SOME ASPECTS OF THE METABOLISM AND DISPOSITION
OF TIQ I NAMIDE IN SEVERAL ANIMAL SPECIES

Being a Thesis Presented for the Award
of a Degree of Doctor of Philosophy in
the University of Surrey
by
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August 1980
## CONTENTS

<table>
<thead>
<tr>
<th>ABSTRACT OF THESIS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER I: INTRODUCTION</td>
<td>3</td>
</tr>
<tr>
<td>Section 1: Peptic ulcer disease and its treatment:</td>
<td>4</td>
</tr>
<tr>
<td>The history and general background to peptic ulcer disease.</td>
<td>5</td>
</tr>
<tr>
<td>A brief summary of gastric physiology relevant to peptic ulcer disease.</td>
<td>5</td>
</tr>
<tr>
<td>The etiology of peptic ulcer disease.</td>
<td>6</td>
</tr>
<tr>
<td>Drug treatment of peptic ulcer.</td>
<td>7</td>
</tr>
<tr>
<td>Section 2: An introduction to tiquinamide:</td>
<td>11</td>
</tr>
<tr>
<td>The sequence of events leading to the development of tiquinamide.</td>
<td>11</td>
</tr>
<tr>
<td>The gastric anti-secretory and anti-ulcer pharmacology of tiquinamide.</td>
<td>13</td>
</tr>
<tr>
<td>Mechanism of anti-secretory activity of tiquinamide</td>
<td>15</td>
</tr>
<tr>
<td>Other pharmacological properties of tiquinamide</td>
<td>17</td>
</tr>
<tr>
<td>Some chemical properties of tiquinamide.</td>
<td>17</td>
</tr>
<tr>
<td>Potential consequences of the physical chemistry of tiquinamide for its absorption, distribution and excretion.</td>
<td>19</td>
</tr>
<tr>
<td>Potential routes of metabolism of tiquinamide.</td>
<td>23</td>
</tr>
<tr>
<td>Section 3: Objectives of the present study:</td>
<td>31</td>
</tr>
<tr>
<td>Some aspects of the metabolism and disposition of tiquinamide potentially relevant to its development as a gastric anti-secretory agent.</td>
<td>37</td>
</tr>
<tr>
<td>CHAPTER II: MATERIALS AND METHODS</td>
<td></td>
</tr>
<tr>
<td>MATERIALS: Chemicals</td>
<td>38</td>
</tr>
<tr>
<td>Thin-layer chromatography: plates and solvents</td>
<td>38</td>
</tr>
<tr>
<td>Animals</td>
<td>40</td>
</tr>
</tbody>
</table>
CHAPTER II (contd)

METHODS: Drug administration
Sample collection
Sample preparation
Determination of radioactivity
Whole body autoradiography
Pharmacokinetic analysis

CHAPTER III: ABSORPTION OF TIQUINAMIDE IN THE PATAS MONKEY

Summary of chapter
Introduction
Materials and Methods
Experimental
(a) Absorption of tiquinamide in anaesthetized, pylorus-ligated and sham-operated patas monkeys
(b) Gastric absorption of tiquinamide in conscious patas monkeys
(c) Effect of tiquinamide on gastric emptying in conscious patas monkeys

Results
(a) Absorption of tiquinamide in anaesthetized pylorus-ligated and sham-operated patas monkeys
(b) Gastric absorption of tiquinamide in conscious patas monkeys
(c) Effect of tiquinamide on gastric emptying in conscious patas monkeys

Discussion

CHAPTER IV: METABOLISM OF TIQUINAMIDE IN THE RAT

Summary of chapter
Introduction
Experimental
(a) The chromatographic pattern of drug-related products in urine
(b) The stability of the thioamide group under the conditions of urine collection and determination of the chromatographic pattern
(c) Hydrolysis of conjugated material
(d) Isolation of drug-related products from urine
CHAPTER IV (contd)

(e) Characterization of urinary end-products 77
(f) The chromatographic pattern of drug-related products in bile 77

Results
(a) The chromatographic pattern of drug-related products in urine 78
(b) The stability of the thioamide group under the conditions of urine collection and determination of the chromatographic pattern 78
(c) Hydrolysis of conjugated material 79
(d) Characterization of urinary end-products 79
(e) The chromatographic pattern of drug-related products in bile 84

Discussion

CHAPTER V: METABOLISM OF TIQUINAMIDE IN THE PATAS MONKEY 110

Summary of chapter 111
Introduction 112
Experimental 113
(a) The chromatographic pattern of drug-related products in urine 113
(b) The stability of the thioamide group under the conditions of urine collection and determination of the chromatographic pattern 113
(c) Hydrolysis of conjugated material 114
(d) Isolation of drug-related products from urine 115
(e) Characterization of urinary end-products 116

Results
(a) Chromatographic pattern of drug-related products in urine 117
(b) The stability of the thioamide group under the conditions of urine collection and determination of the chromatographic pattern 117
(c) Hydrolysis of conjugated material 118
(d) Characterization of urinary end-products 118

Discussion 121
<table>
<thead>
<tr>
<th>CHAPTER VIII (contd)</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Tissue excision studies</td>
<td>189</td>
</tr>
<tr>
<td>(b) Whole body autoradiography</td>
<td>189</td>
</tr>
<tr>
<td>(c) The accumulation of drug-related products in rat stomach wall and contents</td>
<td>189</td>
</tr>
<tr>
<td>(d) The elimination of drug-related products from rat plasma and stomach wall over a 0-21 day period</td>
<td>190</td>
</tr>
</tbody>
</table>

Results

| (a) Tissue excision studies                                                      | 191  |
| (b) Whole body autoradiography                                                   | 192  |
| (c) The accumulation of drug-related products in rat stomach wall and contents  | 193  |
| (d) The elimination of drug-related products from rat plasma and stomach wall over a 0-21 day period | 193  |

Discussion

CHAPTER IX: EXCRETION OF TIQUINAMIDE AND ITS METABOLITES IN RAT, PATAS MONKEY AND MAN

| Summary of chapter                                                               | 208  |
| Introduction                                                                     | 209  |
| Experimental                                                                     | 210  |
| (a) Excretion kinetics in rats                                                   | 210  |
| (b) Excretion kinetics in patas monkeys                                          | 210  |
| (c) Excretion kinetics in man                                                    | 211  |

Results

| (a) Excretion kinetics in rats                                                   | 212  |
| (b) Excretion kinetics in patas monkeys                                          | 213  |
| (c) Excretion kinetics in man                                                    | 214  |

Discussion

CHAPTER X: DISCUSSION

References

| References                                                                       | 237  |
ABSTRACT

Tiquinamide, 5,6,7,8-tetrahydro-3-methylquinoline-8-thiocarboxamide, is a novel tetrahydroquinoline structure with gastric anti-secretory properties. This thesis describes its metabolism and disposition in rat, patas monkey and man.

Gastric absorption was found in anaesthetized, pylorus-ligated patas monkeys, but occurred inconsistently in conscious animals studied by a novel mixed isotope method. Tiquinamide did not significantly alter the rate of gastric emptying in the patas monkey.

Metabolic studies in the rat, patas monkey and man revealed that ω-oxidation of the 3-methyl group to the corresponding carboxylic acid was a common pathway in all three species, as was allylic hydroxylation resulting in a 5-hydroxylated product which was subsequently conjugated with glucuronic acid. Desulphuration of the thioamide group to the nitrile occurred by chemical/metabolic means. Aromatization of the saturated ring occurred in the rat and man, but not in the patas monkey. Some quantitative species differences were also found.

Plasma kinetic studies revealed that tiquinamide was rapidly absorbed and eliminated. However, metabolites were more quickly excreted in patas monkey and man than in the rat. An unidentified metabolite constituted the outstanding component in rat plasma during the slow elimination phase.

Tissue distribution studies revealed no major depots from which slow delivery could have occurred. However, tissue uptake and gastric re-cycling, resulting in high stomach concentrations, may have been factors contributing to slow elimination in the rat.

Rapid urinary excretion occurred in patas monkey and man, whereas a slower phase predominated in the rat. The difference was attributed to the more extensive formation of water-soluble 3-carboxylic acids and glucuronides in the former species. Faecal excretion was extensive only in the rat. No evidence of entero-hepatic circulation was observed.
Factors contributing to the species difference in elimination are reviewed. The significance of the metabolism and disposition of tiquinamide for its development as an anti-secretory agent is discussed.
ACKNOWLEDGEMENTS

I would like to express my appreciation to colleagues at Wyeth Laboratories and others who have willingly assisted with various aspects of the work described. Firstly, I would like to thank Dr. Roger Crossley for the provision of radiolabelled tiquinamide·HCl and Dr. Ken Heatherington for the preparation of i.r. spectra and the interpretation of i.r., n.m.r. and mass spectra. I am also indebted to Mrs. J. Street (Department of Chemistry, University of Southampton) for determination of n.m.r. spectra and to Dr. J. M. Vernon and Dr. C. B. Thomas (Department of Chemistry, University of York) and Dr. M. Nicholson (Physical Chemical Measurements Unit, Atomic Energy Establishment, Harwell) for determination of mass spectra. Thanks are also due to Miss Doreen Beattie and Dr. John Waterfall respectively for providing pharmacological data on the gastrointestinal and cardiovascular effects of the drug. I am grateful also to Dr. P. J. Nicholls and Dr. D. K. Luscombe (Department of Pharmacology, University of Wales Institute of Science and Technology, Cardiff) for preparation of whole body autoradiographs. In addition I would like to thank Mr. Peter Southgate for arranging studies in volunteers. For their excellent technical assistance at different times during the work I am indebted to Mrs. Christine Body (née Smart) and Mrs. Diane Stevenson (née Bramall) and for experiments in monkeys to Mr. Steve Meacham. I am grateful also to my supervisors Dr. Brian Alps, Dr. John Sanford and Dr. Jagadish Chakraborty for their advice and encouragement and to Dr. Richard Franklin for constructive criticism of the manuscript. Thanks are also due to Dr. Sanford for his ligation of the monkey pylorus.

Finally, I would like to extend my thanks to Mrs. Maureen Swaisland for typing the manuscript and to my wife, Vanessa, for her support and encouragement.
CHAPTER I: INTRODUCTION
SECTION 1: PEPTIC ULCER DISEASE AND ITS TREATMENT
THE HISTORY AND GENERAL BACKGROUND TO PEPTIC ULCER DISEASE

The existence of peptic ulcer disease has been known since the earliest period of medical history. It was known to Hippocrates as long ago as 400 B.C. and engravings on the pillars of the temple of Aesculpus, dating back to the end of the 4th century B.C. describe some complications of gastric ulcer. The first comprehensive dissertation on gastric ulceration was by Aetius in the 6th century A.D. Duodenal ulcer, however, was not discovered until much later, being first suggested by Muralt in 1688. (Smith and Rivers, 1953).

In more recent times, it has come to be one of the major afflictions of populations worldwide. For example, at least 1 in 10 Americans is afflicted with the disease during a lifetime (Grossman, Isenberg and Walsh, 1975). Each year in the United States 3½ million people develop the disease (Thompson, 1972). In the United Kingdom according to statistics of the DHSS, published in 1970, the number of gastric ulcer patients annually was 40,000, duodenal ulcer patients 175,000 and patients with peptic ulcers of indeterminate site 115,000.

Thus there is clearly a great need in clinical practice for drugs which make effective contributions to the treatment of peptic ulcer disease.

A BRIEF SUMMARY OF GASTRIC PHYSIOLOGY RELEVANT TO PEPTIC ULCER DISEASE

The chemical function of the stomach is to perform the first stage of proteolysis. To this end, the gastric mucosa, the epithelial layer lining the innermost surface of the stomach wall, contains specialized glands. These secrete the proteolytic enzyme precursor pepsinogen, the hydrochloric acid which releases active pepsin in situ in the stomach lumen, and the mucus which forms a layer over the inner surface of the gastric epithelium, protecting it against the proteolytic action of its own secretions. Cardiac glands in the regions near the oesophageal orifice and pyloric glands covering a transitional zone above the pyloric region contain chiefly cardiac mucous cells and argentaffin cells. They secrete mainly mucus as well as the hormone gastrin. Oxyntic glands which occur widely in the mucosa of the body of the stomach, excluding cardiac and pyloric regions, contain mucous cells,
mucous neck cells and some argentaffin cells, but are characterized by the specific secretory cells, the oxyntic or parietal cells and chief cells, which secrete respectively hydrochloric acid and pepsinogen.

The secretion of acid and pepsinogen in the stomach is primarily under the control of the vagal nerve and the hormone gastrin. The vagal nerve, through release of the neurotransmitter acetylcholine, acts directly on oxyntic glands to stimulate secretion of both acid by parietal cells and pepsinogen by chief cells. It also acts on the pyloric glands to induce release of gastrin, which, on reaching oxyntic glands via the bloodstream, stimulates secretion of acid and pepsinogen. The vagal nerve not only stimulates the secretion of acid and pepsinogen directly, but also sensitizes the oxyntic glands to the action of gastrin (Emas, 1973). Thus the effect of gastrin is enhanced 2-8 fold in the presence of sub-threshold parasympathomimetic stimuli (Davenport, 1966). Histamine is also known to stimulate acid secretion. Although the original view of histamine as the mediator of the effects of acetylcholine and gastrin is no longer accepted (Johnson, 1972), it is believed that all three of these chemostimulants act directly on the parietal cell to stimulate acid secretion and that their receptors may be mutually interactive (Grossman, Isenberg and Walsh, 1975; Debas, 1977).

THE ETIOLOGY OF PEPTIC ULCER DISEASE

The precise mechanism of peptic ulceration is poorly understood. The disease has been conceived of as the result of a conflict between the aggressive and defensive factors vital to peptic digestion (Shay, 1959; Wise, 1972). Optimal functioning of the stomach in peptic digestion involves the secretion of acid and pepsin into the gastric lumen in sufficient concentrations to bring about proteolysis of food without damage to the proteinaceous gastrointestinal epithelium. Factors which contribute to the secretion of acid and pepsin are termed aggressive factors whereas those which increase the protection of the mucosa against proteolysis are termed defensive factors. Aggressive factors which have been discussed as pertinent to ulcerogenesis include vagal overreactivity, excessive gastrin or histamine secretion, disturbed gastric emptying, bile salt reflux and reduced mucosal
blood flow. Defensive factors include reduction in the amount or proteolytic resistance of secreted mucus or poor disposal of acid in the duodenum as a result of reduced bicarbonate secretion. However, one of the few sure facts to have emerged is that the presence of hydrochloric acid is essential for ulcer formation (Grossman et al., 1976). Pepsin secretion is apparently closely linked to that of gastric acid, and its role in promotion of ulcers is probably secondary to that of acid (Ivey, 1974; Grossman et al., 1976). Other possible etiological factors in gastric and duodenal ulcer have been discussed in various reviews of the subject (Rovelstad, 1976; Grossman, Isenberg and Walsh, 1975; Skillman, 1974; Blum, Peter and Krejs, 1975).

**DRUG TREATMENT OF PEPTIC ULCER**

Drug treatment of peptic ulcer disease relies principally on the neutralization of acid stomach contents by antacids and inhibition of acid secretion by anticholinergic drugs or histamine H₂-receptor antagonists. Sedatives and tranquillizers are quite commonly prescribed as an additional part of drug therapy, in order to reduce anxiety which may be a predisposing factor to the peptic ulcer.

**Antacids**

The use of antacids to neutralize gastric acid both relieves pain and produces an environment conducive to ulcer-healing. In addition to reducing the stimulus of acid itself to ulcer formation, this treatment reduces the conversion of the enzyme precursor pepsinogen to active pepsin, which occurs only at pH<4.5. The simplest antacid is sodium bicarbonate. However, because it is rapidly emptied from the stomach and readily absorbed, massive doses of the order of 60-140 g dissolved in 3 litres of milk are necessary to maintain pH 7.4 for 24 h (Müller-Wieland and Ossenberg, 1971). Furthermore, its absorption results in alkalosis, and liberated CO₂ may result in increased pain and even perforation (Thompson, 1972).

The most potent antacids are compounds of magnesium and calcium. The use of magnesium oxide, hydroxide or trisilicate is limited by excessive magnesium absorption (Thompson, 1972).
Calcium carbonate may form concretions in the bowels resulting in constipation and nausea (Müller-Wieland and Ossenberg, 1971).

Aluminium hydroxide delays gastric emptying, resulting in constipation. Also, aluminium chloride formed as a result of acid neutralization may irritate the gastrointestinal tract (Müller-Wieland and Ossenberg, 1971). It can also interfere with absorption of amino-acids, phosphates, glucose and ascorbic acid. Some preparations combine a magnesium-based antacid with one based on aluminium or calcium so as to maintain an acceptable frequency of bowel movement.

