, 433

Kalow, W. & Davies, R.O. (1958) Biochem. Pharmac., 1, 183

Kalow, W. (1959) Anesthesiology, 20, 505

Kamei, T. (1927) J. Biochem., Tokyo, 46, 967

Kapfhammer, J. & Bischoff, C. (1930) Hoppe Seyler's Z. physiol.Chem., 191, 179

Katz, B. (1966) Nerve muscle and Synapse, New York, McGraw-Hill

Katz, B. (1969) The release of neural tansmitter substance Liverpool University Press

Katz, R. L., Ngai, S.H. & Papper, E.M. (1963) Anesthesiology, 24, 18

Katz, R.L. (1965) Anesthesiology, 26, 832

Kennedy, B.R. & Farman, J.V. (1968) Br. J. Anaesth., 40, 773

Kersten, U.W., Meijer, D.K.F. & Agoston, S. (1973) Clin.chim.acta., 44, 59

Kessler, K.M., Lowenthal, D.T., Warner, H., Gibson, T., Briggs, W. & Reidenberg, M.M. (1974) N. Engl. J. Med., 290, 706

King, H. (1935) J. chem. Soc., 2, 1381-1389

Kirsten, W.J. Berggren, A. & Nilsson, K. (1958) Analyt. Chem., 30, 237

Kuhne, W. (1862) Uber die peripherischen Endergon der motorischen noven. Liepzig : Engelmann

Kunin, C.M., Glazko, A.J. & Finland, M. (1959) J. clin.Invest. 38, 1498

Kunin, C.M. & Finland, M., (1959a) Archs. inter. Med., 104, 1030

Kunin, C.M. & Finland, M., (1959b) J. clin. Invest., 38, 1509

Kutscher, F. & Lohman, Z. (1907) Unters Nahr-u. Genussm., 13, 177

Lee, C.M. (1975) Anesth. Analg. curr. Res., 54, 649

Levy, G. (1967) J. pharm. Sci., 56, 1687

Lincoln, P.A. & Chinnick, C.C.T. (1956) Analyst, 81, 100

Logan, D.A., Howie, H.B. & Crawford, J. (1974) Br. J. Anaesth, 46, 69

Loh, L. (1970) Anaesthesia, 25, 356

Lottermoser, A. & Stendel, R. (1938) Kolloid. Ztg., 83, 37

Lowenstein, E., Goldfine, C.& Flacke, W.E., (1970) Anesthesiology, 33, 556

Lowenthal, D.T., Briggs, W.A., Gibson, T.P., Nelson,H., & Cirksena, W.J. (1974a) Clin. Pharmac. Ther., 16, 761

Lowenthal, D.T., Briggs, W.A. & Levy, G. (1974b) J. clin.Invest.,54,1221

Lowenthal, D.T., Oie, S., Van-Stone, J.C., Briggs, W.A., Levy, G. (1976) J. Pharmac. exp. Ther., 196, 570

Lukas, D.S. & De Martino, A.G. (1969) J. clin. Invest., 48, 1041

Lunde, P.K.M. (1973) Proceedings of the 5th congress of Pharmacology, 3, 201

Maclagan, J. (1976). In : Neuromuscular Junction (Ed. Zaimis. E.) Springer Verlag Berlin Heidelberg, N.Y. chapter 4 Br. 421

Maddocks, J.L. Wake, C.J. & Harber, M.J. (1975) Br. J. clin. Pharmac., 2, 339

Mageda, K. & Ogata, M. (1914) Hoppe-Seyler's, Z. physiol.Chem., 89, 251

Manani, G., Galzigna, L., Mammano, S. & Gasparetto, A. (1972) Arzneimittel-Forsch. 22, 1526

Marsh, D.F. (1952) J. Pharmac. exp. Ther., 105, 299

Matteo, R.S., Spector, S. & Horowitz, P.E., (1974) Anesthesiology,41,440

McIntyre, A.R. (1947) In : Curare, its History, Nature and Clinical Use Chicago; Univ. Chicago Press

McLaughlin, A.P., Altwein, J.E., Kessler, W.A. & Gittes, R.F. (1972) J. Urol., 108, 515

McLeod, K., Watson, M.J. & Rawlins, M.D. (1976) Br. J. Anaesth., 48, 341

Merton, P.A. (1954) J. Physiol., Lond., 123, 553

Metcalfe, L.D., (1963) J. Am. Oil. Chem. Soc., 40, 25

Michoel, A.J. & Knight, R.D. (1976) J. Ass.off.anal. Chem., 59, 802

Miller, R.D., Way, W.L., Donlan, W.M., Stevens, W.C. & Eger, E.I.. II (1971) Anesthesiology, 35, 509

Miller, R.D. Stevens, W.C., & Way, W.L. (1973) Anesth. Analg. curr. Res., 52, 661

Miller, R.D. & Cullen, D.J. (1976) Br. J. Anaesth., 48, 253

Miller, R.D., Matteo, R.S., Benet, L.Z. & Sohn, Y.J. (1977) J. Pharmac. exp. Ther., 202, 1

Mitchell, R. & Clarke, B.B. (1952) Proc. Soc. exp. Biol. Med., 81, 105

Modin, R. & Schill, G. (1967) Acta. Pharm. Suecica, 4, 301

Montgomery, J.B. & Bennett-Jones, N. (1956) Lancet, 2, 1243

Mule, S.J., Bostos, M.L., Jukofsky, D. & Saffer, E. (1971) J. chromat., 63, 289

Muscholl, E. & Maitre, L. (1963) Experientia, 19, 658

Mushin. W.W., Wein, R., Mason, D.F.J., Langston, G.T. (1949) Lancet, 1, 726

Oates, J.A., Mitchell, J.R., Feagin, O.T., Kaufmann, J.S. & Shand, D.G. (1971) Ann. N.Y. Acad. Sci., 179, 302