Anti-secretory drugs

Anticholinergics: Anticholinergic drugs are natural or synthetic derivatives of atropine, an alkaloid found as a constituent of deadly nightshade, (Atropa belladonna). These compounds act on the parasympathetic nervous system by attachment to the post-ganglionic receptor and subsequent prevention of access to this site by acetylcholine. Apart from inhibiting acid secretion and reducing gastric emptying rate in a manner analogous to that effected by vagotomy, they also block the effect of the vagus on the cardiovascular system, reduce the flow of saliva and of secretions of mucous membrane in the mouth, nose, pharynx and bronchi, paralyse accommodation of the eye, reduce sweating and relax smooth muscle in the bronchi and bronchioles and in the ureters and bladder (Bowman, Rand and West, 1968). Because of their non-specific mode of action, side-effects of anticholinergic drugs often associated with doses sufficiently high to inhibit acid secretion include dryness of mouth and nose, dryness of skin and flushing, mydriasis, tachycardia and reduction of smooth-muscle tone leading to urinary retention and constipation (Sodeman, Augur and Pollard, 1969). Thus the usefulness of anticholinergic drugs in the treatment of peptic ulcer is controversial. At best, when combined with a suitable antacid, they can help maintain neutralization of gastric acid in those patients able to tolerate the side effects.

Histamine H₂-Receptor Antagonists: Histamine H₂-receptor antagonists represent a new class of drugs which has made a major contribution to the control of peptic ulcer disease. Their invention followed the discovery of two stable conformations for
the histamine molecule, having inter-nitrogen distances 4.55 and 3.6 Å (Kier, 1968) and the suggestion of two receptors, $H_1$ and $H_2$—respectively, corresponding to each of the conformations (Ash and Schild, 1966). Conventional anti-histamine drugs, which did not inhibit acid secretion, had inter-nitrogen distances corresponding to a role as $H_1$-receptor antagonists. Since histamine was known to stimulate gastric acid secretion, it seemed logical to suppose that the reason why known anti-histamines had no effect on this process was that the receptor involved was the $H_2$-receptor.

The first $H_2$-receptor antagonist was burimamide, (Black et al., 1972). This was followed by the more potent metiamide (Black et al., 1973), which was eventually rejected on the grounds that some patients developed a reversible granulocytopenia (Forrest et al., 1975). Since the cause of the toxicity was believed to lie in the thioureido group, this was replaced by a cyanoguanidine moiety to give cimetidine (Brimblecombe et al., 1975) which had no toxic effects on bone-marrow (Brimblecombe and Duncan, 1977).

Cimetidine is a very effective inhibitor of both basal and meal-stimulated acid secretion (Spence, Celestin and McCormick, 1977; Barbezat and Bank, 1977; Pounder et al., 1977; Richardson, Walsh and Hicks, 1976; Pounder et al., 1976). It has also been shown to be extremely effective in healing both gastric ulcers (Ciclitira et al., 1977; Bader et al., 1977) and duodenal ulcers (Bodemar, Norlander and Walan, 1977; Peter et al., 1977).

Since the introduction of cimetidine into clinical practice in 1977, adverse effects reported have included induction of mental confusion in the elderly (Wood, Isaacson and Hibbs, 1978; Flind and Rowley-Jones, 1979), anti-androgenic activity (Winters, Banks and Loriaux, 1979) which may result in gynaecomastia (Sharpe and Hawkins, 1977), inhibition of vitamin B12 absorption (Steinberg, King and Toskes, 1978) and possible interference with immune response as a result of antagonism $H_2$-receptors in T-lymphocytes (Avella et al., 1978). The clinical limitations of these and other adverse effects will only be appreciated as the drug continues to be evaluated in clinical practice.
The successful introduction of cimetidine has led to the search for other $H_2$-receptor antagonists and several similar compounds are now in the later stages of development e.g. ranitidine, a compound four times as potent as cimetidine against pentagastrin-stimulated acid secretion (Domschke, Lux and Domschke, 1979).

Sedatives and tranquillizers: Sedatives (e.g. phenobarbitone, secbutobarbitone) or tranquillizers (e.g. thioprazate, chloridiazepoxide) are prescribed to reduce insomnia and anxiety and so ameliorate predisposing factors. The argument that stress causes increased acid secretion which leads to ulcer development might imply that a tranquillizer alone could bring about ulcer-healing by reducing acid secretion. However, there is no evidence to support this case (Thompson, 1972).

Ulcer-healing drugs: Very few drugs are known which actively promote ulcer-healing rather than inhibiting acid secretion. Carbenoxolone sodium apparently improved the rate of gastric ulcer-healing in ambulatory patients to the same extent that hospitalization would, but did not improve the rate of healing in hospitalized patients (Davis, 1976). Its effectiveness in treatment of duodenal ulcer is still somewhat controversial. However, the enteric-coated formulation, Duogastrone, which releases the drug only in the small intestine, has been shown to be highly effective in healing duodenal ulcers by a recent double-blind controlled trial against placebo (Davies and Reed, 1977).

Carbenoxolone sodium has side-effects of an aldosterone-like nature on salt and water retention (Khan and Sullivan, 1968), resulting in muscle weakness, weight gain, oedema and hypertension due to hypokalaemia and sodium retention.
SECTION 2: AN INTRODUCTION TO TIQUINAMIDE

THE SEQUENCE OF EVENTS LEADING TO THE DEVELOPMENT OF TIQUINAMIDE

Like the development of the H₂-receptor antagonists, the discovery of tiquinamide resulted from fundamental advances in the understanding of the stereochemistry of the common chemical secretagogues. As discussed previously molecular-orbital calculations led Kier (1968) to put forward the idea of two stable histamine conformations in which the inter-nitrogen distances were 4.55 Å and 3.6 Å. Subsequently, Bustard and Martin (1972) found the same critical distance of $3.7 \pm 0.2$ Å between the nitrogen atoms of the C-terminal amino acid amide, phenylalanine amide, of the hormone gastrin, and between the hetero-atoms of a number of compounds found to inhibit gastric acid secretion. Hence an inter-nitrogen distance of 3.7 Å apparently had critical significance for the control of gastric acid secretion at the parietal cell.

"An additional feature common to a number of inhibitors of gastric acid secretion was the presence of a sulphur atom in close proximity to the two nitrogen atoms. The discovery of antigastrin, 2-phenyl-2-(2-pyridyl)-thioacetamide, (Cook and Bianchi, 1967) was followed by reports that a number of the other heterocyclic derivatives of thioacetamide had similar antisecretory properties (Kanno et al., 1973).

The 5,6,7,8-tetrahydroquinoline-8-thiocarboxamide series of antisecretory agents, of which tiquinamide is a member, resulted from attempts to make a thioacetamide derivative in which the distance between the heterocyclic and thiocarboxamide nitrogen atoms was fixed at 3.7 Å by removal of the capacity for free rotation within the molecule. This was to be achieved by substituting the thiocarboxamide function on a coplanar heterocyclic ring system whilst maintaining a separation of two carbon atoms between the heterocyclic nitrogen atom and the point of attachment of the thiocarboxamide group. The first structure designed incorporated a methylene bridge between the phenyl and pyridyl rings of 2-phenyl,2-(2-pyridyl)-thioacetamide to yield a tricyclic structure. However, it proved impossible to synthesize this structure because of steric hindrance at the 8-position."
Antigastrin
(2 phenyl,2-(2-pyridyl)-thioacetamide)

Wy-23995
(5,6,7,8-tetrahydroquinoline-8-thiocarboxamide)

Tiquinamide
(5,6,7,8-tetrahydro-3-methylquinoline-8-thiocarboxamide)

Fig.1.1: The historical development of tiquinamide
Because 2-pyridyl thioacetamide had been shown to have potent anti-
secretory properties (Pascaud, Errard and Blouin, 1974), it was
concluded that the phenyl ring was not vital for antisecretory
activity. Omission of this ring from the above structure resulted
in 5,6,7,8-tetrahydroquinoline-8-thiocarboxamide (Wy 23995), which
could be readily synthesized and was found to be an active anti-
secretory agent. Synthesis of analogues of this fundamental
structure resulted in the discovery of tiquinamide, 3-methyl-
5,6,7,8-tetrahydroquinoline-8-thiocarboxamide as the most potent
member of the series (Curran and Shepherd, 1976; Beattie et al.,
1977). (Fig. I.1).

THE GASTRIC ANTI-SECRETORY AND ANTI-ULCER PHARMACOLOGY OF TIQUNAMIDE

**Animals**

Tiquinamide has been shown to be a potent inhibitor of basal
acid secretion and of gastric secretion induced by a wide variety
of chemical secretagogues (Beattie et al., 1979a). It was more
active than the H₂-receptor antagonist cimetidine against basal
secretion or against secretion stimulated by histamine, penta-
 gastrin or carbachol in pylorus-ligated rats. Thus against basal
secretion, tiquinamide had an ED₅₀ value of 7.8 mg/kg. A 30 mg/kg
intraduodenal dose reduced the total volume of secretion of gastric
contents by 83%, compared with 57% for cimetidine. For antagonism
of histamine-stimulated acid secretion, tiquinamide had an ED₅₀
value of 5.4 mg/kg. At 30 mg/kg intraduodenally, it inhibited
total secretion volume by 85%, as against 39% for cimetidine.
Against pentagastrin-stimulated secretion the ED₅₀ for tiquinamide
was 3.0 mg/kg and reduction of total volume of secretion 94% at
30 mg/kg, compared with 61% for cimetidine. Inhibition of carbachol-
stimulated secretion was marked for tiquinamide (65% at 30 mg/kg
intraduodenally) but cimetidine had no effect on this parameter.
The anti-secretory effect was observed in a wide variety of species.
Thus, basal secretion in the pylorus-ligated guinea-pig was inhibited
70% following a 30 mg/kg intraduodenal dose. Similarly, histamine-
stimulated secretion was inhibited in anaesthetized cats and monkeys
by respectively 80% and 75% after a 20 mg/kg intraduodenal dose
and was virtually obliterated by this dose in the anaesthetized
dog. Pentagastrin-stimulated secretion was inhibited 70% by a
<table>
<thead>
<tr>
<th>Erosion-inducing agent</th>
<th>Tiquinamide dose</th>
<th>Percentage inhibition of erosions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin (20 mg/kg i.p.)</td>
<td>30 mg/kg p.o.</td>
<td>94</td>
</tr>
<tr>
<td>2-deoxy-D-glucose (256 mg/kg s.c.)</td>
<td>30 mg/kg p.o.</td>
<td>94</td>
</tr>
<tr>
<td>5-hydroxytryptamine (50 mg/kg i.p.)</td>
<td>30 mg/kg p.o.</td>
<td>91</td>
</tr>
<tr>
<td>Adrenaline (0.4 mg/kg i.p.)</td>
<td>10 mg/kg intraduodenally</td>
<td>75</td>
</tr>
<tr>
<td>Histamine acid phosphate (0.25 mg/kg i.m.)</td>
<td>30 mg/kg p.o.</td>
<td>92</td>
</tr>
</tbody>
</table>

Table I.1: Inhibition of chemically-induced erosions in rats by tiquinamide
20 mg/kg intraduodenal dose in the anaesthetized cat and secretion stimulated by gastrin tetrapeptide in the conscious, gastric-fistula dog was inhibited 44% by this dose.

Tiquinamide produced more than 90% inhibition of gastric erosion formation induced in rats by a 3 h period of cold-restraint, when administered orally at doses of 10 mg/kg or more. By comparison, metiamide had no significant effect against erosion formation at doses up to 100 mg/kg. Tiquinamide markedly inhibited the formation of gastric erosions induced in rats by a range of chemical agents. Table I.1 shows percentage inhibition by tiquinamide of erosions induced by indomethacin, 2-deoxyglucose, 5-hydroxytryptamine, adrenaline and histamine acid phosphate.

Man

Early investigations in man suggested that significant inhibition of gastric acid secretion could be obtained at quite low doses. In one study, 4 subjects received oral doses of tiquinamide HCl ranging from 10-60 mg. Approximately 50% inhibition of pentagastrin-induced acid secretion was demonstrated at doses of 50 mg (personal communication from Dr. M. Lancaster-Smith, Queen Mary's Hospital, Sidcup to Mr. P. Southgate, Department of Clinical Pharmacology, Wyeth Laboratories). In a second study, 4 subjects received 25, 50 and 75 mg oral doses of tiquinamide HCl. At the 25 mg dose, 30% inhibition of pentagastrin-induced acid secretion was observed and at 75 mg 60-70% (personal communication from Dr. Parkins, Charing Cross Hospital, London to Mr. P. Southgate, Department of Clinical Pharmacology, Wyeth Laboratories). Thus the potency of the compound apparently compared very favourably with that of cimetidine, which elicited a maximum inhibition of acid secretion of more than 75% after a single intraduodenal dose of 200 mg (Burland et al., 1975).

MECHANISM OF ANTI-SECRETORY ACTIVITY OF Tiquinamide

Attempts to elucidate the mechanism of action of tiquinamide have so far involved a thorough study of its effects on the autonomic nervous system and on gastric mucosal blood flow. However, no plausible explanation for the potent gastric anti-secretory activity of this compound has yet been discovered (Beattie et al., 1979b).
Many conventional anti-secretory compounds used in clinical practice, such as atropine and ambutonium, are anti-cholinergic drugs. However, in studies using the guinea-pig isolated ileum preparation, non-competitive antagonism of cholinergic receptors by tiquinamide was demonstrable only at concentrations as high as $10^{-3}$M. Thus for all practical purposes tiquinamide was devoid of anti-cholinergic activity.

The newer class of anti-secretory compounds, typified by cimetidine and its precursor compounds burimamide and metiamide, are inhibitors of histamine $H_2$-receptors. Whilst tiquinamide demonstrated some weak $H_2$-receptor antagonism in the isolated guinea-pig atrial preparation, the $pA_2$ value for this effect was only 4.06 compared to 5.11 and 5.85 for burimamide and metiamide respectively. Since tiquinamide had been found to be a more potent inhibitor of acid secretion than these compounds, it is unlikely that its $H_2$-receptor antagonism accounted for more than a small part of its anti-secretory activity.

Some drugs which inhibit gastric acid secretion do so by enhancing gastric mucosal blood flow. However, in studies to examine the effect of tiquinamide on gastric mucosal blood flow, aniline clearance was reduced in parallel with acid secretion. Therefore it is unlikely that tiquinamide exerted a primary effect on gastric mucosal blood flow.

Investigations into other possible autonomic effects of tiquinamide revealed an absence of ganglion-blocking or local anaesthetic activity and showed no evidence of antagonizing effects of either noradrenaline or 5-hydroxytryptamine. Thus it seemed unlikely that tiquinamide exerted its effect via the peripheral nervous system. Possibly elucidation of its mechanism of action will depend on further development of fundamental knowledge about the control of gastric acid secretion.

In view of the absence of central or autonomic effects for tiquinamide coupled with its ability to antagonize the stimulation of acid secretion by a wide range of chemical secretagogues (e.g. pentagastrin, histamine, carbachol) deemed to act close to the site of acid secretion in the parietal cell, it seems likely that tiquinamide too exerts its effect here.
OTHER PHARMACOLOGICAL PROPERTIES OF TIQUINAMIDE

It emerged from early volunteer studies that tiquinamide possessed some undesirable side-effects. Doses in excess of about 40 mg apparently resulted in nasal congestion and sinusitis-type headaches. Furthermore, tachycardia was frequently observed, usually at doses of around 100 mg, but in some subjects at lower doses and in one subject at as low as 10 mg. The tachycardia normally occurred at about 2-4 h after dosing and was associated with postural hypotension (Coleman, A. J. personal communication to Mr. P. Southgate, Department of Clinical Pharmacology, Wyeth Laboratories).

Serious consideration was given to the nature and mechanism of the tachycardia, this being a potentially lethal side-effect. A model for the tachycardia was established in conscious, normotensive rats. When blood pressure in the rat tail vein was monitored using pressure transducers linked to a polygraph and heart rates were derived from the blood pressure traces, it was possible to show that doses of 10-50 mg/kg tiquinamide HCl administered orally promoted increases in heart rate of up to 40% of the pre-dose value and intravenously up to 30%. Marked falls in blood pressure were associated with the tachycardia in orally dosed animals. Onset of action occurred more rapidly after oral administration (6-10 min at 10 mg/kg) than after intravenous dosing (≈30 min). Once evident the tachycardia persisted until at least 2 h after dosing when the experiment was terminated (Waterfall, 1978).

The more rapid onset of tachycardia in orally-dosed animals than in those administered the drug intravenously prompted the suggestion that the effect could be mediated by a metabolite.

SOME CHEMICAL PROPERTIES OF TIQUINAMIDE

pKa

Tiquinamide (5,6,7,8-tetrahydro-3-methylquinoline-8-thiocarboxamide) is a weak base of pKa 5.2.

Solubility in water and organic solvents

The aqueous solubilities of tiquinamide and its hydrochloride salt are respectively 0.84 and 364 mg/ml.
Partitioning of \(^{14}\)C-tiquinamide between pH 7.4 0.1 M phosphate buffer and various organic solvents when mixed for 12 h at ambient temperature was as shown in Table I.2. The initial concentration of \(^{14}\)C-tiquinamide HCl in buffer was 200 \(\mu\)g/ml.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solvent/aqueous partition coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heptane</td>
<td>0.17</td>
</tr>
<tr>
<td>Bensene</td>
<td>5.53</td>
</tr>
<tr>
<td>Chloroform</td>
<td>17.3</td>
</tr>
<tr>
<td>1-octanol</td>
<td>15.4</td>
</tr>
</tbody>
</table>

Table I.2: Partition coefficients of \(^{14}\)C-tiquinamide between various organic solvents and pH 7.4 0.1 M phosphate buffer

Because of the known tendency of the thioamide group to degrade to a nitrile function (see below), the determined values may not have been the true partition coefficients of the thioamide, but may have included a contribution from the corresponding nitrile.

Stability

Tiquinamide HCl is unstable in aqueous solution. In the dark at 25°C, a 1.6 mg/ml solution underwent oxidative degradation (10% after 10 days). The main products of the degradation were the amide derivative, Wy 24117 and elemental sulphur. Lesser amounts of the nitrile derivative, Wy 24146, were also formed. The compound was no less stable in acid solution (0.1 N HCl) but in alkali (0.1 N NaOH) degradation was accelerated, such that only 58% of a 5 mg/ml solution remained unchanged after 1 week. (Davidson, 1974). More importantly, at lower concentrations (i.e. <100 \(\mu\)g/ml) likely to occur in biological fluids after administration of \(^{14}\)C-tiquinamide HCl, desulphuration to the nitrile derivative occurred. Such degradation made manipulation of drug in biological fluids difficult. Certain manipulations enhanced the extent of degradation. Thus extraction of tiquinamide into toluene from a 2 \(\mu\)g/ml solution in plasma basified to pH 9 resulted in virtually complete degradation. However, the
degradation could in this case be almost completely overcome for the purpose of analyzing samples containing radio-labelled drug, by adding unlabelled tiquinamide HCl to a concentration $\geq 100 \mu g/ml$ before extraction. Thus whenever unchanged tiquinamide was to be assayed, it was necessary to adopt this procedure and to make allowance for the extent of desulphuration by taking standards containing known concentrations of tiquinamide through the entire assay. Further complications were introduced by the possibility of thioamide-containing metabolites and these will be discussed more fully in the chapters concerned with the metabolism of the drug.

**POTENTIAL CONSEQUENCES OF THE PHYSICAL CHEMISTRY OF TIQUINAMIDE FOR ITS ABSORPTION, TISSUE DISTRIBUTION AND EXCRETION**

**Absorption**

Since the work of Schanker et al., (1957) and Hogben et al., (1957), it has become a well established principle that drugs are usually absorbed by passive diffusion and that their rates of absorption are dependent on the lipid-solubilities of their most lipophilic forms. For most drugs, the unionized forms have much greater lipid solubility than do their ionized counterparts, and therefore it is normally found that unionized drugs are absorbed rapidly, ionized ones more slowly.

It follows that weak bases which are extensively unionized at the pH of intestinal contents (5-8) are well absorbed from the small intestine but poorly absorbed from the stomach (pH 1-3), where they are largely ionized. Weak acids are unionized in the stomach and therefore more likely to be absorbed from this site than are basic compounds.

Tiquinamide is a weak base (pKa 5.2), expected to be almost completely unionized in the contents of the small intestine. In addition it has a moderately high lipophilicity. Its octanol/aqueous buffer partition coefficient of 12 was similar to the value of 10 found to be optimal for the intestinal absorption of carbamates (Houston, Upshall and Bridges, 1974 and 1975). Furthermore, its partition coefficients between, respectively, heptane, benzene or chloroform and pH 7.4 aqueous buffer were 0.17, 5.5 and 17.3, of a similar order to those of aniline (0.55, 0.90 and 17) and aminopyrine (0.15, 0.40 and 73) (Brodie, 1964), which are weak bases (pKa 5.0) known to traverse biological membranes rapidly.
It thus seemed very likely that this drug would be well absorbed from the small intestine. However, there existed the additional possibility that it would be significantly absorbed from the stomach. Although basic drugs such as tiquinamide would not normally be expected to be absorbed from the stomach because of their extensive ionization at this site, Schanker et al. (1957) did show that bases could be absorbed from the stomach provided that the pH of gastric contents was maintained above the pKa of the drug, so promoting the presence of significant concentrations of unionized drug. Because tiquinamide was a potent inhibitor of gastric acid secretion believed to act directly on the parietal cell, it was envisaged that the pH of gastric contents would begin to rise as soon as sufficient drug had been absorbed to initiate the anti-secretory effect, following its uptake into parietal cells from the systemic circulation. Then, as a result of the low pKa of this drug, at pH's greater than about pH 4, a significant proportion of the drug would be unionized and hence available for absorption. Furthermore, there was an indication from experiments in rats that it inhibited gastric emptying (Beattie et al., 1979b). Thus subsequent absorption might occur directly from gastric contents as a consequence of the combination of the low pKa of tiquinamide with its inhibitory effect on acid secretion and gastric motility.

**Tissue distribution**

The factors governing the transfer of drugs from blood into tissues are similar to those governing their absorption. Thus it has been shown that the permeability constants for drugs passing from plasma into cerebro-spinal fluid are related firstly to their pKa's and secondly to their partition coefficients between various organic solvents and aqueous buffer (pH 7.4) (Mayer, Maickel and Brodie, 1959; Brodie, Kurz and Schanker, 1960). This model represented the case in which the pH on both sides of the membrane is identical i.e. pH 7.4, as is true at the interface between plasma and many tissues. In some cases, however, a lipid membrane separates plasma from a tissue or fluid in which the pH differs substantially from that of plasma. Then the pH may markedly affect drug distribution in a manner dependent on the pKa of the drug concerned. Thus weak bases are known to be rapidly transferred from plasma into gastric juice (pH 1-3) and hence secreted into
the gastric lumen (Shore, Brodie and Hogben, 1957). Similarly, the localization of basic drugs in the lung is believed to result from the intracellular trapping of these drugs in their ionized forms because the intracellular pH in lung is approximately 0.5 pH units below that in plasma (Waddell, 1973).

Thus the moderately high lipophilicity of tiquinamide suggested that it would be rapidly taken up into tissues and maybe also into gastric contents. It was a possibility, however, that the elevation of the pH of gastric contents, as a result of the anti-secretory activity of tiquinamide, would reduce the extent of gastric secretion of the drug.

A possible site of accumulation of tiquinamide was the thyroid in view of the presence of a thioamide group in its structure. A number of thioureas and thiouracils are accumulated at this site and are in fact active as anti-thyroid agents e.g. thiourea (Maloof and Soodak, 1965), thiouracil (Maloof and Soodak, 1957), propyl thiouracil (Marchant et al., 1971) and methimazole (Marchant and Alexander, 1972). Furthermore, the thioamide anti-tubercular compound ethionamide has been found to exert an anti-thyroid effect in some subjects (Moulding and Fraser, 1970).

Excretion

Drugs and their metabolites are normally excreted most extensively in the urine and following biliary secretion, in the faeces, though elimination may also take place through secretion into other body fluids such as sweat and saliva and, in the case of volatile compounds, by exhalation from the lungs. The extent and rate of excretion of a given compound via each of the major routes is determined by physical and structural properties of the molecule. Drugs in solution in plasma pass freely into urine by glomerular filtration. Passage is through the pores of the membrane and thus the process is independent of the ionization state of the molecule or of its solubility in lipoidal membranes. However, the concentration of drug in the filtrate will often greatly exceed that in capillaries intimately associated with the renal tubule and thus reabsorption may take place in a manner dependent on pKa and lipid solubility. Thus lipophilic drugs unionized in urine (i.e. at pH 4.5-8) will tend to be reabsorbed, whereas ionized species will usually be
too poorly soluble in lipoprotein for this process to occur significantly (Brodie and Hogben, 1957). Variations in urinary pH may substantially affect the extent of renal reabsorption of weak acids and bases, and in so doing profoundly affect the rate of their renal elimination. Thus, for example, it was found that the weakly basic drug amphetamine was excreted substantially unchanged in human urine but that the rate of excretion fluctuated as urinary pH varied in the range 4.9-8.3. Under controlled acid conditions (pH 5) 60% of the dose was excreted in urine in 16 h, whereas when urine was kept alkaline (pH 8) only 2-3% of the dose was excreted in urine in the same period (Beckett and Rowland, 1965). An additional mechanism by which some compounds may enter the urine involves active secretion into the proximal kidney tubule. Separate specific mechanisms exist for the active secretion of strong organic bases such as N-methylnicotinamide, tolazoline and hexamethonium (Peters, 1960) and of organic acids such as salicylic acid, hippuric acid and glucuronide conjugates (Sperber, 1959).

The largest single factor governing the extent of biliary secretion of a drug is its molecular weight. It is now well established that there is a molecular weight threshold for biliary secretion which varies from species to species. Low molecular weight compounds are poorly excreted in bile. For compounds of molecular weight above the threshold value the proportion of the dose excreted in bile increases dramatically with the increase in molecular weight until, for compounds of molecular weight 200 or so greater than the threshold value, elimination occurs almost entirely by biliary secretion (Millburn, Smith and Williams (1967; Aziz et al., 1971).

It has already been established that tiquinamide is a weak base likely to readily traverse biological membranes in its unionized state. Furthermore, its pKa (5.2) falls within the normal range of urinary pH (4.5-8) and thus it was probable that the drug would exist in substantially unionized form in urine. Therefore it was predictable that if the drug was extensively excreted in urine unchanged, it would be prone to reabsorption by passive diffusion in the renal tubules. The absence of strongly basic or acidic groups in the molecule suggested that active renal tubular
secretion of tiquinamide was unlikely. Extensive biliary secretion of the unchanged drug was precluded on the grounds of its low molecular weight (206), which fell well below the lowest known threshold value of 325 ± 50 in the rat (Millburn, Smith and Williams, 1967; Aziz et al., 1971). The combination of its lipophilicity with low pKa, resulting in a high degree of unionization at physiological pH (7.4), would have predisposed it to passive entry into other body fluids e.g. sweat and saliva (Stowe and Plaa, 1968). However, these do not normally constitute predominant routes of excretion. It was thus possible that excretion of tiquinamide from the body in unchanged form would prove difficult. Thus elimination might be dependent on metabolism to more polar products.

POTENTIAL ROUTES OF METABOLISM OF TIQUINAMIDE

Reactions involved in the metabolism of drugs by the mammalian liver include, for example, hydroxylation, N- or S- oxidations, O-, N- or S- dealkylations, hydrolyses and reductions as well as conjugation with a variety of polar endogenous compounds e.g. sulphate, glucuronic acid or glycine. These fundamental pathways have been extensively reviewed by Williams (1959a) and Parke (1968a). In addition a series of monographs covering the routes of biotransformation of a very large number of different foreign compounds has been published in recent years (Hathway, 1970, 1972, 1975, 1977 and 1979). Whilst the liver is the major site of metabolism it has become obvious in recent years that microsomal oxidases of a similar nature to those in liver occur also in a number of different extra-hepatic tissues, including lung, intestinal tract, placenta, kidney, adrenals, skin, brain and testes (Murphy, 1972; Lake et al., 1973). In addition, a wide range of metabolic reactions have now been attributed to gastrointestinal microflora. These tend to be reductive and degradative rather than oxidative and synthetic. They include reductions, hydrolyses, decarboxylations, dealkylations, dehalogenations, deaminations, heterocyclic ring fissions, aromatizations, acetylations and esterifications. These reactions and their significance have been discussed by Scheline (1973), Williams (1972) and Goldman (1978).
It is not the intention to dwell on the basic routes of metabolism, since these have been well covered in the literature and thus further discussion will be confined to reactions which could have been directly concerned in the biotransformation of tiquinamide.

Since 5,6,7,8-tetrahydroquinolines were not previously known prior to the synthesis of tiquinamide and related compounds (Curran and Shepherd, 1976), the metabolism of such compounds has not been described. Some work on 1,2,3,4-tetrahydroquinolines and 1,2,3,4-tetrahydroisoquinolines has been reported, but has not lead to any general conclusions as to how this type of ring structure is metabolized. For example, Bernhard (1939) reported that 1,2,3,4-tetrahydroquinoline underwent in the dog aromatization and hydroxylation at the 2-position, resulting in 2-hydroxyquinoline. On the other hand, a derivative of this structure, the anti-schistosomal compound 2-isopropylaminomethyl-6-methyl-7-nitro-1,2,3,4-tetrahydroquinoline, was in mouse, rat, rabbit and rhesus monkey, not metabolized in the tetrahydroquinoline ring, but purely by oxidation of methyl and isopropylaminomethyl substituents (Kaye and Woolhouse, 1972). Still other metabolic routes predominated in the metabolism of the 1,2,3,4-tetrahydroisoquinoline compound debrisoquine by rat, dog and man. This compound was metabolized principally by allylic hydroxylation yielding 4-hydroxydebrisoquine. Another significant route involved opening of the saturated ring and subsequent oxidation to yield carboxylic acids (Allen et al., 1975; Allen, Brown and Marten, 1976). Thus reports on the metabolism of compounds possessing partially saturated heterocyclic ring systems similar to that of tiquinamide were rare and yielded a diversity of possible metabolic routes. (Fig. 1.2). In order to assist speculation about possible biotransformation routes for tiquinamide, the ring structure was considered as fused cyclohexene and pyridine rings. Thus knowledge of the metabolism of aromatic heterocyclic amines, cycloalkenes, methyl-substituted aromatic compounds and thioamides was all pertinent to the possible metabolism of tiquinamide.

**Heterocyclic aromatic amines**

**Aromatic hydroxylation:** Pyridine and quinoline and related compounds have been quite commonly found to undergo aromatic hydroxylation. Quinoline, for example, is hydroxylated by the
rabbit at a number of different positions, notably at the 2-, 3-, 5- and 6- positions (Williams, 1959b) (Fig. 1.3).

**N-methylation:** The vitamin nicotinic acid has been found to undergo extensive N-methylation in a wide range of species e.g. rat (Lin and Johnson, 1953), dog, hamster, mouse (Leifer et al., 1951) and man (Reddi and Kodicek, 1953) (Fig. 1.3). However, this reaction has been found to be only a minor one for exogenous compounds such as pyridine (Williams, 1959c), nicotine (Hansson, Hoffmann and Schmiterlöw, 1964) and quinoline (Williams, 1959d).

**N-oxidation:** A number of aromatic, heterocyclic amines have been found to undergo N-oxidation e.g. nicotinamide, nikethamide, 3'-acetyl-pyridine and cotinine (Gorrod, 1973) (Fig. 1.3). For pyridine the reaction was found to occur quite extensively (e.g. 40% of the dose in mice), but 3-methyl substitution markedly reduced its quantitative significance (Gorrod and Damani, 1980).

**Ring scission:** Ring scission is an uncommon reaction of pyridine or quinoline derivatives. Apart from the cleavage of the 1,2,3,4-tetrahydroisoquinoline ring of debrisoquine mentioned above, there exists only one or two examples for instance, 8-methylquinoline was extensively degraded in dogs and rabbits such that quinoline-related structures could not be traced (Cohn, 1895, cited by Williams, 1959).

**Cycloalkenes**

**Allylic hydroxylation:** Cycloalkene rings, whether independent or fused to an aromatic ring system, are commonly found to undergo allylic hydroxylation i.e. at a position adjacent to the double bond. This reaction has only recently been established for cyclohexene itself (Leibman and Ortiz, 1978) (Fig. 1.3), but has long been known for derivatives such as the cyclohexenyl barbiturates (Williams, 1959e). It is sometimes accompanied by further oxidation to the corresponding keto-derivative. Thus, for example, hexobarbital was found to be metabolized in the rabbit and dog principally by 3'-hydroxylation in the cyclohexene ring, yielding 3'-hydroxyhexobarbital and by further oxidation to keto-hexobarbital (Holcomb, Woodside and Bush, 1969; Gerber et al., 1970; Holcomb, Gerber and Bush, 1974).
Aromatization: Evidence for aromatization of cycloalkenes or of saturated rings fused to aromatic systems is scarce. As mentioned previously, Bernhard (1939) reported the aromatization of 1,2,3,4-tetrahydroquinoline in the dog, which, in combination with hydroxylation, yielded 2-hydroxyquinoline (Fig. I.2). Shikimic acid is known to undergo aromatization, but it has been established that this only occurs as a result of initial reduction by intestinal micro-organisms (Brewster, Jones and Parke, 1977a) followed by aromatization of the resulting cyclohexane carboxylic acid (Brewster, Jones and Parke, 1977b). The mechanism is specific for saturated rings with carboxylic acid substituents, since it requires the formation of an acylcoenzyme A intermediate (Babior and Bloch, 1966).

Methyl-substituted aromatic compounds

Oxidation: Methyl substituents to aromatic rings are oxidized to the corresponding carboxylic acid derivatives. This reaction has been known since the late 19th century, and numerous examples of compounds oxidized in this manner are cited by Williams (1959f) e.g. toluene, α-methylpyridine, 2,5-dimethylfuran, 6-methylquinoline. It is now known that the complete metabolic pathway involves the formation first of the hydroxymethyl derivative and subsequently the corresponding aldehyde, oxidation of which yields the carboxylic acid (Gillette, 1963) (Fig. I.3).