Odar-Cederlof, I. & Borga, O. (1974) Eur. J. clin. Pharmac., 7, 31

Ogg, C.S., Toseland, P.A. & Cameron, J.S. (1968) Br. med. J., 2, 283

Okuda, Y. (1959a) J. Biochem., Tokyo, 46, 73

Okuda, Y. (1959b) J. Biochem., Tokyo, 46, 967

Organe, G.S.W. (1956) Can. Anaesth. Soc. J., 3, 5

Pantuck, E.J. (1967) In : Advances in anaesthesiology Muscle Relaxants. Chapter 5, p.63 N.Y. : Hoeber

Paton , W.D.M. & Zaimis, E., (1949) J. Physiol., Lond., 4, 381

Paton, W.D.M. & Perry, W.L.M. (1951) Br. J. Pharmac., 6, 299

Paton, W.D.M. & Waud, D.R. (1967) J. Physiol., Lond., 191, 59

Payne, J.P. (1958) Br. J. Anaesth., 30, 206

Payne, J.P. (1959) Acta anaesth. Scand., 3, 53

Payne, J.P. (1960) Acta anaesth. Scand., 4, 83

Pierides, A.M., Alvarex-Ude, F., Kerr, D.N.S. & Skillen, A.W. (1975) Lancet, 2, 1279

Pohlmann, L.W. (1977) J. Chromat., 131, 297

Popescu, D.T. (1972) In : Anaesthesia and Pharmaceutics (Eds. J. Spierdijk & S.A. Feldman) p.74. Leiden : Leiden University Press

Powell, M.J.D. (1968) Harwell report, AERE-R5947. H.M. Stationery Office

Prescott, A.B. (1896) J. Am. chem. Soc., 18, 91

Prescott, L.F. (1969) Clin. Pharmac. Ther., 10, 383

Prescott, L.F. (1971) Scott. med. J. 16, 122

Prudhomme, M.R.D. (1938) Bull.Soc. path. exot., 31, 929

Prudhomme, M.R.D. (1940) J. pharm. chim., 9, 8

Rabkin, R., Simon, N.M. & Steiner, S. (1970) N. Engl. J. Med., 282, 182

Reddi, K.K. & Kodicek, E. (1953) Biochem. J., 53, 286

Reid, W.D., Hanbrich, D.R., & Krishna, G. (1971) Analyt.Biochem., 42, 390

Reidenberg, M.M. Odar-Cederlof, I., Von Bahr, C., Borga, O & Sjoqrist, F. (1971) N. Engl. J. Med., 285, 264

Reidenberg, M.M. (1972) Proceedings of the 5th congress on Pharmacology 3, 174

Reidenberg, M.M., James, M. & Dring, L.G. (1972) Clin. Pharmac. Ther., 13, 279

Reidenberg, M.M. & Affrime, M. (1973) Ann. N.Y. Acad. Sci., 226, 115

Reidenberg, M.M. Sheer, L. & Cohen, R.V. (1973) Am. Rev. Resp. Dis., 108, 1426

Reidenberg, M.M. & Drayer, D.E. (1978) Drug Metab. Rev., 8, 293

Riegelman, S., Loo, J.C.K. & Rowland, M. (1968) J. pharm. Sci., 57, 117

Riff, L.J. & Jackson, G.G. (1971) J. infec. Dis., 124, (suppl), 98

Riggs, D.S., (1963). Mathematical Approach to Physiological Problems p. 203 Baltimore : Williams and Wilkins Co.

Riker, W.F. & Wescoe, W.C. (1951) Ann. N.Y. Acad. Sci., 54, 373

Riordan, D.D. & Gilbertson, A.A. (1971) Br. J. Anaesth., 43, 506

Roe, R.B. (1962) Anaesthesia, 17, 179

Rouse, J.M., Galley, R.L.A., Bevan, D.R., (1977) Anaesthesia, 32, 247

Savarese, J.J. & Kitz, R.J. (1975) American society of Anesthesiologists. Refresher courses in Anesthesiology. Volume III Lippincott, Philadelphia

Schanker, L.S. (1962a) Pharmac. Rev. 14, 501

Schanker, L.S. (1962b) Biochem. Pharmac., II, 253

Schill, G. (1965) Acta Pharm. Succicca, 2, 13

Shanks, C.A., Somogyi, A.A. & Triggs, E.J., (1978) Br. J. Anaesth., 50, 235

Shaw, F.H. (1938) Biochem, J., 32, 1002

Shindo, H., Nakajima, E., Miyakoshi, N. & Shigelaia, E. (1974) Chem. pharm. Bull., Tokyo, 22, 2502

Simpson, B.R., Strunin, L., Savege, T.M., Walton, B., Foley, E.I., Maxwell, M.P., Ross, L.A. & Harris, D.M. (1972) Lancet, 1, 516

Singer, M.M., Dutton, R. & Way, W.L., (1971) Br. J. Anaesth., 43, 404

Soloman, H.M. (1973) Introductory remarks. Ann. N.Y. Acad. Sci. 226, 5

Somogyi, A.A., Shanks, C.A. & Triggs, E.J. (1976) Eur. J. clin. Pharmac., 10, 367

Somogyi, A.A., Shanks, C.A. & Triggs, E.J. (1977) Eur. J. clin. Pharmac., 12, 23

Stevens, H.M. & Moffat, A.C. (1974) J. forens. Sci. Soc., 14, 141

Stovner, J. & Lund, I. (1970) Br. J. Anaesth., 42, 235

Szeto, H.H., Inturrisi, C.E., Houde, R., Soal, S., Cheigh, J. & Reidenberg, M.M. (1977) Ann. intern. med., 86, 738

Takahashi, M. (1927) Hoppe-Seyler's Z. physiol. Chem., 169, 297

Tamura, S. (1924) Acta Sch. med. Univ. Kioto, 6, 449, 459

Tanaka, K., Hioki, M. & Shindo, H. (1974) Chem. pharm. Bull., 22, 2599

Testa, B. & Jenner, P. (1976). Drug Metabolism - chemical and biochemical aspects. In : Drugs and the Pharmaceutical Sciences., Vol.1 New York, Marcel Dekker.

Theslef, S. (1951) Nord. med., 46, 1045

Thompson, J.M. (1976) Anaesthesia, 31, 219

Tomita, M. (1921) Biochem. Z., 116, 55

Totani, G. & Hoshiai, Z. (1910) Hoppe-Seyler's, Z. physiol. Chem., 68, 83

Wagner, J.G., (1971) Biopharmaceutics and relevant pharmacokinetics Hamilton, Illinois, Drug Intelligence publications

Wall, E.N. & McKenna, J. (1970) J. chem. Soc. B, 318

Walts, L.F., Lebowitz, M. & Dillon, J.B. (1967) Br. J. Anaesth., 39, 845

Walts, L.W. & Dillon, J.B. (1968) Anesthesiology, 29, 499

Warrington, H.P., (1961) Analyt. Chem., 33, 1898

Waud, B.E. & Waud, D.R. (1971) Anesthesiology, 35, 456

Waud, B.E. & Waud, D.R. (1972a) Anesthesiology, 37, 413

Waud, B.E. & Waud, D.R. (1972b) Anesthesiology, 37, 417

Waud, B.E. (1975) Anesthesiology, 43, 381

Waser, P.G. & Luthi, V. (1968) J. nucl. Biol. Med., 12, 4

Watson, M.J. & McLeod, K. (1977) Clin.Chim.Acta, 79, 511

West, R. (1932) Proc. Roy. Soc. Med., 25, 1107

Westgate, H.D. & Van Bergen, F.H. (1962) Can. Anaesth. Soc. J., 9, 497

White, R.D., De Wreed, J.H. Dawson, B. (1971) Anesth. Analg. curr. Res., 50, 11

Williams, R.T. (1959) In : Detoxication Mechanisms, 2nd Ed. Chapman & Hall Ltd., Lon.

Wilson, J.B. (1943) J. Assoc. off. agric. Chem., 29, 310

Wilson, J.B. (1952a) J. Assoc. off. agric. Chem., 35, 455

Wilson, J.B. (1952b) J. Assoc. off. agric. Chem., 35, 459

Wilson, J.B. (1954) J. Assoc. off. agric. Chem., 37, 379

Wingard, C.B. & Cook, D.R. (1976) Br. J. Anaesth., 48, 839

Yoshima, K. & Morita, M. (1955a) Bull.natn.hyg.Lab., Tokyo, 73, 141

Yoskima, K. & Morita, M. (1955b) Pharm. Bull., Tokyo, 3, 432

Zaimis, E.J., Cannard, T.H. & Price, H.L. (1958) Science, 128, 34

Zaimis, E. & Head, S. (1976). In : Neuromuscular Junction (Ed. Zaimis, E.) Chapter 4a. Pg. 365. Springer-Verlag, Berlin, Heidelberg, New York

Zeh, D.W. & Katz, R.L. (1978) Anesth. Analg. curr. Res., 57, 13

A rapid radiometric method for the determination of pancuronium and other quaternary ammonium compounds in biological fluids

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Mono- and bis-quaternary ammonium compounds are of considerable biological interest. Various methods have been described for their assay in biological fluids, including spectro photometric and fluorimetric procedures (Cohen, 1963; Buzello, 1974), gas chromatography (Chan, Williams, Baty & Calvey, 1976) and radioimmunoassay (Horowitz & Sector, 1973).

We have developed a simple, convenient adaption of the original method of Cohen (1963) for (+)-tubocurarine which involves the interaction of the quaternary ammonium compound with Rose Bengal to form a fluorescent complex, readily extractable into organic solvent, whose fluorescence is then measured. In our modified method, urine or plasma containing the quaternary ammonium compound is treated with [^{125}I]-rose bengal. The two interact to form a lipid-soluble radiolabelled ion-pair, readily extractable into an organic solvent. The extractable ^{125}I is then determined by scintillation counting and this, after subtraction of background, is directly proportional to the concentration of pancuronium.

The recoveries of known amounts (10 ng–1 μg) of pancuronium, added to plasma or urine and assayed radiometrically were 88% (range 78–101) and 94% (range 89–109) respectively.

Investigation of other quaternary ammonium compounds showed that tetraethylammonium, neostigmine, succinylcholine and decamethonium did not form extractable radiolabelled complexes with [^{125}I] rose bengal while (+)-tubocurarine and fazadinium formed extractable labelled complexes.

The radiometric method for pancuronium was applied to the determination of its pharmacokinetic parameters after its intravenous administration to two patients undergoing surgery. The plasma concentration-time curves showed a bi-exponential decay pattern which was analysed assuming a two-compartment open model. The various pharmacokinetic parameters of this model in the two subjects were:

A (ng/ml), 803, 1388; α (min^{-1}) 0.180, 0.345;
B (ng/ml), 217, 258; β (min^{-1}) 0.0058, 0.020;
V_1 (ml/kg), 131, 65; V_2 (ml/kg) 362, 180;
$V_{d\ area}$ (ml/kg), 549, 315; K_{12} (min^{-1}) 0.118, 0.197;
K_{21} (min^{-1}) 0.043, 0.071;
K_{el} (min^{-1}) 0.024, 0.097; $T_{\frac{1}{2}\alpha}$ (min) 3.86, 2.00;
$T_{\frac{1}{2}\beta}$ (min), 120, 80; AUC (μg ml^{-1} min^{-1}) 43, 17.

These values are broadly in agreement with those of Somogyi, Shanks & Triggs (1976) and McLeod, Watson & Rawlins (1976) who used the fluorimetric complexing method for analysis. Radiometric analysis of the urine showed that both patients excreted about 70% of the dose in the urine within 30 h, some 30–40% being eliminated within the first 4 h. A similar radiometric method for the estimation of fazadinium in biological fluids has been developed and it is being used to evaluate the pharmacokinetics of this drug.