Thioamides

Sulphoxidation: One established route of thioamide metabolism involves sulphoxidation. Thus, for example the anti-tubercular compound ethionamide was metabolized in the man, rat, mouse and dog to ethionamide sulphoxide, which was in dynamic equilibrium with the unchanged drug. (Bieder and Mazeau, 1964; Bieder, Brunel and Mazeau, 1966; Johnston, Kane and Kibby, 1967). Other metabolites arising from further oxidation of ethionamide and its sulphoxide were 2-ethylisonicotinamide and its hydrolysis product 2-ethylisonicotinic acid (Fig. I.4).

Desulphuration: An alternative route of metabolism of thioamides has been found to involve desulphuration to the nitrile. For example, 2,6-dichlorothiobenzamide was desulphurized to 2,6-dichlorobenzonitrile in the rat to the extent that the metabolic patterns following oral administration of the thioamide or nitrile...
could not be distinguished. The major urinary metabolite, representing about 30% of the administered dose was 2,6-dichloro-3-hydroxybenzonitrile and its conjugates (Griffiths et al., 1966) (Fig. 1.4).

Conclusions

In conclusion, the evidence from the literature suggested that tiquinamide might undergo hydroxylation in the saturated ring at one of the allylic positions adjacent to the points of fusion of the saturated and aromatic rings and/or in the aromatic ring. Methyl group oxidation was another likely possibility. The thioamide group seemed certain to be metabolized, probably by one of two alternative routes: either by oxidation to the sulphoxide or by desulphuration to the corresponding nitrile. N-oxidation, N-methylation and aromatization represented other possibilities.

The nature of any subsequent conjugation reaction would obviously be dictated by the routes of Phase I metabolism. Thus hydroxyl metabolites might be conjugated with glucuronic acid or sulphate or carboxylic acids with glucuronic acid or amino acids such as glycine, glutamine or taurine, for example (Parke, 1968b).
Fig. I.2: Metabolism of some tetrahydroquinolines and tetrahydroisoquinolines

Formed after derivatization of acidic metabolites with acetylacetone and diazomethane.
Fig. 1.3: Reactions potentially relevant to the metabolism of tiqunamide.

1. Reactions of the ring system

- Reactions of the ring system

- 2-Hydroxyquinoline

- 1,2,3,4-Tetrahydrazo-

- Quinoline

- 2-Hydroxyquinoline

- 1,2,3,4-Tetrahydrazo-

- N-Oxidation

- N-Methylation

- N-Methylated

- N-Methylated

- N-Oxidation

- Aromatization

- Allylic Hydroxylation

- Hydroxyquinolines

- 2,3,5 and 6-

- Quinoline

- Aromatic Hydroxylation
Methyl group oxidation

\[
\begin{align*}
\text{p-nitro-toluene} & \quad \rightarrow \quad \text{p-nitro-benzyl alcohol} \quad \text{benzaldehyde} \quad \text{benzoic acid} \\
\text{CH}_3 & \quad \downarrow \quad \text{CH}_2\text{OH} \quad \downarrow \quad \text{CHO} \quad \downarrow \quad \text{COOH}
\end{align*}
\]

Thioamide group

1. Sulphoxidation

\[
\begin{align*}
\text{Ethionamide} & \quad \rightarrow \quad \text{2-ethyl-iso-nicotinamide} \quad \rightarrow \quad \text{2-ethyl-iso-nicotinic acid} \\
\text{CSNH}_2 & \quad \downarrow \quad \text{CONH}_2 \quad \downarrow \quad \text{COOH}
\end{align*}
\]

2. Desulphuration

\[
\begin{align*}
\text{2,6-dichloro-thiobenzamide} & \quad \rightarrow \quad \text{2,6-dichloro-benzonitrile} \\
\text{Cl} & \quad \downarrow \quad \text{Cl} \quad \downarrow \quad \text{CN}
\end{align*}
\]

Fig.I.4: Reactions potentially relevant to the metabolism of tiquinamid:
2. Reactions of methyl and thioamide substituents
SECTION 3: OBJECTIVES OF THE PRESENT STUDY:
SOME ASPECTS OF THE METABOLISM AND DISPOSITION OF
TIQUINAMIDE POTENTIALLY RELEVANT TO ITS DEVELOPMENT
AS A GASTRIC ANTI-SECRETORY AGENT

Before speculating on ways in which study of the metabolism and disposition of tiquinamide might aid its development as an anti-secretory agent useful in the treatment of peptic ulcer disease, it is perhaps pertinent to give some thought to the properties which are desirable in such a compound. In general, these are not very different from what would be expected of any drug, whatever its intended use - namely that it should reach its site of action rapidly, that it should remain there at effective concentrations for a period likely to be significant with respect to the time-course of the condition to be treated and that it should be eliminated at a rate which makes possible the design of a dosage regime appropriate to this condition.

Thus the primary consideration for an anti-secretory drug deemed to act directly on the parietal cell is that, once administered, it should be taken up rapidly and efficiently into the gastric mucosa. The most appropriate route of administration would appear to be the oral route. Hospital confinement of peptic ulcer patients normally gives way to the ambulatory state during the course of treatment. Furthermore, daily administration of the drug over a period of months after the return of the patient to normal life may be necessary if recurrence of the ulcer is to be prevented. An oral dosage form constitutes a convenient means of administration of a drug to a non-hospitalized patient. In addition, the oral route may be particularly appropriate in diseases of the gastrointestinal tract because it offers the opportunity to introduce the drug close to the intended site of action. Thus it is particularly desirable for a gastrointestinal agent of this type that it should have good oral bioavailability. In this respect, cimetidine scored, having an oral bioavailability of 72% in man (Griffiths, Lee and Taylor, 1977). It was of interest to know whether tiquinamide would perform as well. On a dose-for-dose basis in animals this compound was more potent after oral administration than was cimetidine. Whilst it seemed probable that tiquinamide would be well absorbed, it was not known whether
it would be more extensively metabolized than cimetidine on its first passage through the intestinal wall and the liver.

The site of gastrointestinal absorption also has particular significance for an agent acting directly on the gastric mucosa. An ideal compound would be taken up directly and rapidly from the stomach contents. In this way, the possibility of a low active site availability resulting from high first-pass metabolism and dissipation of drug among other tissues would be circumvented. Indeed, allied to extensive gastric absorption, a high first-pass effect could be positively advantageous for a drug acting on the gastric mucosa, since the resultant low systemic drug concentrations would mean a minimal risk of side-effects, provided that metabolites were innocuous. Gastric absorption had not been suggested as a significant feature of cimetidine. It was less likely for this compound than for tiquinamide in view of its higher pKa (7 as compared with 5.2), but in any case was perhaps of little significance in this case because of the high bioavailability after intestinal absorption. For tiquinamide, extensive gastric absorption might prove useful if the reduction in bioavailability by first-pass metabolism should prove high.

The nature of the association of a direct-acting anti-secretory compound with the gastric mucosa may be significant. Particularly important is the question of whether this association is a readily reversible one in which drug in the mucosa rapidly equilibrates with that in the systemic circulation. Under these circumstances, the time-course of drug in plasma is representative of that in the gastric mucosa and monitoring plasma concentrations may help the clinician to adhere to the most effective therapeutic regimen for a given subject. Otherwise the kinetics of release of the drug from the receptor site may differ markedly from those of elimination from plasma, and thus pharmacodynamics will not be related to plasma kinetics. Studies with cimetidine in normal volunteers had indicated a relationship between plasma concentration of drug and inhibition of acid secretion (Burland et al., 1975). It was of interest to see whether such a relationship would also exist for tiquinamide.

In the event that plasma concentrations did mimic those at the receptor site, what would represent an optimal plasma elimination half-life? In defining the shortest acceptable half-life for an
anti-secretory drug, one must take account of the fact that a major limiting factor in the use of antacids is the rapidity with which they are emptied from the stomach. However, it is possible to effectively control ulcer pain by maintaining pH of gastric contents $> 4$ with hourly doses of sodium bicarbonate (1 g), even in quite severe cases (Card, 1966). Thus there is little advantage over conventional antacids of a drug which has to be administered more than, say, four times a day. Another factor which determines the ideal half-life for an anti-secretory compound is related to nocturnal acid secretion. Ideally, the half-life of the drug should be sufficiently high to maintain therapeutic plasma concentrations throughout an average sleep period. Otherwise the ulcer patient will be awakened by pain during the night. This is the time when he is most vulnerable to gastric acid secretion because natural buffering by food in the stomach is minimal.

A rapidly absorbed drug of half-life equal to the average sleep duration of 8 h, will result in plasma concentrations which fluctuate between a maximum of 25% above the steady state level to a minimum of 25% below this value. Thus, if the dose magnitude is adjusted so that the minima exceed the concentration required to control the rate of acid secretion, the nocturnal ulcer pain can be avoided. Maxima of 25% above the steady-state concentrations should be tolerable provided that the drug in question has a satisfactory therapeutic index. A drug with a half-life of 8 h fits neatly into a 3 times daily regimen of equal doses. By comparison, consider the case of a rapidly absorbed drug of half-life 2 h. A 8-hour sleep represents 4 half-lives, during which time the plasma concentration has dropped to only 6.25% of its maximum value. Therefore very high maximum plasma concentrations must be achieved if the minima are still be exceed the minimum therapeutic concentration. Such peaks could conceivably be associated with side effects for a drug which does not have a very high therapeutic index. Cimetidine has rather a short half-life for an anti-secretory compound, around 2 h in man (Griffiths, Lee and Taylor, 1977; Burland et al., 1975). In order to cope with nocturnal acid secretion, the bedtime dose administered is 400 mg, which is twice the dose given three times during the day. In this case, no acute side-effects are known to result.
Indeed single oral doses as high as 800 mg have been administered without ensuing difficulty (Blackwood and Northfield, 1977).

However, risk of side-effects would obviously be minimized if wide fluctuations in plasma concentration could be avoided, and therefore it was felt that an ideal half-life for an anti-secretory compound such as tiquinamide would be of the order of 8 h.

Metabolism of a drug can lead to the formation of active metabolites. Thus, for example, 4-hydroxypropranolol is a metabolite of the β-receptor antagonist propranolol formed after oral dosing and contributing quite substantially to the anti-hypertensive activity of this compound (Paterson et al., 1970). It is important that such metabolites should be defined so that their contributions to the overall pharmacological activity of the drug can be assessed. Attempts to correlate drug plasma concentrations with pharmacological effect may need to take into account the plasma concentrations and relative potencies of active metabolites in order to arrive at a meaningful relationship. Thus it was of interest to know whether active metabolites of tiquinamide existed. The most likely candidates to show anti-secretory activity would be compounds containing the thioamide group since the nitrile compound corresponding to tiquinamide showed substantially diminished activity and the analogous amide was inactive (Beattie et al., 1977).

Safety evaluation forms a critical part of the development of a potential drug. Animal species chosen for the study of a compound’s toxicity should be appropriate models for man. Thus it is necessary to compare the rates and extents of absorption and elimination in candidate species with those in man so as to know how the dosage regime applied in animal toxicity studies relates to the anticipated therapeutic regime and to establish either that they metabolize the drug in the same manner as man or that the differences are not significant. Candidate species for the study of the toxicity of tiquinamide were the rat and patae monkey, these being chosen from among a relatively few species of animal for which an adequate data bank of normal values for haematological, biochemical and histological parameters was available. It remained to be seen whether these species would resemble man in the manner of metabolism and disposition of tiquinamide.
One aspect of a drug's disposition which may be particularly important with respect to its potential safety is the extent and nature of the localization in tissues of the drug or its metabolites. Compounds may accumulate to high concentrations in certain tissues as a result, for example, of covalent binding to tissue components such as proteins, nucleic acids, or pigments. Such localization may lead to toxic lesions. Thus, for example, high concentrations of the anti-malarial compound chloroquine in the uveal tract which result from binding of the compound to melanin have been associated with the retinopathies produced by this drug (Bernstein et al., 1963).

The possibility of active metabolites has been discussed in connection with their potential contribution to the pharmacological activity of the drug. However, it may also be the case that metabolites have acute or chronic toxic effects. Thus, for example the severe hepatic necrosis which results from paracetamol over-dosage has been attributed to a reactive N-hydroxy-metabolite which, at low doses, is conjugated with glutathione and safely eliminated, but which at higher doses exceeds the supply of glutathione, binds covalently to liver macromolecules and induces centrilobular necrosis (Healey et al., 1978). Thus evaluation of the toxicities of identified metabolites may contribute greatly to confidence in the safety of a drug or may identify potentially limiting side-effects or adverse reactions. The most obvious threat of toxicity from tiquinamide was the risk of generation of hydrogen sulphide during the desulphuration of the compound. As discussed previously (p.26), desulphuration to the nitrile was a likely route of metabolism for such a thioamide and it was possible that free hydrogen sulphide would be released by this reaction. Hydrogen sulphide is an extremely toxic compound, having LD50 values in the rat of 0.27-0.55 mg/kg intravenously and 2.3-2.8 mg/kg intraperitoneally (Smith and Williams, 1961). It was implicated as the primary agent responsible for the high acute toxicity of monosubstituted arylthioureas e.g. phenylthiourea, which underwent desulphuration to the nitrile in a manner at least superficially analogous to that proposed for tiquinamide (Smith and Williams, 1961). Thus evaluation of the rate and extent of this reaction as related to the acute toxicity of the drug formed one of the
objectives of the study. It was possible also that evaluation of the toxicological properties of the major metabolites of tiquinamide would prove desirable. Thus to conclude, at the outset of the present study, there was nothing known about the extent or nature of the metabolism of tiquinamide in animals or man, on rates of disposition processes such as absorption or elimination, or on tissue accumulation or localization of drug-related products. Furthermore, tiquinamide represented an example of a novel class of compounds, the 5,6,7,8-tetrahydroquinolines. The metabolism and disposition of this type of compound had not previously been studied. Information on the metabolic behaviour of other partially saturated quinoline or isoquinoline compounds was very scarce. The objective, therefore, was to furnish fundamental information about the metabolism and disposition of this compound and, wherever possible, to make use of this information to aid the development of the drug.
CHAPTER II: MATERIALS AND METHODS
The preparation of $^{14}$C-tiquinamide base was performed by The Radiochemical Centre, Amersham. The synthetic routes involved are shown in Fig. II.1.

Conversion to the hydrochloride salt was carried out by Dr. R. Crossley, Department of Medicinal Chemistry, Wyeth Laboratories (U.K.). The final product was shown by thin-layer chromatography to be 97% radiochemically pure. Further purification was deemed inadvisable in view of the tendency of the thioamide to undergo spontaneous dissociation to the nitrile 8-cyano-5,6,7,8-tetrahydro-3-methylquinoline, such that it always existed in the presence of a small proportion of the latter compound.

Specific activities of various batches of the hydrochloride ranged from 7.7-8.8 \( \mu \)Ci/mg. Dilution with unlabelled drug was carried out as dictated by the needs of individual experiments. Unless otherwise stated, solvents were of reagent grade, obtained from May and Baker Limited, Dagenham, Essex.

**THIN-LAYER CHROMATOGRAPHY: PLATES AND SOLVENTS**

For thin-layer chromatography, plates were of silica gel, 0.25 mm with fluorescent marker (E. Merck & Co. Ltd., Darmstadt, West Germany, supplied by Anderman & Co., East Moseley, Surrey). Plates with a pre-adsorbent zone made by Quantum Industries Ltd., Fairfield, New Jersey, U.S.A. were supplied by Microbio Laboratories, London. Similarly, preparative layer plates were either 2 mm silica gel (E. Merck & Co. Ltd.) or 1 mm silica gel with pre-adsorbent zone (Quantum Industries Ltd).

The following solvents were used for thin-layer chromatography of end-products of tiquinamide in plasma, urine and bile.

**Acidic solvents**

Solvent 1: Chloroform/methanol/acetic acid (18:2:1)
Solvent 2: Chloroform/acetic acid (19:1)
Solvent 3: Toluene/ethyl formate/formic acid (60:35:5)
Solvent 4: Toluene/ethyl formate/formic acid (5:4:1)
Solvent 15: Ethyl acetate/acetic acid/methanol/water (70:10:20:5)
Cy clohexanone

\[ \begin{align*}
\text{H}_3\text{C} & \text{C} = \text{CHO} \\
\text{H}_2\text{N} & \text{C} \text{H}
\end{align*} \]

3-amino-2-methacryl aldehyde

120°C 16 h
Distilled

\[ \begin{align*}
\text{CH}_3
\end{align*} \]

5,6,7,8-tetrahydro-3-methylquinoline

Ether soln. treated with 9% (w/v) BuLi/hexane

\[ \begin{align*}
\text{CH}_3
\end{align*} \]

5,6,7,8-tetrahydro-8-lithio-3-methylquinoline

1. Trimethylsilyl thiocyanate in ether, 0°C
2. 4h at norm temp.
3. Acidified (2N HCl)
4. Basified with sodium carbonate (pH 10); extracted with chloroform

\[ \begin{align*}
\text{CH}_3
\end{align*} \]

\[ ^{14}\text{C}-\text{TIQUINAMIDE} \]

(5,6,7,8-tetrahydro-3-methylquinoline-8-thiocarboxamide)

Conversion to hydrochloride

* Asterisk denotes position of "C-label

Fig. II.1 Radiosynthesis of \(^{14}\text{C}-\text{tiquinamide. HCl}\)
Neutral solvents
Solvent 5: Dichloromethane/acetone (5:4)
Solvent 6: Toluene/ethanol (4:1)
Solvent 7: Ethyl acetate/methanol (95:5)
Solvent 8: Chloroform/acetone (1:1)
Solvent 16: Ethyl acetate
Solvent 19: Chloroform/methanol (95:5)

Basic solvents
Solvent 9: Chloroform/methanol/ammonia (5:4:1)
Solvent 10: Chloroform/isopropanol/ammonia (90:10:1)
Solvent 11: Dichloromethane/methanol/ammonia (65:35:5)
Solvent 12: Ether/ethanol/ammonia (6:4:1)
Solvent 13: Ether/ethanol/ammonia (8:2:1)
Solvent 14: Ethyl acetate/methanol/diethylamine (70:20:15)
Solvent 17: Cyclohexane/chloroform/diethylamine (5:4:1)
Solvent 18: Chloroform/methanol/ammonia (90:10:1)

ANAESTHETIC
Halothane anaesthetic was supplied by ICI Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire.

ANIMALS
Rats: Male and female COBS* rats, CD strain, descended from Sprague-Dawley were supplied by Charles River (U.K.) Ltd., Marston, Kent. They were maintained on a diet of Oxoid 41B pellets and weighed between 200-300 g at the time of use unless otherwise stated. The same 4 male and 4 female animals were used throughout plasma kinetic, excretion balance and tissue excision studies. Rats for biliary excretion studies were larger, circa 400 g.

Monkeys: Male and female red patas monkeys (Erythrocebus patas), weighing 2-5 kg were supplied by Shamrock Farms Ltd., Brighton, Sussex or Animal Suppliers (London) Ltd., Welwyn, Herts. They were kept on a diet of Oxoid 41B pellets, supplemented daily by fresh fruit. The same 4 monkeys were used to investigate plasma kinetics and excretion. Two of these animals were subsequently used for study of tissue distribution by tissue excision. Plasma kinetic data was subsequently supplemented by results in two further animals.

* Caesarian-originated, barrier sustained
Human subjects: Human subjects were two healthy male volunteers, ML and DM, weighing respectively 80 and 95 kg. They were fasted overnight but allowed to drink at will, though asked to refrain from strong tea or coffee on the day of the study.
METHODS

DRUG ADMINISTRATION

All animals were starved overnight and for approximately 4 h after drug administration, but were allowed water ad libitum.

For oral administration to rats, $^{14}$C-tiquinamide HCl was given by gastric intubation as a solution in saline (10 ml/kg). The usual dose given was 10 mg/kg unless otherwise stated. This was chosen as a dose which had a median anti-secretory effect.

For oral administration to monkeys, the drug was given by gastric intubation as a solution in saline (5 ml/kg). The dose was 10 mg/kg unless otherwise stated.

For administration to man, the drug was given in No 4 soft gelatin capsules filled out with lactose. Each volunteer received initially a 20 mg dose containing 5 $\mu$Ci $^{14}$C-tiquinamide HCl. After a period of 7 days, the same volunteers were subsequently administered a 50 mg dose containing 50 $\mu$Ci $^{14}$C-tiquinamide HCl. The 5 $\mu$Ci dose was of sufficiently high radioactivity to allow approximate determination of the rate of elimination such that the potential radiation exposure to a subject receiving a 50 $\mu$Ci dose could be assessed. The 50 $\mu$Ci dose was adequate for more precise delineation of the pharmacokinetic profile over a period of several days and also allowed determination of the chromatographic pattern of radioactive end-products in 0-24 h urine.

SAMPLE COLLECTION

Blood: Blood (0.1-0.5 ml portions) was collected from the tail vein of conscious rats. When larger volumes (6-8 ml per animal) were required, animals were exsanguinated from the inferior venae cavae while under ether anaesthesia.

Monkeys were bled from the femoral vein (2-3 ml) and human volunteers from the cephalic vein (10-20 ml). In all cases, blood was collected in pre-heparinized tubes. Plasma and red cells were separated by centrifugation using either a Mistral 4L centrifuge (MSE Ltd., Crawley, Surrey) or for small volumes, a Beckman Microfuge, Beckman Instruments Ltd., Croydon, Surrey.

Tissues: Small portions (100-200 mg in triplicate) of a wide range of tissues of rats and monkeys were removed by excision.
Urine, faeces and expired air: For collection of urine and faeces, rats were housed in all-glass metabolism cages (Jencons Ltd., Hemel Hempstead, Herts). Expired $^{14}$CO$_2$ was trapped by drawing air into the cages and through Nilox columns containing 33% ethanolamine in ethanol.

Monkeys were housed either in specifically constructed primate metabolism cages (National Iron and Wire Co. Ltd., Manchester) or in conventional primate cages (E. K. Bowman Ltd., London) modified by the addition of sloping urine collection trays draining into suitable collecting vessels.

Human subjects voided urine samples directly into plastic collection bottles and faecal samples into small round polythene tubs (2.5 litre capacity, supplied by Solmedia Ltd., Walthamstow).

Bile: Bile was collected from the cannulated bile-ducts of female rats anaesthetized with halothane. Anaesthesia was induced with a 5% mixture of halothane in oxygen at 2 l/min and maintained for periods of up to 6 h on 1.5-2% mixture at 500 ml/h. In each animal, an abdominal incision was made and the bile duct located and freed from surrounding connective tissue. An incision was made in the duct wall with scissors and a cannula (Green Luer, 2 FG, Portex Ltd., Hythe, Kent) was inserted. The cannula was pushed into the duct in the direction of the liver until bile flowed freely along it and was then secured in position by two ligatures. The cannula was cut to a suitable length and the secreted bile allowed to drain into a 2 dram glass vial.

In monkeys, bile was collected from the gall bladders of the animals killed for tissue excision studies.

SAMPLE PREPARATION

Blood and tissues: Whole blood and tissues were prepared for scintillation counting by an in-vial wet oxidation method similar to that described by Mahin and Lofberg (1966). Up to 250 µl blood or 250 mg tissue was placed in a suitable scintillation vial (Packard, low potassium glass vials, type 6001050) 0.5 ml 70% V/v perchloric acid and 1.0 ml 100 vol hydrogen peroxide were added and the vial sealed with a cap containing a polythene scive. The vial was heated in an oven at 70°C for 1 h and subsequently cooled at -20°C for 20 min.
**Bile:** Bile was prepared for liquid scintillation counting by direct incorporation of aliquots (20 μl) into NE260 scintillant. For thin-layer chromatography, bile was diluted with ethanol, evaporated to dryness under reduced pressure and residues dissolved in small volumes of methanol.

**Faeces:** Rat faeces were homogenized in methanol and the supernatant after centrifugation decanted. The pellet was dried overnight in an oven at 70°C. Portions of the dried pellet were treated by the in vial wet oxidation method of Mahin and Lofberg (1966), as described previously. Monkey and human faeces were homogenized to a stiff sludge in a 0.1% aqueous solution of the bacteriostat chlorhexidine ("Hibitane", ICI Pharmaceuticals Ltd., Macclesfield, Cheshire), using a Colworth Stomacher paddle homogenizer (A. J. Seward & Co. Ltd., London), as described by Morrison and Franklin (1978). Aliquots of the homogenate were treated by the modified Mahin and Lofberg acid digestion procedure described above.

**Carcasses:** Carcasses were dissolved by overnight incubation at 60°C in SHT solubilizer (1 litre/rat containing 80 g NaOH, 100 ml Triton X-405, 300 ml methanol and 600 ml water), according to the method of Dent and Johnson (1974).

**Extraction from plasma or urine of a toluene-soluble fraction containing unchanged tiquinamide:** The search for a specific assay for tiquinamide was made difficult by its readiness to degrade to the corresponding nitrile derivative during simple extraction procedures from biological fluids at initial concentrations below 100 μg/ml approx. This meant that the unchanged drug could only be specifically assayed in plasma of animals dosed with radio-labelled drug by a procedure involving loading of plasma to the appropriate concentration with unlabelled drug, solvent extraction of basified plasma with toluene and subsequent thin-layer chromatography of the extract, followed by quantitation of the band corresponding to unchanged drug by liquid scintillation counting. Such a procedure was adopted in order to determine unambiguously the kinetics of unchanged tiquinamide in animals. However, the method was relatively insensitive, requiring large volumes of plasma which necessitated, at least in rats, working
with pooled samples from several animals. Thus it imposed limitations on experimental design. Therefore, in some experiments in which it was more important to monitor the general kinetics of tiquinamide and related metabolites rather than the absolute concentrations of unchanged drug, a simplified procedure was utilized in which toluene-soluble radioactivity was used without subsequent t.l.c. as a measure of the maximum concentration of unchanged drug present.

In a glass scintillation vial, plasma (0.1-2.0 ml) was made alkaline by addition of 1M bicarbonate buffer (pH9). Toluene-based scintillant (10 ml; 8.5 g butyl PBD* in 1 litre BDH "Analar" toluene) was added and after agitation for 15 sec on a vortex mixer and phase separation effected by gravity, 8 ml of the toluene scintillant was transferred to a clean glass vial. The aqueous phase and residual toluene were dissolved in 15 ml NE260 scintillant and total radioactivity determined as the sum of toluene-soluble and residual radioactivity. The method was based on the in vial extraction procedure described by Hess et al., 1972.

Tiquinamide was extracted quantitatively by this procedure, but extracts contained in addition the nitrile derivative and two other metabolites. The specificity of this assay will be discussed in more detail in Chapter VII.

DETERMINATION OF RADIOACTIVITY

Liquid scintillation counting: The universal scintillant for incorporation of all samples except toluene extracts was NE260 (Nuclear Enterprises Ltd., Edinburgh). Up to 2.0 ml of plasma or urine, 1 ml of SHT digests, 20 ul of bile, or the total product of acid digestion of blood, tissues or faeces (up to 250 mg) were dissolved in 15 ml NE260 prior to liquid scintillation counting.

Determination of radioactivity was in a Packard "Tri-Carb" 3380 liquid scintillation spectrometer, equipped with automatic external standardization. Counting efficiency correction curves (external standard ratio vs efficiency) were determined following chemical quenching by chloroform of scintillant to which had been added known amounts of a labelled standard (\(^{14}\text{C}-\text{hexadecane}\)).

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* 2-(4-tert-Butyl phenyl)-5-(4'-biphenylyl)-1,3,4-oxadiazole
Comparison of the efficiency correction curves prepared by chemical or colour quenching (using Sudan Yellow in toluene) showed them to be very similar except at 14C efficiencies less than 30%.

In counting mixed isotope samples containing 14-carbon and tritium, a restricted "window" setting was used which virtually eliminated inclusion of tritium counts in the carbon channel. For determination of tritium, however, a correction for spillover of carbon into the tritium channel had to be made, based on a calculation using the isotope ratio.

Isotope ratio =

\[
\frac{\text{efficiency of counting tritium in tritium channel}}{\text{efficiency of counting carbon in tritium channel}}
\]

The isotope ratio was determined at different levels of quench and its application allowed the calculation of the actual dpm in the tritium channel due to tritium. The calculation was made by a dedicated computational facility. (Packard Automatic Activity Analyzer). The use of the isotope ratio method makes use of the assumption of negligible spillover of tritium into the carbon channel. This assumption was valid except in the gastric absorption studies in conscious monkeys, when the specific activity of 3H-inulin greatly exceeded that of 14C-salicylic acid at the later time-points. Therefore, in these experiments, the actual dpm due to carbon and tritium had to be calculated manually from simultaneous equations using the carbon and tritium cpm values and the known efficiencies for counting carbon and tritium in each channel.

Detection and quantitation of radioactivity on thin-layer plates: Radioactivity on thin-layer plates was detected by use of a thin-layer scanner (Panax Equipment Ltd., Redhill, Surrey) or by apposition autoradiography. Autoradiography was performed by placing thin-layer plates in contact with X-ray film (X-O-Mat RP, Kodak Ltd., London) in suitable light-proof X-ray cassettes (Kodak Ltd., London) for periods of 7 days or more. At the end of the exposure period, films were removed and developed in an X-ray developer (Ilford DX-80, diluted with 4 parts water). After 3 min in the developer, each film was transferred to a stop-bath containing 3% acetic acid and finally to a third bath containing fixative (Ilford FX-40, diluted with 4 parts water). Films were washed in cold, running water for 15 min each and hung up to dry.
Quantitation of radioactivity on thin-layer plates was by scraping and subsequent liquid scintillation counting. The area of the plate between 1 cm below the origin and the solvent front was segmented as described in the relevant Experimental section. Each segment was scraped off with a straight-edged scalpel blade, powdered and transferred to a polypropylene scintillation vial. Desorption of radioactivity was effected by addition of 2 ml methanol/water (1:1), and assay of radioactivity was by liquid scintillation counting, following the addition of 15 ml scintillant.

WHOLE BODY AUTORADIOGRAPHY

Whole body autoradiography was performed by Dr. P. Nicholls and Dr. D.K. Luscombe (Department of Pharmacology, University of Wales Institute of Science and technology, Cardiff). The animal carcass was pinned to a board and rapidly frozen in a mixture of hexane and CO₂. The frozen rats were then embedded in frozen 1% carboxymethylcellulose and sagittal sections at 20 μ thickness were cut from the left side of the animal. The frozen sections were transferred to 3M’s tape and dehydrated at -20°C for 4 days. The sections were then exposed to Kodak X-ray film for either 4 weeks (Kodirex, Kodak Ltd., London) or 3 months (Industrex, Ilford Ltd., London). After this time the sections were removed from the photographic emulsion in air in the darkroom. The X-ray film was developed in Ilford X-ray developer for 4 min at 20°C to yield the autoradiograms.

PHARMACOKINETIC ANALYSIS

Analysis of plasma kinetics assumed first order, linear kinetics and was performed according to methods described, for example, by Wagner (1971). Plasma concentrations of unchanged drug, toluene-soluble radioactivity or total radioactivity were logarithmically transformed (log₁₀) and plotted against time. If the resulting plot was linear, representing a monoexponential relationship, the best-fit straight line was determined by regression analysis, when the elimination half-life could be determined from the slope of the line, thus:

\[
\text{Elimination rate constant, } k_e = 2.303 \times \text{slope}
\]

\[
\text{Elimination half-life, } t_{1/2} = \frac{0.693}{k_e}
\]

In those cases when the plot was not linear throughout the entire time-course, the process of curve-stripping was performed, in order to distinguish two exponential components, a fast (α) phase and a slow (β) phase. It was not found necessary in these experiments
to resort to more than two exponential components to define the time-course of any elimination process. The half-life of each phase was determined after regression analysis as in the monoexponential case.

Areas under plasma concentration - time curves were calculated by means of the trapezoidal rule and were expressed as dose/area ratios for the purposes of comparison at different doses.

Analysis of urinary excretion kinetics was by the sigma-minus method described by Martin (1967). The total proportion of the radioactive dose excreted by the urinary route was summed and the amount excreted by a given time after dosing expressed as a percentage of this total. The percentage of the total activity remaining to be excreted in urine was then determined by subtraction. Plots of $\log_{10}$ (percentage total remaining to be excreted in urine) against time resulted in curves which could be represented as the sum of two exponential components, separable by curve-stripping as described above. Regression analysis performed to define the best-fit straight lines representing the individual component phases ($\alpha$ and $\beta$) made possible the calculation of the half-life for each phase from the slope of the line, as described above. The proportions of total excretion occurring in each phase were represented by the respective y-intercepts.

For analysis of urinary excretion kinetics in the rat, a modification of the sigma-minus method was required in order to allow for the fact that excretion was incomplete at 7 days when the last collections of urine and faeces were made. Since carcass residue studies revealed the proportion of the dose remaining to be excreted at this time, the proportion of this amount which would be ultimately eliminated in urine could be determined from the ratio of urinary/faecal excretion occurring during the slow phase in the previous 7 days, found to be fairly constant. Thus a value for the true total percentage of the dose eliminated in urine in infinite time could be determined so as to provide the basis for sigma-minus analysis.
CHAPTER III: ABSORPTION OF TIQUINAMIDE IN THE PATAS MONKEY
SUMMARY

The extent of gastric absorption of tiquinamide in the patas monkey was investigated in view of the possibility that the combination of the low pKa with the relatively high lipophilicity of this compound promoted absorption by this route. Studies in conscious animals employed a novel mixed-isotope method which allowed determination of gastric absorption rates in the presence of normal gastric emptying. The gastric absorption rate was determined as the rate of decline of the ratio of concentration of $^{14}$C-labelled drug to that of an inert marker, $^{3}$H-inulin.

Results showed that whilst tiquinamide was apparently well absorbed from the stomachs of anaesthetized, pylorus-ligated monkeys, it was inconsistently gastrically absorbed in conscious animals with uncontrolled starting pH of gastric contents. Reasons for this difference were discussed.