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References

BUZELLO, W. (1974). Die Kolorimetrische Bestimmung von Pancuronium und seinen Desacetylierungsderivaten in Korperflussigkeiten des Menschen. *Anaesthesist*, **23**, 443–449.

CHAN, K., WILLIAMS, N.E., BATY, J.D. & CALVEY, T.N. (1976). A quantitative gas-liquid chromatographic method for the determination of neostigmine and pyridostigmine in human plasma. *J. Chromat.*, **120**, 349–358.

COHEN, E.N. (1963). Fluorescent analysis of d-tubocurarine hydrochloride. *J. Lab. clin. Med.*, **61**, 338–345.

HOROWITZ, P.E. & SPECTOR, S. (1973). Determination of serum d-tubocurarine concentration by radioimmunoassay. *J. Pharmac. exp. Ther.*, **185**, 94–100.

McLEOD, K., WATSON, M.J. & RAWLINS, M.D. (1976). Pharmacokinetics of pancuronium in patients with normal and impaired renal function. *Br. J. Anaesth.*, **48**, 341–345.

SOMOGYI, A.A., SHANKS, C.A. & TRIGGS, E.J. (1976). Clinical pharmacokinetics of pancuronium bromide. *Eur. J. clin. Pharmac.*, **10**, 367–372.

^{125}I-labelled Rose Bengal in the quantitative estimation of fazadinium and other quaternary ammonium compounds in biological fluids

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In the original Rose Bengal ion-complexing method of Cohen (1963) for the estimation of bis-quaternary ammonium compounds such as fazadinium, the drug interacts with Rose Bengal to form an ion-pair complex which is readily extractable into an organic solvent and whose fluorescence is measurable. The fluorescent complex is not stable though this can be improved by the use of ethanol in the assay (Watson & McLeod 1977), and difficulties may be encountered when drug concentrations are low, or with small volumes of biological fluids. The instability can be overcome by replacing the dye as the complexing agent by ^{125}I-labelled Rose Bengal which allows the complex to be determined by scintillation counting.

Materials. [^{125}I] Rose Bengal (specific activity 0·7mCi mg^{-1}) was purchased as a sterile aqueous solution of its sodium salt from Radiochemical Centre, Amersham, U.K. For the assay, this was diluted with carrier Rose Bengal to provide a stock solution of the dye 100 μg ml^{-1}. Fazadinium bromide (Fazadon) m.p. 215 °C (uncorr) was a gift from Allen & Hanburys Research Ltd., Ware, Herts., U.K.

Assay of fazadinium in human plasma. For calibration, an aqueous solution of fazadinium bromide was added to plasma samples to provide concentrations from 0·02–100 μg ml^{-1}. To each sample (0·5 ml in duplicate) in a 15 ml stoppered extraction tube was added 0·2 M phosphate buffer pH 7·8 (1 ml) and [^{125}I] Rose Bengal solution (0·5 ml containing approx. 2×10^6 d min^{-1}), then the solutions were mixed by vortexing for 30 s, extracted with chloroform (5 ml) for 15 min on a rotary mixer to avoid emulsions and centrifuged at 2500 rev min^{-1} for 10 min. The upper aqueous layer was removed with a Pasteur pipette attached to a water pump and aliquots (2×1 ml) of the chloroform extract transferred by automatic pipette to a 5 ml scintillation counter insert tube. The chloroform was then evaporated off at room temperature with nitrogen, the whole process taking about 10–15 min. The residue was then dissolved in 4 ml of scintillation fluid (Bray 1960) and the ^{125}I determined using a Packard Tri-Carb liquid Scintillation Spectrometer (Model 3385). The efficiency of counting was about 80%. Blank plasma samples (without added fazadinium) were similarly treated for estimation of background extractable ^{125}I. The calibration curve is linear over a concentration range of 0·01–10 μg, with a correlation coefficient of 0·99. The reproducibility of the assay over this concentration range was ±5% (n = 5).

Fazadinium in plasma samples from patients, was measured by taking three aliquots (0·5 ml) of each sample through the above procedure and determining the concentration of drug by reference to the calibration curve.

The drug was not appreciably bound to red blood cells, since addition of known concentrations (0·01–50 μg ml^{-1}) to whole blood gave a recovery of 89% (range 85–102) from plasma. The assay appears to measure only the unbound fraction of the drug in plasma, since after ultrafiltration of plasma to which fazadinium had been added, the ultrafiltrate contained the same amount of the drug as was measured by assay of the whole plasma.

Table 1 shows that the ability to form a chloroform-soluble ion-pair complex with Rose Bengal is not restricted to fazadinium. Although the simple mono-quaternary ammonium compounds, tetraethylammonium, neostigmine and MIP did not complex, higher

Table 1. Formation by some quaternary ammonium compounds of chloroform-soluble ion-pair complexes with [^{125}I] Rose Bengal. 1 μg of each drug was added as an aqueous solution of the appropriate salt to 0·2 M phosphate buffer pH 7·8 (1 ml) then complexed and treated as described in the text.

Compound	Mol. wt of cation	Chloroform-extractable ^{125}I (counts min^{-1}) formed from interaction of the compounds (1 μg) with [^{125}I] Rose Bengal
Mono-quaternary ammonium		
Tetraethylammonium	136	0
2-Methyl-3-phenylimidazole (1, 2a)pyridinium (MIP)	210	24
Neostigmine	223	0
Dibenzyldimethyl-ammonium	226	7720
Cetyltrimethylammonium	285	29 180
Tribenzylmethyl-ammonium	302	6900
Bis-quaternary ammonium		
Succinylcholine	290	0
Decamethonium	258	0
Paraquat	186	9020
Diquat	184	3750
Fazadinium	444	78 300
Pancuronium	572	16 098
(+)-Tubocurarine	625	14 800

* Correspondence.

molecular weight mono-quaternary ammonium compounds, dibenzyldimethyl-, tribenzylmethyl- and cetyltrimethyl-ammonium did so. All, but succinylcholine and decamethonium, of the bis-quaternary ammonium compounds examined, formed extractable complexes, the most extensive extraction being with the higher molecular weight compounds, (+)-tubocurarine and fazadinium, suggesting that molecular weight may be a factor influencing the extent of formation of lipid-soluble complexes with the dye.