Tiquinamide was found not to significantly alter the rate of gastric emptying in the patas monkey.
INTRODUCTION

Gastric absorption of the weakly basic tiquinamide was a finite possibility because of the combination of its anti-secretory properties with its low pKa and moderately high lipophilicity, as discussed in Chapter I. It was of interest to establish the extent of gastric absorption of tiquinamide because it could be advantageous for an inhibitor of acid secretion to be absorbed through the gastric mucosa, especially if the compound were subject to a high degree of first-pass metabolism. Because delayed gastric emptying would enhance the extent of any gastric absorption which occurred, it was important also to determine the effect of tiquinamide on the rate of gastric emptying. There had been a previous indication that the compound inhibited gastric emptying in rats (Beattie et al., 1979b), but it was more relevant to establish its effect in a primate because the normal rate of gastric emptying in primates, and particularly in man, was considerably slower than in rats. Thus gastric emptying half-lives in patas monkey and man are respectively approximately 30 min (Franklin, 1977) and 47-70 min (Van Dam, 1974; Griffith et al., 1968; Harvey et al., 1970) compared with around 10 min only in the rat (Feldman, Wynn & Gibaldi, 1968; Franklin, 1977).

The method most frequently used for investigating the gastric absorption of drugs is that employed by Schanker et al., (1957), which involves measurement of the decline in concentration of drug in the pylorus-ligated stomach. In the absence of adsorption to the stomach wall, this provides a means of studying the potential for gastric absorption of a drug in relation to its lipophilicity and pKa and for comparing this potential with those of other drugs. However, the method is subject to the limitation that it does not permit evaluation of the significance of gastric absorption in the presence of normal gastric emptying. Furthermore, the presence of an anaesthetic as an accessory to the pylorus-ligation may itself influence the course of gastric absorption by altering gastric motility and blood flow to the stomach. Hence, if the significance of gastric absorption of a given drug under normal physiological conditions is to be properly evaluated, this process should be examined in conscious animals with a functioning pylorus.
Several workers have described the use of non-absorbable markers in absorption studies to make an allowance for loss of drug by gastric emptying and also for dilution of stomach contents by gastric secretion. For example, Cooke and Hunt (1970) used the marker phenol red when studying the gastric absorption of acetylsalicylic acid in man. The method relies on the principle that changes in drug concentration resulting from gastric emptying and dilution of stomach contents are paralleled by similar changes in the concentration of the marker. Any additional decline in drug concentration must then result from gastric absorption. In all previous studies, the non-absorbable marker was unlabelled and was determined spectrophotometrically by an assay separate from that used to measure concentrations of the drug in stomach contents. The low sensitivity of the assay methods necessitated the use of relatively high concentrations of non-absorbable markers. Under these conditions, the method is subject to the criticism that the marker itself may influence the absorption of the drug (Beerman, Groschinsky-Grind and Rosén, 1976). Because of these limitations, a novel mixed isotope procedure was developed during the present work to investigate absorption under normal physiological conditions. The ratio of concentration of a $^{14}$C-labelled drug to that of an $^3$H-labelled marker was used to make automatic compensation for effects of volume changes and gastric emptying. The high specific activity of the marker made possible the use of a much lower concentration than that of drug, so minimizing the possibility of interference with absorption. The rate of gastric absorption was indicated simply by the rate of decline of the $^{14}$C/$^3$H ratio in gastric contents.
MATERIALS AND METHODS

Materials

\(^{14}\text{C}}\)-salicylic acid (specific activity 87.2 \(\mu\text{Ci/mg}\)), \(^{14}\text{C}}\)-polyethylene glycol 4000 (PEG 4000; 34.7 \(\mu\text{Ci/mg}\)) and \(^{3}\text{H}}\)-inulin (500 \(\mu\text{Ci/mg}\)) were obtained from The Radiochemical Centre, Amersham and diluted as dictated by the needs of individual experiments.
EXPERIMENTAL

(a) Absorption of tiquinamide in anaesthetized, pylorus-ligated and sham-operated patas monkeys

Surgery was performed under halothane anaesthesia (0.5-4.0%). Animals were kept warm by means of a thermal blanket. Rectal temperature and respiration rates were monitored throughout. Depth of anaesthesia was adjusted as necessary by varying the halothane/oxygen ratio.

Tracheotomy was performed to assist breathing under anaesthesia and abdominal incisions were made. In three animals, the pylorus was subsequently ligated. In the other two animals, sham-operation was performed by passing a ligature underneath the pylorus but leaving it untied and the pylorus unrestricted. A solution of $^{14}$C-tiquinamide HCl in isotonic saline was warmed to 37°C to reduce the probability of regurgitation, and was then administered by gavage at a dose of 10 mg/kg (5 ml/kg).

Blood samples (circa 2 ml) were collected from the femoral vein at various times up to 5 h after dosing as detailed in Fig. III.1. Concentrations in plasma of total radioactivity and toluene-soluble radioactivity containing unchanged drug were determined by liquid scintillation counting, as described previously. (Chapter II p.45).

(b) Gastric absorption of tiquinamide in conscious patas monkeys

Salicylic acid was chosen for the validation of the mixed isotope method because it was readily available in radiolabelled form and, like many other weak acids, it had been shown to be rapidly absorbed from the stomach (Schanker et al., 1957; Hogben et al., 1957).

$^{14}$C-salicylic acid (1.60 μCi/mg) was administered at 0.5 mg/kg by gastric intubation using a solution (5 ml/kg) of the drug in saline containing 1 μg/ml $^{3}$H-inulin (500 μCi/mg). Alternatively, $^{14}$C-tiquinamide HCl (0.21 μCi/mg) was administered in the same manner at 10 mg/kg. The dose solution in this case was in isotonic saline containing 6 μg/ml $^{3}$H-inulin (500 μCi/mg).

For a period of 1 h after drug administration, small aliquots (0.5-1.0 ml) of gastric contents were withdrawn at 10 min intervals. At the end of this time, stomach contents were aspirated so that
the amount of remaining $^3$H-inulin could be assayed. Because of
the possibility of incomplete recovery of stomach contents in the
final gastric wash, the efficiency of this recovery was checked
by introduction of an additional non-absorbable marker, $^{14}$C-PEG
4000. It was administered by gastric intubation of 20 ml of a
12 $\mu$g/ml solution (8.15 $\mu$Ci) in isotonic saline and was recovered
within 3 min of dosing by washing out with $3 \times 50$ ml isotonic
saline. Radioactivity administered as $^{14}$C-PEG 4000 was in large
excess over that of any remaining $^{14}$C-salicylic acid, which thus
did not interfere with the determination of the $^{14}$C-labelled marker.

All samples of gastric contents were centrifuged to precipitate
any solid matter. An estimate of the pH of each sample was made
using pH papers (Whatman BDH narrow range, graduated in 0.5 pH
units). $^{14}$Carbon and tritium activities of 0.1-0.5 ml aliquots
were assayed by liquid scintillation counting as described.
(Chapter II p.45).

The half-life of gastric absorption of salicylic acid was
calculated from the slope of the regression lines relating log$_{10}$
$^{14}$C/$^3$H ratio to time after dosing. The gastric emptying half-life
was determined from the amount of $^3$H-inulin not emptied from the
stomach, assuming exponential emptying, as established by Hunt
and MacDonald (1954) from studies in man. The method of calculation
used the formula:

$$\text{Gastric emptying t/2} = \frac{0.301 \cdot t}{\log_{10}\left(\frac{X_0}{X_t}\right)}$$

where $\left(\frac{X_0}{X_t}\right)$ was the fraction of the dose of $^3$H-inulin remaining
in the stomach at time t.

The total percentage of the administered dose absorbed from
the stomach was calculated from the rate constants for gastric
absorption and gastric emptying, $k_{ga}$ and $k_{ge}$ respectively, as
follows:

$$\text{Percentage dose absorbed} = \frac{k_{ga}}{k_{ga} + k_{ge}} \times 100\%$$

where rate constant, $k = \frac{0.693}{t_{1/2}}$
(c) Effect of tiquinamide on gastric emptying in conscious patas monkeys

The half-life of normal gastric emptying was determined using $^{14}$C-PEG 4000 (21.4 μCi/mg) as an 11 μg/ml solution in isotonic saline. Each animal received by oral intubation 20 ml containing 5 μCi (0.06 mg/kg). Unlabelled tiquinamide HCl was administered at 10 mg/kg as a solution in the above vehicle (5 ml/kg). The experiment was performed twice in each monkey. In each experiment every monkey received a control dose containing $^{14}$C-PEG 4000 alone in the morning followed by a dose containing also tiquinamide HCl in the afternoon.

At 30 min after dosing, $^3$H-inulin (89.7 μCi/mg) at 6 μg/ml in saline (20 ml) was introduced into the stomach and gastric contents were washed out with 3 x 50 ml isotonic saline. All gastric washes from each animal were pooled and replicate 1 ml aliquots were assayed for $^{14}$-carbon and tritium content by the liquid scintillation counting procedure, described in Chapter II p. 45.

Gastric emptying half-lives were determined by the method described above.
RESULTS

(a) Absorption of tiquinamide in anaesthetized, pylorus-ligated and sham-operated monkeys

In Fig. III.1 are expressed plasma concentrations of toluene-soluble radioactivity and total radioactivity in anaesthetized, pylorus-ligated or sham-operated patas monkeys.

It was evident from the detection of the significant concentrations of toluene-soluble and total radioactivity in the plasma of pylorus-ligated monkeys that gastric absorption had occurred in these animals.

Toluene-soluble radioactivity concentrations in plasma increased at similar rates in pylorus-ligated and sham-operated animals for a period of at least 2 h. Thus concentrations in pylorus-ligated animals were 0.7-1.2 μg equivalents ¹⁴C-tiquinamide HCl/ml at 1 h after dosing and in sham-operated animals were 0.7-0.8 μg equivalents/ml at this time. Similarly at 2 h plasma concentrations in pylorus-ligated animals were 1.1-2.2 μg equivalents/ml and in sham-operated animals 1.1-1.4 μg equivalents/ml. Thus absorption rates were similar in the two groups of animals over this period.

Concentrations of total radioactivity in plasma confirmed these observations, despite somewhat greater inter-animal variations in this parameter among the pylorus-ligated animals. Thus at 1 h after dosing total radioactivity concentrations in plasma of pylorus-ligated animals were 1.2-4.4 μg equivalents/ml and in sham-operated animals were 1.7-1.9 μg equivalents/ml. At 2 h after dosing, the concentrations were 2.1-5.3 μg equivalents/ml in pylorus-ligated animals and 2.9-3.0 μg equivalents/ml in sham-operated animals.

Absorption occurred more slowly after about 2 h, as it approached completeness. Thus in pylorus-ligated animals, the maximum value (2.2 μg equivalents/ml) of toluene-soluble radioactivity was achieved in one animal (♀ 309) at 2 h after dosing and in the other two animals, a plateau value of 1.3-1.6 μg equivalents/ml was approached from 3 h after dosing onwards.

The maximum proportion of unabsorbed drug at the termination of the experiment was 12-35%, as represented by total radioactivity in the gastric contents, which, however, might have included some absorbed drug secreted back into the stomach. In sham-operated

* The unit μg equivalent ¹⁴C-tiquinamide.HCl is derived by division of the determined radioactivity (dpm) by the specific activity (dpm/μg tiquinamide.HCl).
animals, less than 3% of the dose remained in the gastric contents at the termination of the experiment, but there may, of course, have been unabsorbed drug in the small intestine of these animals. Areas under the plasma-concentration time curves indicated that there was not a substantial difference in the total extent of absorption over the 0-4 h period between pylorus-ligated and sham-operated animals. Thus the areas under the plasma concentration time curve from 0-4 h for toluene-soluble radioactivity were 3.9-5.4 and 4.4-6.2 µg h.ml⁻¹ respectively. Similarly, for total radioactivity, areas calculated over the same time period were 7.4-20.1 and 13.9-14.4 µg h.ml⁻¹ respectively (Table III.1).

The pH of gastric contents at the time of death was relatively high in all animals, ranging from 4.1-6.4 in pylorus-ligated monkeys and from 6.7-7.7 in sham-operated animals.

(b) Gastric absorption of tiquinamide in conscious patas monkeys

Validation of the method for salicylic acid: In Fig. III.2 are expressed the ratios of $^{14}$C-salicylic acid/$^3$H-inulin in samples of gastric contents withdrawn at various times up to 60 min after a 0.5 mg/kg oral dose of $^{14}$C-salicylic acid administered simultaneously with an 0.05 mg/kg dose of $^3$H-inulin to 3 patas monkeys. Ratios declined monoexponentially with time (mean correlation coefficient = 0.996), indicating that gastric absorption of salicylic acid had occurred. In Table III.2 are presented the half-lives of gastric absorption and gastric emptying and the percentages of dose absorbed from the stomach. The half-life of gastric absorption was approximately 13 min and of gastric emptying 37 min.

The percentage of the administered dose absorbed from the stomach would therefore have been of the order of 70%.

Tiquinamide: In Fig. III.3 are expressed the ratios of $^{14}$C-tiquinamide/$^3$H-inulin and the approximate pH values in samples of gastric contents withdrawn at various times up to 60 min after a 10 mg/kg oral dose of $^{14}$C-tiquinamide HCl administered simultaneously with a 0.05 mg/kg dose of $^3$H-inulin to five conscious patas monkeys.

The change in the $^{14}$C/$^3$H ratio was more complex and showed greater inter-animal variation for tiquinamide than for salicylic acid. The major factor in the inter-animal variation appeared to be the pH of gastric contents. In two animals ($f^351$ and $f^354$),
the $^{14}C/^{3}H$ ratio declined within 1 min to 80-90% of the initial value, but subsequently remained approximately constant in this range for the following 60 min. The pH was found to decline from the initial starting value of 4.7 in the dose solution in saline to pH 2.5 ($\delta^351$) and pH 3.5-4 ($\delta^354$) at 1 min after dosing and subsequently did not rise above pH 2.5 in either monkey over the 60 min period of monitoring.

In two other monkeys (♀ 312 and ♀ 322), the $^{14}C/^{3}H$ ratio declined respectively to 31% at 10 min and 23% at 20 min after dosing and subsequently rose to settle around the range 40-50% and 50-60% respectively of the starting value. In these animals, the pH changes were less marked, remaining in the range 4.5-5.5, close to the pH of the dose solution, throughout the 60 min after dosing.

In the fifth monkey, $\delta^355$, the $^{14}C/^{3}H$ ratio declined rapidly over the first 30 min to a value of less than 2% of the starting value and remained below 10% of this value for the remainder of the experiment. There was evidence of gastric bleeding in this animal, and hence gastric mucosal damage may have contributed to the more rapid absorption of tiquinamide. However, it was also observed that the pH in gastric contents of this animal rose to pH 7 at 10 min after dosing and to pH 8.5-9.0 subsequently.

In view of the wide inter-animal variation and the absence of a simple linear relationship between log ($^{14}C/^{3}H$ ratio) and time, no sensible attempt could be made to determine half-lives of gastric absorption of tiquinamide in these animals. Consequently, the percentage of drug absorbed from the stomach could not be determined.

(c) Effect of tiquinamide HC1 on the rate of gastric emptying in conscious patas monkeys

In Table III.3 are expressed percentage recoveries of $^{14}C$-polyethylene glycol, percentage recoveries of $^{3}H$-inulin and gastric emptying half-lives in patas monkeys in the presence and absence of a 10 mg/kg oral dose of tiquinamide HC1.

Whilst the majority of values of gastric emptying half-life were below 50 min, there were some very high values indicative of gastric stasis. It has been suggested that depressed psychological states can result in delayed gastric emptying (Mayersohn, 1971). Possibly the trauma associated with removal
of animals from cages and restraint during dosing induced a delay in the onset of gastric emptying on some occasions. In view of the short experimental period of 30 min, quite a brief delay in the onset of emptying would have resulted in an apparent gastric emptying half-life substantially longer than normal. The distinction between normal gastric emptying and gastric stasis was made on the basis of a frequency distribution (Table III.4) which clearly demonstrated an approximately normal Gaussian distribution for 19 values in the range 0-50 min with an additional 9 observations distributed randomly over an interval from 70 to 100. Gastric stasis did not occur any more frequently in tiquinamide-dosed monkeys (5 observations) than under control conditions (4 observations). When these values were excluded from the statistical analysis, the mean gastric emptying half-life in control animals, averaged over two experiments, was $17.6 \pm 4.0$ min. In the presence of a 10 mg/kg oral dose of tiquinamide HCl, the mean half-life in the same 7 animals was $26.6 \pm 4.3$ min. Hence there was an indication that a 10 mg/kg oral dose of tiquinamide HCl delayed gastric emptying in the patas monkey to a minor extent. However, the difference was not significant at the 5% level ($p = 0.1-0.2$, as determined by Student's t-test).
DISCUSSION

A novel mixed isotope method for studying the gastric absorption of drugs

The effectiveness of this novel mixed isotope method for demonstrating the occurrence of gastric absorption was quite clearly established with reference to the well-known example of salicylic acid absorption. The only other circumstance which could have accounted for the monoexponential decline of the $^{14}C/^{3}H$ ratio in this experiment was concentration-dependent adsorption of the drug to the stomach wall. However, the absence of significant adsorption of salicylic acid to stomach wall had already been demonstrated by Schanker et al., (1957), who first observed the gastric absorption of this compound. The half-life of 13 min observed for the gastric absorption of salicylic acid in the present studies was very similar to the value of 14 min in man calculated from the data of Hogben et al., (1957) as presented by Levy and Leonards (1966).

In the rhesus monkey, Nayak and Benet (1974) demonstrated quite rapid gastric absorption of salicylic acid. Though it was not possible to determine an accurate half-life from their data, the appearance of maximum plasma concentrations within 1.5 h of drug administration suggested a half-life of a similar order to that determined in the present experiments. Thus the new mixed isotope method developed during the current work proved to be a useful simplification of previously described procedures for demonstrating the phenomenon of gastric absorption in conscious animals.

For acidic compounds such as salicylic acid not subject to secretion from plasma back into gastric contents, the method allowed simultaneous determination of rates of gastric absorption and gastric emptying and thus permitted a simple assessment of the total extent to which gastric absorption occurred under conditions of unimpaired gastric emptying.

The gastric absorption of tiquinamide

In conscious animals, the extent of gastric absorption was equivocal. Differences between animals in the degree of gastric absorption were apparently related to the wide variation of gastric pH. Gastric absorption was insignificant in those animals (2351 and 2354) with low gastric pH (2-2.5), moderate in those animals
(♀ 312 and ♂ 322) with pH at 4.5-6 in the range of the pKa of tiquinamide (5.2), and very extensive in one animal (♂ 355) in which the pH rose as high as 8.5-9. The reasons for the pH variation are not known. Theoretically, the pH of the gastric contents immediately after dosing would have been that of the dose solution i.e. pH 4.6. However, it may be speculated that the low values in two animals resulted from acidification by residual acid in the stomach. The high value in one animal may, conversely, have resulted from reflux of the duodenal secretions, particularly bile, into the stomach. Whatever the reasons for the pH variation, it was a necessary consequence predicted by Schanker's pH-partition hypothesis that tiquinamide would be more extensively gastrically absorbed at higher than at lower gastric pH's with a pKa of 5.2, it would have been only 0.5% unionized at pH 2, but 67% unionized at pH 6 and 98% at pH 9. It is a generally valid assumption of the above hypothesis that drugs are much more readily absorbed in their unionized than in their ionized states. The tendency of the $^{14}$C/$^3$H ratio in the two animals with median gastric pH to increase again, after significant initial gastric absorption, possibly reflected back-secretion of absorbed drug into the gastric contents as a result of the greater degree of ionization in gastric contents at pH 5-6 than in plasma at pH 7.4. It is common for basic compounds to be secreted into the normally very acidic (pH 1-3) gastric contents because the extensive and instantaneous ionization which occurs at such pH's ensures that there is always a concentration gradient in favour of the passage of unionized drug from plasma into gastric contents (Shore, Brodie and Hogben, 1957). This would occur, though at a lower rate, even at higher pH's unless the gastric contents achieved pH 7.4.

The enhancement of gastric absorption in halothane-anaesthetized, pylorus-ligated animals was evidently a consequence of the artificiality of the conditions. Pylorus-ligation would have retained in the stomach drug which might otherwise have been emptied into the small intestine, making it no longer available for gastric absorption. Furthermore, it was possible that halothane anaesthetic had facilitated gastric absorption by enhancing gastric mucosal blood flow, since it is known to be a vaso-dilator (Johnstone, 1956).
Ethanol has been shown to promote drug absorption by such a mechanism (Magnussen, 1968). In addition, halothane inhibits gastric emptying, and therefore could have promoted gastric absorption in the sham-operated animals by increasing the residence time in the stomach. (Marshall, Pittinger and Long, 1961).

Finally, there is evidence to suggest that halothane elevates the pH of gastric contents by itself inhibiting acid secretion. MacKrell and Schwartz (1969) reported a dose-dependent decrease in hydrogen ion secretion in the isolated gastric mucosa of frogs anaesthetized with halothane. Furthermore, Christensen and Skovsted (1975) observed elevated pH of gastric contents in halothane-anaesthetized patients undergoing non-abdominal surgery. Thus, it seems possible that in halothane-anaesthetized monkeys in the present experiment, the pH of gastric contents may have been already significantly raised before absorption of tiquinamide took place. In view of the pH-dependence of gastric absorption of tiquinamide in conscious animals, it seems likely that it was the elevating effect of Halothane on the pH of gastric contents which was primarily responsible for the enhancement of gastric absorption in anaesthetized, pylorus-ligated monkeys.

In conclusion, it seems evident, in view of the variable and pH-dependent gastric absorption of a single 10 mg/kg dose that gastric absorption of tiquinamide in a normally secreting stomach would be of low significance. However, the possibility still remains that once inhibition of acid secretion was established, subsequent doses would be more extensively gastrically absorbed. Results of investigations into the effect of tiquinamide on the rate of gastric emptying have suggested that any contribution to the overall extent of gastric absorption made by inhibition of gastric emptying would be minimal.
Fig. III.1:
Toluene-soluble and total radioactivity in plasma of anaesthetized, pylorus-ligated and sham-operated patas monkeys following oral administration of $^{14}$C-tiquinamide HCl. at 10mg/kg.
Fig. II.2. Gastric absorption of salicylic acid in the patas monkey.

Time after dosing (hr)

14C/3H Ratio

Results represent the ratio of 14C-salicylic acid to 3H-tartrazine.

Each result represents the mean ± SEM of determinations in 3 animals. Each value expresses as a percentage of the initial value.
Fig. 3. Gastric absorption of $^{14}$C-liquinamide in the patas monkey.

$^{14}$C/$^3$H ratios after co-administration of $^{14}$C-liquinamide with $^3$H-malin.*

* pH of gastric contents
<table>
<thead>
<tr>
<th>Toluene-soluble radioactivity</th>
<th>Total radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>Pylorus-ligated</td>
</tr>
<tr>
<td>Monkey No</td>
<td>Area (ug.h.ml⁻¹)</td>
</tr>
<tr>
<td>+311</td>
<td>6.2</td>
</tr>
<tr>
<td>+316</td>
<td>4.4</td>
</tr>
<tr>
<td>+310</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Table III.1: Areas under plasma concentration time curves for toluene-soluble and total radioactivity following oral administration of ¹⁴C-tiquinamide HCl at 10 mg/kg to anaesthetized, pylorus-ligated and sham-operated *patas* monkeys
<table>
<thead>
<tr>
<th>Monkey no</th>
<th>Gastric absorption t½ (min)</th>
<th>Gastric emptying t½ (min)</th>
<th>% dose absorbed from stomach</th>
</tr>
</thead>
<tbody>
<tr>
<td>♂ 354</td>
<td>13.1</td>
<td>22.7</td>
<td>63.4</td>
</tr>
<tr>
<td>♀ 356</td>
<td>9.6</td>
<td>53.8</td>
<td>80.9</td>
</tr>
<tr>
<td>♀ 358</td>
<td>15.7</td>
<td>35.4</td>
<td>64.3</td>
</tr>
<tr>
<td>Mean</td>
<td>12.8</td>
<td>37.3</td>
<td>69.0</td>
</tr>
<tr>
<td>± SEM</td>
<td>± 1.5</td>
<td>± 9.0</td>
<td>± 6.1</td>
</tr>
</tbody>
</table>

Table III.2: Half-lives of gastric absorption and gastric emptying and percentages of dose absorbed from the stomach following 0.5 mg/kg administration of $^{14}$C-salicylic acid to conscious rhesus monkeys.
Table 111: Effect of 10 mg/kg 14C-tiquinamide HCl on the half-life of gastric emptying in conscious patas monkeys.

<table>
<thead>
<tr>
<th>Monkey No</th>
<th>CONTROL</th>
<th>TTIQUINAMIDE HC1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14C-PEG</td>
<td>3H-inulin</td>
</tr>
<tr>
<td>σ'306</td>
<td>12.1, 24.2</td>
<td>82.4, 26.6</td>
</tr>
<tr>
<td>σ'307</td>
<td>14.9, 33.5</td>
<td>81.8, 77.1</td>
</tr>
<tr>
<td>φ 313</td>
<td>83.5, 10.1</td>
<td>85.2, 59.6</td>
</tr>
<tr>
<td>φ 314</td>
<td>6.42, 1.22</td>
<td>68.8, 73.6</td>
</tr>
<tr>
<td>φ 291</td>
<td>91.2, 58.6</td>
<td>90.4, 61.4</td>
</tr>
<tr>
<td>φ 312</td>
<td>59.3, 59.3</td>
<td>92.7, 10.8</td>
</tr>
<tr>
<td>φ 322</td>
<td>17.6, 15.6</td>
<td>85.6, 81.1</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>22.0 ± 9.5</td>
<td>83.8 ± 2.9</td>
</tr>
<tr>
<td>Overall Mean ± SEM</td>
<td>23.1 ± 6.0</td>
<td>78.4 ± 2.6</td>
</tr>
</tbody>
</table>

Each animal received a control dose of 20 ml 14C-polyethylene glycol 4000 (PEG) in isotonic saline in the morning. After 30 min, a solution of 3H-inulin in saline was introduced to aid quantitation of the completeness of recovery of gastric contents. Gastric contents were washed out with 3 x 50 ml saline. Results expressed are the percentage recoveries of each radiolabelled marker and the half-life of gastric emptying of 14C-PEG in saline.

In the afternoon each animal received a 10 mg/kg dose of tiquinamide HCl dissolved in the 20 ml 14C-PEG saline solution. The experiment was performed twice in each animal on different days.
<table>
<thead>
<tr>
<th>Gastric emptying half-life (min)</th>
<th>No. of observations in range</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>2</td>
<td>(</td>
</tr>
<tr>
<td>10-20</td>
<td>7</td>
<td>Normal</td>
</tr>
<tr>
<td>20-30</td>
<td>3</td>
<td>gastric</td>
</tr>
<tr>
<td>30-40</td>
<td>2</td>
<td>emptying</td>
</tr>
<tr>
<td>40-50</td>
<td>3</td>
<td>(</td>
</tr>
<tr>
<td>50-60</td>
<td>0</td>
<td>(</td>
</tr>
<tr>
<td>60-70</td>
<td>0</td>
<td>(</td>
</tr>
<tr>
<td>70-80</td>
<td>1</td>
<td>(</td>
</tr>
<tr>
<td>80-90</td>
<td>1</td>
<td>Gastric</td>
</tr>
<tr>
<td>90-100</td>
<td>0</td>
<td>stasis</td>
</tr>
<tr>
<td>100-1000</td>
<td>3</td>
<td>(</td>
</tr>
<tr>
<td>&gt; 1000</td>
<td>4</td>
<td>(</td>
</tr>
</tbody>
</table>

Table III.4: Frequency distribution of gastric-emptying half-lives in conscious patas monkeys
CHAPTER IV: METABOLISM OF TIQUINAMIDE IN THE RAT
SUMMARY

A study of the nature of end-products in 0-24 h urine of rats revealed the presence of at least three major pathways of bio-transformation. w-Oxidation of the 3-methyl group resulted in the formation of 8-cyano-5,6,7,8-tetrahydro-3-hydroxymethylquinoline and, after further oxidation, 8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid. Allylic hydroxylation at the 5-position and subsequent glucuronide conjugation gave rise to 5-hydroxy,8-cyano-5,6,7,8-tetrahydro-3-methylquinoline and its glucuronide conjugate. Aromatization of the tetrahydroquinoline ring resulted in 8-cyano-quinoline-3-carboxylic acid. Desulphuration was probably a fourth metabolic pathway, but some uncertainty remained about the extent to which this reaction occurred enzymically because the thioamides would have degraded chemically to the corresponding nitriles.

Preliminary chromatographic characterization of products in 0-6 h bile suggested that 8-cyano-5,6,7,8-tetrahydro-3-methylquinoline, 8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid and the glucuronide conjugate of 5-hydroxy,8-cyano-5,6,7,8-tetrahydro-3-methylquinoline, as well as a polar product absent from urine, were all excreted in small amounts.

Possible mechanisms of formation of the major metabolites are discussed.
INTRODUCTION

As previously discussed (Chapter I, p.31), the extent and nature of the metabolism of tiquinamide had potential significance for its pharmacology and toxicology. Furthermore, it was of interest for its own sake because the metabolism of 5,6,7,8-tetrahydroquinolines had not previously been studied.

Possible routes of biotransformation of tiquinamide, reviewed previously in Chapter I (p. 23) included desulphuration of the thioamide and/or sulphoxidation, \( \omega \)-oxidation of the 3-methyl group, aliphatic or aromatic hydroxylation in the tetrahydroquinoline ring system, aromatization and N-oxidation or N-methylation. It remained to be seen which of these would prove most significant in vivo in the species of interest, or indeed whether previously unsuspected routes of metabolism would be revealed.

Methods used to study the nature of the major metabolites in 0-24 h urine were based on the establishment of a chromatographic pattern in a single suitable solvent system, combined with the testing of the homogeneity of individual components by further t.l.c. in different solvent systems. Some of the components present in the highest proportions were then isolated by preparative layer chromatography and characterized by physico-chemical means. Because of the inherent instability of the thioamide group of tiquinamide, combined with the knowledge that thioamide-containing metabolites were potentially pharmacologically active, attempts were made to establish the extent of urinary excretion of such compounds.
EXPERIMENTAL

(a) The chromatographic pattern of drug-related products in urine

Three female rats, weighing 180-200 g, each received 10 mg/kg \( ^1 \)C-tiquinamide HC1 as a solution in saline. Rats were housed in all-glass metabolism cages (Jencons Ltd., Hemel Hempstead, Herts) and urine collected over the following intervals: 0-3, 3-6.5, 6.5-24, and 24-48 h. Collection vessels were surrounded by solid CO\(_2\) for the period up to 6.5 h, representing the remainder of a working day, but returned to room temperature on standing overnight. A pooled 0-24 h urine sample was prepared for each animal. The chromatographic pattern was determined by t.l.c on silica plates (Quantum Industries) in solvent 1 (p.38). Preliminary investigations of different solvents had revealed that this solvent system separated the largest number of individual components. After location of radioactivity on t.l.c. plates by thin-layer scanning and autoradiography, the clearly definable bands were designated RT1-RT6 in order of increasing mobility, which was determined relative to 5,6,7,8-tetrahydroquinoline-3-carboxylic acid (THQ-COOH). This was chosen as the reference compound after preliminary studies because it was a readily available compound which in solvent system 1 ran to a median position on the plate near to several major radioactive components and was thus preferable to unchanged drug which ran to a position near the solvent front. The distribution of radioactivity among the major and minor bands on the plate was quantitated by scraping 0.5 cm strips into scintillation vials followed by elution and assay by liquid scintillation counting.

As a check on the stability of the observed products, the determination of the chromatographic pattern was repeated on a portion of the 0-24 h urine allowed to remain at ambient temperature for 7 days prior to analysis.

In an examination of homogeneity, components separated by chromatography in solvent 1 were scraped off, eluted with methanol and certain of the most prominent ones subjected to further thin-layer chromatography.

Bands RT3 and RT4 were chromatographed on silica plates (Merck) in solvents 2, 3, 11 and 12.
Band RT5 was separated into two components, RT5A and RT5B by t.l.c. in dichloromethane/acetone (5:4). RT5A was subjected to further t.l.c. in solvents 4, 6, 10 and 13 and RT5B in solvents 6, 10 and 13.

(b) The stability of the thioamide group under the conditions of urine collection and determination of the chromatographic pattern

As has been previously stated (Chapter I), tiquinamide was predisposed to chemical desulphuration at concentrations below approximately 100 µg/ml in aqueous solution, resulting in formation of the corresponding nitrile derivative. Because of the possibility that thioamide-containing metabolites had been excreted in urine but had degraded during work-up to nitrile-containing end-product, it was necessary to establish what proportion of any thioamides present would have degraded to nitriles under the conditions of determination of the chromatographic pattern.

Solutions of $^{14}$C-tiquinamide HCl (50 µg/ml) in control rat urine (5 ml) were prepared in triplicate and stored in flasks surrounded by solid CO$_2$ for 0-6.5h, but were subsequently allowed to return to room temperature over the 6.5-24 h period. These represented conditions similar to those pertaining during the collection of urine used to determine the chromatographic pattern of urinary end-products.

At the end of the 24 h period, unchanged tiquinamide was separated from any products of degradation by thin-layer chromatography in dichloromethane/acetone (5:4). Tiquinamide and its nitrile derivative were run for comparative purposes. Major radioactive areas were located by scanning, scraped into scintillation vials and eluted with methanol/H$_2$O (1:1; 2 ml). Radioactivity was assayed by liquid scintillation counting.

(c) Hydrolysis of conjugated material

A portion of 0-24 h rat urine was incubated at 37°C for 18 h with molluscan $\beta$-glucuronidase/sulphatase (Sigma Chemical Co., Poole, Dorset; Helix pomatia Type H-1) at 20,000 units/ml in pH 4.5 0.1M acetate buffer.