The assay was used to determine the pharmacokinetic parameters of fazadinium in man. Five patients (2M, 3F; ages 52–74 years, wt 53–80 kg), with normal hepatic and renal function, after induction of anaesthesia, had an indwelling cannula placed in a vein in the antecubital fossa. A blood sample (25 ml) was taken immediately before the administration of the drug (i.v.) and this was used as a blank for subsequent analysis and to establish the calibration curve. Further blood samples (5 ml) were taken into heparinized tubes at regular intervals up to 3 h after the drug had been given. The plasma was separated by centrifugation and the concentration of drug present determined. Plasma concentration data were analysed assuming a two-compartment open model (Reigelman et al 1968). Data were processed using a digital computer with the E04FBF program (Nottingham Algorithms Group) which requires initial estimates of the zero-time intercepts (A, B) of the two components of the bi-exponential decay and the slopes of these lines (α, β). These parameters were obtained from log plasma concentration-time plots by feathering. The dimensions of the compartments, the rate constants and other parameters of the model were calculated from A, α, B and β using the equations of Greenblatt & Koch-Weser (1975). Attempts were also made to fit the data to a three-compartment open model using the above program.

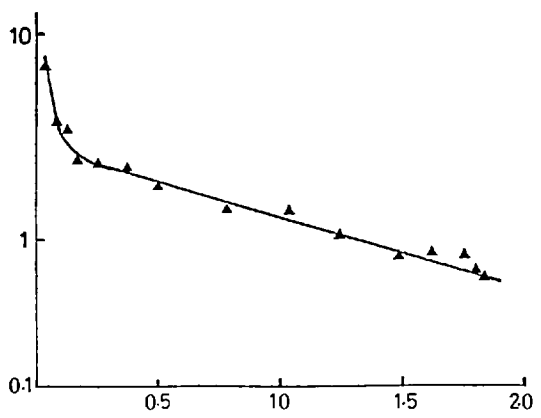

FIG. 1. A representative log plasma concentration-time curve of fazadinium (▲) after i.v. injection of 70 mg. Ordinate: plasma concentration (μg ml^{-1}). Abscissa: time (h).

The disappearance of the drug from plasma exhibited a bi-exponential decay pattern (Fig. 1), and this was analysed assuming a two-compartment open model, the parameters of which (mean ± s.e.) are shown in Table 2.

In all cases studied, plasma concentrations at 2 min post-injection were greater than 10 μg ml^{-1}, and declined rapidly over 10 min. A slower elimination phase followed, and at 1 h after injection the plasma value was 2·2 ± 0·5 μg ml^{-1} (mean ± s.e., n = 5).

The present results are similar to those of Blogg et al (1973) who used [^{3}H]-labelled fazadinium and measured plasma radioactivity after its intravenous administration.

Comparison of our results with those of other workers for other neuromuscular blocking drugs shows broad similarities between fazadinium and related compounds. Thus, the diquaternary ammonium compound, pancuronium, also shows a biphasic plasma elimination curve which is fitted best by a two-compartment open model (Somogyi et al 1976) the important dimensions of which, i.e. volumes of distribution, microconstants, half lives, were of the same order as those we report.

There has been some controversy over whether the pharmacokinetics of neuromuscular blocking drugs are better described by a three-compartment rather than a two-compartment model. Thus, Agoston et al (1973) claimed that the plasma level-time curve of pancuronium in man is triphasic, but Somogyi et al (1976), who fitted similar data to both two and three-compartment open models, showed that three compartments had no advantages with this drug. However Agoston et al (1978) show a triphasic plasma elimination curve for the triquaternary ammonium compound gallamine, although the only dimensions of the model given are the half lives of the three phases.

When we attempted to fit our data to a three-compartment open model in three cases there was a slight improvement, but in one case, the two-compartment

Table 2. Pharmacokinetic parameters of fazadinium in patients.

	Mean	s.e.
A (μg ml^{-1})	92·02	45·84
α (min^{-1})	0·509	0·11
B (μg ml^{-1})	5·30	1·74
β (min^{-1})	0·015	0·002
V_1 (ml kg^{-1})	41	15
V_2 (ml kg^{-1})	143	52
V_d area (ml kg^{-1})	226	76
k_{12} (min^{-1})	0·29	0·04
k_{21} (min^{-1})	0·07	0·01
k_{el} (min^{-1})	0·17	0·09
Area under curve (ml min^{-1})	410	113
Plasma clearance (μg ml^{-1} min^{-1})	185	49
$t_{\frac{1}{2}}\alpha$ (min)	1·58	0·28
$t_{\frac{1}{2}}\beta$ (min)	50	6

model was better and in one case the data could not be fitted satisfactorily to a three-compartment model. We therefore conclude that the simplest model consistent with our data is the two-compartment open model.

We have found our adaptation of the method rapid, sensitive and convenient such that large numbers of biological samples can be handled simultaneously.

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REFERENCES

Agoston, S., Vermeer, G. A., Kersten, U. W., Meijer, D. K. F. (1973) Acta Anaesthesiol. Scand. 17: 267

Agoston, S., Vermeer, G. A., Kersten, U. W., Scaf, A. H. J. (1978) Br. J. Anaesth. 50: 345

Blogg, C. E., Simpson, B. R., Martin, L. E., Bell, J. A. (1973) Ibid., 45: 1233–12

Bray, G. A. (1960) Analyt. Biochem. 1: 279–285

Cohen, E. N. (1963) J. Lab. Clin. Med. 61: 338–345

Greenblatt, D. J., Koch-Weser, J. (1975) New Engl. J. Med. 293: 702–705

Reigelman, S., Loo, J. C. K., Rowland, M. (1968) J. Pharm. Sci. 57: 117–123

Somogyi, A. A., Shanks, C. A., Triggs, E. J. (1976) Eur. J. Clin. Pharmacol. 10: 367–372

Watson, M. J., McLeod, K. (1977) Clin. Chim. Acta 79: 511–512

Steady state plasma concentration of clonidine and its relation to the effects on blood pressure in normotensive and hypertensive rats

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In a previous study it was found that clonidine given intravenously as bolus doses of less than 20 μg kg^{-1} produced a dose-dependent hypotensive response in conscious normotensive rats. When the intravenous dose was increased, an initial short hypertensive phase became more and more dominant and in doses from 40–500 μg kg^{-1} a clear dose-dependent increase of blood pressure was obtained. The subsequent fall in blood pressure was delayed up to 2 h in the dose of 500 μg kg^{-1} (Paalzow & Edlund 1978). Obviously, in a certain dose range clonidine produces a hypotensive response, while in a higher dose-range the hypertensive effects dominate. It has been suggested that the hypotensive response of clonidine is induced by an inhibition of the sympathetic outflow from the brain (Schmitt et al 1968; Klupp et al 1970; Kobinger 1973; Haeusler 1974). The intial rise in blood pressure after i.v. bolus doses which has been observed both in man and in animals, has been suggested to be due to a stimulation of peripheral α-adrenoceptors (Schmitt et al 1971, 1973; Finch 1974; Ozawa et al 1977) as well as due to a central mechanism (Trolin 1975). We have aimed to evaluate the relationship between the steady state plasma concentrations of clonidine and the effects on arterial blood pressure in conscious normotensive and spontaneous hypertensive rats (SHR).

Male Sprague Dawley normotensive rats, 162–315 g, and SHR/Okamoto rats, 191–287 g, were used. Blood pressure was recorded in conscious rats through an indwelling carotid arterial catheter (Silastic o.d. 0·025 in) exteriorized at the back of the neck and connected to a pressure transducer (Statham P23DC) writing on a Grass Polygraph.

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Blood pressure was expressed as mean arterial blood pressure. Clonidine was infused through a catheter in the jugular vein exteriorized together with the arterial catheter. The two consecutive intravenous infusions technique described by Wagner (1975) was used to rapidly attain steady state plasma concentrations of clonidine. Variables needed for a given drug to be used in this technique are plasma clearance and half-life. These data were obtained from a previous study (Paalzow & Edlund 1979). The first more rapid infusion lasted for 30 min; this was then abruptly changed to a lower rate, which was maintained for the 2 h experiment. 15 min after the end of the first infusion, steady state plasma concentrations of clonidine were obtained and to check that these were maintained and in agreement with theoretically calculated values, plasma concentrations were determined from blood samples withdrawn from the jugular vein. Clonidine concentrations were assayed by gas liquid chromatography according to Edlund & Paalzow (1977). The initial infusion rate (Q_1) is given by equation 1 and the final rate (Q_2) by equation 2.

$$Q_1 = \frac{Q_2}{1-e^{-\beta \cdot 30}} \quad (\text{ng min}^{-1}\,\text{kg}^{-1}) \qquad (1)$$

$$Q_2 = \frac{\text{Dose} \cdot C_{p_{ss}}}{A/\alpha + B/\beta} \quad (\text{ng min}^{-1}\,\text{kg}^{-1}) \qquad (2)$$

A, B, α, β are the coefficients and exponents of the bi-exponential equation describing the disposition of clonidine in plasma after an intravenous bolus dose (Edlund & Paalzow 1977; Paalzow & Edlund 1979).

In normotensive rats, a steady state value above 0·5 ng ml^{-1} was needed to obtain a decrease in blood

The urinary excretion of ketamine and its metabolites in the rat

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The effect of altering urinary pH of adult male Wistar rats (250–300 g.b.wt.), on the duration of sleeping time following a single injection of ketamine (75 mg kg i.p.) and the urinary excretion of ketamine and its metabolites was measured. The rats were divided into three groups of ten animals. The first group (A) were given 2% ammonium chloride to drink for 5 days, the second group (B) 2% sodium bicarbonate for 5 days and the third group (C) were given water. After this period the urine from animals in group A had a pH of 5.50 ± 0.34 (s.e. mean), group B had a pH of 8.80 ± 0.05 and the control animals a pH of 6.90 ± 0.23.

The rats were injected with ketamine and the sleeping time, that is the time between the loss and regaining of the righting reflex, was measured. The urine was collected for the two hour period following the injection and assayed for ketamine and its metabolites by a gas-liquid chromatographic method based on that described by Chang & Glazko (1972). There was no difference in the onset time of anaesthesia between the three groups, but the sleeping time was significantly prolonged ($P < 0.001$) in the group A animals to 31.1 ± 0.9 min compared to the control values of 21.9 ± 0.6 minutes. Group B were not significantly different to control with a sleeping time of 24.0 ± 1.3 minutes. The rate of excretion of ketamine, metabolite I (the demethylated metabolite) and metabolite II (the subsequent oxidation product) in the urine of control rats (Group C) was 1.00 ± 0.13, 3.19 ± 0.36 and 0.93 ± 0.13 μg/min respectively whilst in the group A rats these rates were 0.35 ± 0.03, 0.94 ± 0.15 and 0.34 ± 0.05 μg/min, all of which were significantly lower. In group B the rates of excretion were 1.81 ± 0.24, 4.90 ± 0.55 and 1.14 ± 0.15 μg min, all of which were significantly higher than control.

However, the volumes of urine production were significantly lower in Group A than in both Groups B and C, and it was found that there was a great deal of variation in the concentration of ketamine and its metabolites in the urine of the various groups. It appeared that the low rate of excretion in Group A could be associated with the low volume of urine production. In order to test if the prolongation of sleeping time in Group A was associated with the low volume of urine produced an experiment was set up where one group of rats received an injection of vasopressin tannate (250 mu rat i.m.) four hours before the injection of ketamine (75 mg kg, i.p.) whilst the other group received an injection of the vasopressin vehicle only, the sleeping times were measured as before and no significant difference between the groups could be found, although the dose of vasopressin used abolished all urine production over the period of the experiment.

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Reference

CHANG, T. & GLAZKO, A.J. (1972). A gas chromatographic assay for ketamine in human plasma. *Anesthesiology*, **36**, 401–404.

Influence of impaired renal function on the disposition of [^{14}C]fazadinium in the anaesthetized greyhound

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Drugs and their metabolites are generally ultimately cleared from the body by excretion in the urine and/or bile. Some compounds are cleared almost exclusively by one of these routes while others show a mixed elimination pattern, being excreted both in urine and bile. In such cases, the two pathways can be complementary to each other, so that deficiencies in elimination by one route are compensated by increased use of the other (Hirom, Millburn & Smith, 1975). The inter-relationship of the two elimination pathways for compounds showing mixed elimination pattern can be of clinical significance in patients with defects of one of the pathways. This obtains in the choice of neuromuscular blockers used for renal transplantation surgery. Using the dog as a model species,

we have examined the inter-relationship of urinary and biliary excretion for the elimination of fazadinium (Fazadon), a diquaternary ammonium neuromuscular blocker, and the consequences of alternatively occluding the two excretion routes for the disposal of the drug.

Adult female greyhounds (weight 25 ± 2.5 kg) were anaesthetized with thiopentone (30 mg/kg i.v.) and intermittent ketamine (10 mg kg i.m.) and ventilated with O_2/air. The common bile duct was cannulated above the gall bladder, the ureters catheterized and cannulae placed in the femoral artery and vein. [^{14}C]-fazadinium dihromide (2.5 mg; 2.5 μCi) was injected i.v. and blood samples taken at frequent intervals for 1 h and then every 0.5 h for a further 4 hours. Urine and bile were collected hourly. The [^{14}C] content of plasma, urine and bile was determined by liquid scintillation spectrometry. In other experiments, the above was repeated except that the renal pedicles were ligated to prevent urine formation (5 dogs) or the common bile duct ligated above the cystic duct (3 dogs).

Table 1 shows the urinary and biliary excretion of [^{14}C]-fazadinium in the three groups of dogs. In 5 h, the control animals excreted some 65% of the dose (bile 47%; urine 19%). Ligation of the renal pedicles did not increase the biliary excretion of the drug, and similarly there was no increase in urinary excretion when the common bile duct was ligated. In both cases, the total excretion of drug was less than in the control dogs. The distribution half-lives for the plasma [^{14}C] in the three groups were similar, however, there were differences in the plasma elimination half-lives, which were as follows: 20 min (control), 22 min (renal ligated) and 30 min (bile duct ligated). The increase in plasma elimination half-life for the latter group can be attributed to decreased drug clearance due to prevention of biliary excretion. Examination of the urine and bile by thin-layer chromatography showed that the major excretion product was unchanged fazadinium together with smaller amounts of a metabolite formed by scission of the tetrazene link, 3-methyl-2-phenylimidazo-(1,2α)-pyridinium (Blogg, Simpson, Martin & Bell, 1973).

Thus, for the dog, the main pathway of elimination of the drug is via the bile, the ratio of hepatic to renal excretion being approximately 3:1. Furthermore, the two pathways are not complementary since one pathway does not compensate when the other is occluded. If biliary excretion of fazadinium predominates in man, the dog data suggests that its disposition should be relatively unaffected by impaired renal function, as does occur with *d*-tubocurarine (Miller, Mateo, Benet & Sohn, 1977; McLeod, Watson & Rawlins, 1976). This would suggest that from the drug disposition viewpoint fazadinium has advantages for patients with impaired renal function.

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References

BLOGG, C.E., SIMPSON, B.R., MARTIN, L.E. & BELL, J.A. (1973). Metabolism of ^{3}H AH 8165 in man. *Br. J. Anaesth.*, **45**, 1233–1234.

HIROM, P.C., MILLBURN, P. & SMITH, R.L. (1975). Bile and urine as complementary pathways for the excretion of foreign organic compounds. *Xenobiotica* **6**, 55–64.

McLEOD, K., WATSON, M.J. & RAWLINS, M.D. (1976). Pharmacokinetics of pancuronium in patients with normal and impaired renal function. *Br. J. Anaesth.*, **48**, 341–345.

MILLER, R.D., MATTEO, R.S., BENET, L.Z. & SOHN, Y.J. (1977). Pharmacokinetics of *d*-tubocurarine in man with and without renal failure. *J. Pharmac. exp. Ther.* **202**, 1–7.

Table 1 Urinary and biliary excretion of [^{14}C]-fazadinium in dogs

	% Dose (± s.e. mean) excreted in 5 h		
	Urine	*Bile*	*Total*
Control (n = 4)	18.6 ± 4.6	47.1 ± 4.0	65.7 ± 2.8
Ligated renal pedicles (n = 5)	—	52.8 ± 3.3	52.8 ± 3.3*
Ligated common bile duct (n = 3)	21.7 ± 5.0	—	21.7 ± 5.0**

* $P < 0.025$ cf control.
** $P < 0.005$ cf control; all others n.s.

The interaction of antibiotics with ethinyloestradiol in the rat and rabbit

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Conjugation is a major route of metabolism of the synthetic oestrogen ethinyloestradiol (EE_2). Conjugates formed in the liver and gut wall may be subsequently available for enterohepatic circulation (EHC). Tritiated EE_2 conjugates were obtained from the bile of 'donor' rats and were then infused into the caecum of 'recipient' rats. Bile was collected from the 'recipient' rats over a period of 6 hours. Radioactivity appearing in the bile of 'recipient' rats is a measure of the extent of deconjugation in the gastrointestinal tract, since only unconjugated steroid can be reabsorbed across the intestinal mucosa. The influence of various antibiotics on the EHC of EE_2 was then studied following pretreatment of 'recipient' animals with ampicillin, a combination of neomycin + lincomycin, or cefoxitin. There was a reduction in the biliary excretion of the radiolabelled drug of 83%, 79% and 81% respectively, with a concomitant supression of the gut microflora (Table 1).

Following the intravenous administration of EE_2 to rabbits, a biphasic decline in plasma concentration of the steroid was found. However, after 7 h a secondary peak was observed in all animals. Pretreatment with the antibiotic combination of neomycin + lincomycin (100 + 100 mg kg^{-1} day^{-1} for 4 days) resulted in a significant decrease ($P < 0.01$) in the area under the plasma concentration time curve ($AUC_{control}$ 61.3 ± 6.2; $AUC_{antibiotic}$ 37.4 ± 5.3 ng ml^{-1} h). Not only was there a reduction in the secondary peak consistent with a reduced EHC, but also a change in the initial disposition of EE_2.

Table 1 Effect of chronic antibiotic treatment on the EHC of EE_2 and the gut microflora

Treatment	*% excretion in bile*	*Caecal flora*
Control	32.6 ± 2.3	LFC + + +; M.An. + + +
Ampicillin (200 mg kg^{-1} day^{-1} for 4 days)	***8.1 ± 2.6	LFC +*; M.An. ±
Neo + Linco (100 + 100 mg kg^{-1} day^{-1} for 4 days)	***6.9 ± 1.7	No LFC; M. An. ±
Cefoxitin (100 mg kg^{-1} day^{-1} for 4 days)	***6.2 ± 1.3	—

LFC—Lactose fermenting coliforms (e.g. E. Coli; Strep. faecalis) M.An.—Mixed Anaerobes (e.g. Clostridia spp., Bacteroides spp.) ± < 10^3/ml; + 10^3–10^5/ml; + + + 10^7–10^{10}/ml.
*** Significantly different from controls, $P < 0.001$.

Cefoxitin was generously donated by Merck Sharp & Dohme Limited. We are grateful to Dr Elizabeth Thomas for the bacteriological analysis.

C.16

Species variations in the *N*-methylation and quaternization of [^{14}C]-pyridine *in vivo*

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It is well known that many phenols, catechols and amines undergo biological methylation *in vivo* and that this reaction is of importance for their activity and disposition. By contrast the *N*-methylation and quaternization of nitrogen functions is much less well understood and explored despite the fact that it was first observed by His in 1887. The biological methylation and quaternization of nitrogen could be of pharmacological interest since the metabolic conversion of tertiary nitrogen to quaternary functions may be expected to be associated with a marked change in both physico-chemical and pharmacological properties. Accordingly, we have made a study of the biological methylation *in vivo* in a range of species of the simple heterocycle, pyridine as well as with respect to dose and route of administration.

Table 1 Species Variations in the *N*-Methylation and Quaternization of [^{14}C]Pyridine *in vivo*

Species	% *dose excreted in 0–24 h urine*	% *dose excreted as N-methylpyridinium*
Man	63	<3
Rat	48	5
Mouse	66	12
Rabbit	57	21
Gerbil	52	26
Hamster	67	26
Guinea-pig	66	30
Cat	75	41

Dose of pyridine base was 7 mg/kg given by intraperitoneal injection of an aqueous solution of the hydrochloride except for the two human volunteers where the oral dose was 5 mg. Results are means for 2–5 animals.

[^{14}C]-pyridine hydrochloride was administered orally or by intraperitoneal injection to various species including the rat, mouse, guinea pig, rabbit, hamster, gerbil, cat and two human volunteers. The urine was collected for 24 h post-dosing and analyzed for ^{14}C and *N*-methylpyridinium by reverse isotope dilution. *N*-methyl-pyridinium excretion was shown to be a valid parameter of its formation since in separate experiments the ^{14}C-labelled quaternary ammonium compound was shown to be eliminated unchanged in the urine. Table 1 shows the species variations in the urinary excretion of *N*-methylpyridinium following the administration of [^{14}C]-pyridine. The species excreted some 50–75% of the dose of ^{14}C in the urine within 24 h of administration. There occur however marked variations in the extent of elimination of *N*-methylpyridinium, the product of *N*-methylation, this being low in man, rat and mouse (3–12% dose) and relatively high in the hamster, gerbil, rabbit, guinea pig and cat (20–40% dose).

The *N*-methylation of pyridine in the rat and guinea-pig was independent of route of administration since it occurred when the compound was given both intra-peritoneally and orally. In the rat the extent of methylation declined with increase in dose suggesting that this pathway in this species is readily saturable.

The findings show that marked species variations occur with respect to the *N*-methylation and quaternization of pyridine. Similar species differences may exist with respect to the metabolic quaternization of nitrogen heterocycle drugs.

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C.17

Pharmacokinetic studies on disodium cromoglycate in the piglet

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As a preventive drug, disodium cromoglycate has found extensive use in bronchial asthma and allergic rhinitis. However, its absorption from the lung and passage to the blood is still poorly understood. Nevertheless, this absorption in the lung will determine the pharmaco-kinetic profile of the drug. To assess the pharmacokinetic behaviour we have designed an animal model study. For this, the piglet is our animal of choice. The rationale for this is a recent study (Rendas, Branthwaite & Reid, 1978) that shows the similarity in functional and structural development of piglet and human lung. Plasma concentration curves and renal excretion are studied following intravenous and intratracheal administration of ^{14}C-labelled disodium cromoglycate.

Reference

RENDAS, A., BRANTHWAITE, M. & REID, L. (1978) Growth of pulmonary circulation in normal pig-structural analysis and cardiopulmonary function *J. Appl. Physiol.*, 45, 806–817.