Additionally, a non-mobile component, RT1, eluted from t.l.c. plates after chromatography in chloroform/methanol/acetic acid (18:2:1) was treated at 37°C for 18 h with both the molluscan
β-glucuronidase/sulphatase and with bacterial β-glucuronidase (E. Coli. Type I, Sigma Chemical Co.) at 20,000 units/ml urine in pH 7.4 0.1M phosphate buffer saturated with chloroform.

In each case, incubation mixtures were centrifuged to remove proteins and the products of hydrolysis recovered from the supernatants by reduction to dryness and dissolution in methanol. Characterization of aglycones was by comparative t.l.c. in solvent 1 against components RT4, RT5B and authentic 5,6,7,8-tetrahydroquinoline-3-carboxylic acid.

(d) Isolation of drug-related products from urine

Eight rats (300-400 g), housed in all-glass metabolism cages, were fasted overnight before dosing. Each animal received daily for 5 days a 100 mg/kg dose of 14C-tiquinamide HCl as a solution in saline. Urine was collected daily and pooled from all animals over the whole collection period before extraction. Isolation of the major unconjugated drug-related products was as shown in Scheme V.1.

The fractionation procedure involved extraction with diethyl ether under basic and acidic conditions. Urine was adjusted to pH 9 and extracted with diethyl ether (3 x 2 vol). The aqueous phase was adjusted to pH 2 and extracted again with diethyl ether (2 x 2 vol). The aqueous residue after this extraction was neutralized, taken to dryness and subsequently incubated with β-glucuronidase/sulphatase (Helix pomatia, Type H1, Sigma Chemical Co., 20,000 units/ml urine) at 37°C for 24 h. The hydrolysate was adjusted to pH 9 and extracted with diethyl ether (2 x 2 vol and 2 x 4 vol). The extracts were pooled with those obtained from the first pH 9 ether extraction.

The basic ether extract contained principally band RT5 which was separated from RT6 by preparative layer chromatography in solvent 1. Band RT5 was separated into two components, RT5A and RT5B, by subsequent thin-layer chromatography in solvent 5. Each of these components was further purified, free of endogenous contaminants, by thin-layer chromatography in solvent 13 and subsequent chloroform extraction. RT5A was extracted at pH 9 into 2 x 4 vol chloroform and RT5A at pH 2 into 2 x 5 vol chloroform.
The acidic ether extract contained components RT3 and RT4, which were separated by thin-layer chromatography in solvent 1. Each of these components was further purified by extraction into chloroform (2 x 5 vol).

(e) Characterization of urinary end-products

u.v. Spectrophotometry: All u.v. spectra were obtained on a Perkin-Elmer double-beam spectrophotometer from solutions in ethanol (10 µg/ml approx).

i.r. spectrometry: i.r. spectra were determined using a Perkin-Elmer 521 spectrometer. RT3 was prepared as a KBr disc and RT5A as a solution in chloroform.

N.m.r. spectrometry: N.m.r. spectra were obtained in deuterated chloroform, using a Varian XL-100 nuclear magnetic resonance spectrometer.

Mass spectrometry: The electron impact mass spectra of RT3, RT4 and RT5A were determined using an AEI MS30 mass spectrometer and that of RT5B using an AEI MS50 mass spectrometer.

Thin-layer chromatography: Chromatographic characterization of RT6 was carried out on 0.25 mm silica plates (Merck) in solvents 1, 2, 5, 6, 10, 12 and 16. Authentic Wy-24146 (8-cyano-5,6,7,8-tetrahydro-3-methylquinoline) was run for comparative purposes.

(f) The chromatographic pattern of drug-related products in bile

Bile was collected over hourly intervals for periods of 6 h from three bile-duct cannulated rats maintained under halothane anaesthesia and administered 14C-tiquinamide HCl (2-4 µCi/mg) at 10 mg/kg orally. Radioactivity in bile was assayed by liquid scintillation counting. A portion of the pooled 0-6 h sample of bile from each rat was subjected to thin-layer chromatography in solvent 1. Radioactive areas were subsequently located by thin-layer scanning.
RESULTS

(a) The chromatographic pattern of drug-related products in urine

The 0-24 h urine of three female rats administered tiquinamide HCl orally at 10 mg/kg contained 40% of the administered dose (Table IV.1). Chromatography of this fraction in chloroform/methanol/acetic acid (18:2:1) revealed the presence of 6 radioactive bands (RT1-RT6) which were readily distinguishable (Fig. IV.1). The fifth of these bands, in order of increasing distance from the origin contained two components. A clear shoulder on the peak was evident, and 2 bands were distinguished by autoradiography (Fig. IV.2).

Percentages of the administered dose found to be present in each of components RT1-RT6 are expressed in Table IV.1. The most prominent bands were RT3 (8.5%) and RT4 (7.4%). Band RT1, remaining on the origin, constituted a significant proportion, 6.8% of the dose. Bands RT2 and RT5B (3.8 and 4.5% respectively) were lesser but still significant components. The other bands, RT5A and RT6 (0.9% and 1.3% respectively) represented only minor components. No change was observed in the proportions of individual components when urine was allowed to remain at ambient temperature for 7 days prior to analysis.

On subjection to t.l.c. in several further solvent systems bands RT3, RT4 and RT6 were found to be essentially homogeneous, Band RT5 was more readily separated into two components, RT5A and RT5B by t.l.c. in dichloromethane/acetone (5:4). Each of these components was found subsequently to be essentially homogeneous by further thin-layer chromatography in several additional solvent systems.

(b) The stability of the thioamide group under the conditions of urine collection and determination of the chromatographic pattern

\[ ^{14}C \text{-Tiquinamide HCl present in urine at 50 \mu g/ml degraded to its nitrile derivative under conditions which would have occurred during the collection, storage and subsequent work-up of urine from rats dosed with 10 mg/kg tiquinamide HCl for the purpose of determination of the chromatographic pattern of} \]
metabolites. Following an overnight thaw after a period of 6.5 h surrounded by dry ice the degree of degradation of tiquinamide to the nitrile was as much as 67%. No other significant degradation products were observed in either case.

(c) Hydrolysis of conjugated material

The polar fraction not extractable into diethyl ether consisted of conjugates. Treatment of the 0-24 h urine of tiquinamide-dosed rats with molluscan β-glucuronidase/sulphatase followed by t.l.c. in chloroform/methanol/acetic acid (18:2:1) revealed the absence of the immobile component RT1 seen in untreated urine accompanied by a greatly enhanced presence of a component corresponding to RT5B (Figs. IV.1 and IV.2).

When RT1 was isolated from untreated 0-24 h urine, and subsequently subjected to treatment with sulphatase-free bacterial β-glucuronidase, further t.l.c. of the hydrolysate revealed virtually complete hydrolysis. The product was a single radioactive component which chromatographed identically with authentic RT5B in the solvent system. It could be reasonably concluded from these results that RT1 represented predominantly the glucuronide conjugate of RT5B.

(d) Characterization of urinary end-products

Basic, ether-soluble fraction

RT5A: Characterization of RT5A was by means of a combination of analyses by ultra-violet, infra-red, nuclear magnetic resonance and mass spectrometry.

The u.v. absorption peak at 270 nm with a shoulder at 277 nm was characteristic of the pyridine ring of the 5,6,7,8-tetrahydroquinoline system (Fig. IV.3). It also suggested that the cyclohexene ring remained saturated, if intact. The absence of any influence of pH changes on the u.v. spectrum suggested the absence of aromatic hydroxyl groups.

The presence of bands in the region 3000-3500 cm⁻¹ of the i.r. spectrum suggested the presence of one or more hydroxyl groups. A weak band at 2240 cm⁻¹ could only be attributed to a nitrile group (Fig. IV.6).

In the n.m.r. spectrum of RT5A (Fig. IV.7), complex multinlets at δ 1.5-2.5 ppm and δ 2.75-2.95 ppm were indicative of the cyclohexane ring protons of a tetrahydroquinoline system and thereby
suggested absence of substitution in the 5,6 or 7 positions. The approximate triplet at δ 4.0-4.2 ppm indicated the presence of a single proton in the 8-position. The appearance of a strong singlet at δ 4.75 ppm instead of about 2.2 ppm was indicative of a 3-hydroxymethyl substituent instead of the 3-methyl group. Doublets at δ 7.5 ppm and δ 8.45 ppm were indicative respectively of the aromatic protons at the 4- and 2-positions of the pyridine ring, confirming an absence of substitution at these positions.

Hence the combination of u.v., i.r. and n.m.r. spectra suggested that RT5A was 3-hydroxymethyl-5,6,7,8-tetrahydroquinoline-8-nitrile.

The mass spectrum of RT5A (Fig. IV.9) was entirely consistent with the proposed structure. The most abundant ion at m/e 188 corresponded to the parent ion and another fragment at m/e 187 (M-1) to the loss of a single proton. Fragments at m/e 160 (M-28) and 159 (M-29) possibly arose as a result of the loss of -CO from the parent ion and the corresponding ion lacking in a single proton. A fragment at m/e 148 (M-40) corresponded to loss of -CH2CN from the parent ion. This was an unusual fragmentation, but one found to occur also in the mass spectrum of the authentic reference compound 8-cyano-5,6,7,8-tetrahydro-3-methylquinoline (personal communication, C. B. Thomas to K. Heatherington). A fragment at m/e 132 (M-56) could be attributed to the loss of -HCN in addition to -CO from the parent ion.

RT5B: The characterization of RT5B is based on u.v., n.m.r. and mass spectra.

The presence of a u.v. absorption maximum at 269 nm with a shoulder at 276 nm (Fig. IV.3) was characteristic of the pyridine ring of the 5,6,7,8-tetrahydroquinoline system. It also suggested that the cyclohexane ring remained saturated, if intact. The lack of any effect of change of pH on the u.v. spectrum was indicative of an absence of hydroxyl substitution of the pyridine ring.

In the n.m.r. spectrum of RT5B (Fig. IV.7), the complex multiplet at δ 1.7-2.5 ppm was indicative of the cyclohexane ring protons at the 6 and 7-positions of a tetrahydroquinoline system. The presence of a strong singlet at δ 2.35 ppm was indicative of the 3-methyl substituent. The multiplet at δ 3.9-4.2 ppm suggested the presence of a single proton in the 8-position. The presence of another multiplet at δ 4.7-5.0 ppm together with the absence of a multiplet at about δ 2.8 ppm was indicative of a single proton.
in the 5-position. Doublets at δ 7.65 ppm and δ 8.4 ppm, not quite resolved, were characteristic, respectively, of the aromatic protons at the 4 and 2-positions.

On the basis of these spectra, RT5B could tentatively be identified as 5-hydroxy-5,6,7,8-tetrahydro-3-methyl-quinoline-8-nitrile, a structural isomer of RT5A.

The mass spectrum of RT5B (Fig. IV.9) was consistent with the assigned structure. The presence of a parent ion at m/z 188 and a fragment at m/z 187 corresponding to the loss of one proton were indicative of a structure of molecular weight identical with that of RT5A. There were, however, differences in the details of the fragmentation pattern. The presence of a fragment at m/z 173 (M-15) was possibly attributable to the loss of -CH₂, suggesting that the 3-methyl group was intact. The presence of the nitrile group was indicated by observation of fragments at m/z 161 (M-27) and m/z 160 (M-28) which could be attributed to loss of HCN respectively from the parent ion and that resulting from the loss of a single proton. Prominent fragments at m/z 170 (M-18 and M-19) could have resulted from the loss of H₂O from each of the latter two ions. This fragmentation was indicative of an alcoholic hydroxyl group. Fragments at m/z 133 (M-55) and m/z 132 (M-56) possibly arose from the loss of both -HCN and -CO from the parent ion and that resulting from the loss of a single proton.

Hence the overall conclusion, on the basis of its u.v., n.m.r. and mass spectra was that RT5B could be fairly certainly identified as 5-hydroxy-5,6,7,8-tetrahydro-3-methylquinoline-8-nitrile.

RT6: The characterization of RT6 was performed by comparative t.l.c. with Wy-24146 (8-cyano-5,6,7,8-tetrahydro-3-methylquinoline) following the finding of correspondence between the compounds in solvent 1 (Fig. IV.1).

Comparative t.l.c. in 7 different solvent systems tentatively identified this component as the nitrile derivative (Table IV.2).

Acidic, ether-soluble fraction

RT3: The characterization of RT3 rests principally on the evidence obtained from its u.v., i.r. and mass spectra.

Evidence of the acidic character of this product was obtained initially from the fact that it could be readily extracted from urine into diethyl ether under acidic, but not under basic conditions.
Furthermore, it showed chromatographic properties similar, though not identical, to those of the acidic reference compound THQ-COOH (Table IV.3). Treatment of RT3 with ethereal diazomethane resulted in a product which behaved on t.l.c. in chloroform/methanol (4:1) very similarly to 5,6,7,8-tetrahydroquinoline-3-methyl carboxylate (relative Rf 0.95) but very differently from unesterified THQ COOH (relative Rf 5.75).

The u.v. spectrum of RT3 (Fig. IV.5) showed obvious signs of increased aromaticity. Whereas unchanged tiquinamide and the closely related nitrile derivative, Wy-24146, showed the characteristic u.v. spectrum of 5,6,7,8-tetrahydroquinoline with a major absorption maximum at 268 nm and a secondary peak at 276 nm, the spectrum of RT3 had a major absorption peak at 283 nm with a shoulder at 290 nm. In addition, the u.v. spectrum of RT3 contained minor bands at 312 nm and 326 nm. These minor bands were reminiscent of those observed in the spectrum of quinoline, but not 5,6,7,8-tetrahydroquinoline. The appearance of these bands at longer wavelengths in the spectrum of RT3 than in the spectrum of quinoline (300 and 313 nm respectively) presumably reflected bathochromic shifts resulting from aromatic substitution. Comparison of the u.v. spectra of quinoline, quinoline-8-nitrile and quinoline-3-carboxylic acid made possible the prediction of the absorption maxima for the anticipated three bands in the spectrum of 8-cyano-quinoline-3-carboxylic acid, according to the principle of additive bathochromic shifts e.g. Scott, 1964. The predicted values of 283 nm, 312 nm and 327 nm were in very close agreement with those observed for RT3 (Fig. IV.5).

In the i.r. spectrum of RT3 (Fig. IV.6), a sharp band at 2220 cm\(^{-1}\) could be unambiguously attributed to anitrile group. A broad band at 1600-1800 cm\(^{-1}\) was probably indicative of a carboxylic acid. The nitrile group almost certainly existed at position 8 as a result of desulphuration of the 8-thiocarboxamide group of tiquinamide. Therefore the carboxylic acid moiety was likely to be at position 3. Thus the combination of u.v. and i.r. spectra suggested that RT3 was 8-cyano-quinoline-3-carboxylic acid.

In the mass spectrum of RT3 (Fig. IV.10) the most abundant ion at \(m/e\) 198 was consistent with the parent ion of the proposed structure. Another fragment, at \(m/e\) 153 (M-45) corresponded to the loss of the carboxyl group.
RT4: The evidence for the proposed structure of RT4 comprises principally u.v., n.m.r. and mass spectra.

Initial evidence of the acidic nature of this compound came from the finding that it could be extracted into diethyl ether under acidic, but not basic conditions. Furthermore, it showed chromatographic properties similar, though not identical, to those of the acidic reference compound THQ-COOH (Table IV.3).

The presence of a u.v. absorption maximum at 272 nm with a shoulder at 279 nm was indicative of the pyridine portion of the 5,6,7,8-tetrahydroquinoline ring (Fig. IV.4), though the small (3-4 nm) shift to longer wavelength suggested aromatic substitution by an electron withdrawing group and the total spectrum was more closely identical with that of THQ COOH than with that of the parent compound. The absorption maximum was, however, close enough to that of 5,6,7,8-tetrahydroquinoline to suggest that the second ring remained saturated, if intact. The absence of any significant influence by alkalinization on the u.v. spectrum of RT4 suggested the absence of aromatic hydroxyl substitution.

In the n.m.r. spectrum (Fig. IV.8), a complex multiplet at \( \delta 1.5-2.5 \) ppm and an approximate triplet at \( \delta 2.75-3.1 \) ppm were characteristic of the cyclohexane ring protons in the tetrahydroquinoline system. The multiplet at \( \delta 4.1-4.3 \) ppm suggested the presence of a single proton at position 8 of this system. The lack of a strong singlet signal at about \( \delta 2.2 \) ppm showed the absence of the 3-methyl substituent. Nevertheless, the pair of doublets with coupling constants 2Hz in the low field region was characteristic of a pyridine ring substituted at the 3-position. However, the shift of these signals to \( \delta 8.15 \) and \( \delta 9.15 \) ppm from the position \( \delta 7.5 \) ppm and \( \delta 8.2 \) ppm seen with tiquinamide was indicative of the presence of an electron-withdrawing group at position 3. On the basis of the u.v. and n.m.r. spectra, the structure tentatively assigned to RT4 was 8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid.

The mass spectrum (Fig. IV.10) confirmed this structure. The most abundant ion at \( m/e 202 \) corresponded to the parent ion of the postulated acid. A fragment at \( m/e 201 \) (M-1) corresponded to the loss of a single proton. Fragments at \( m/e 175 \) (M-27) and \( m/e 174 \) (M-28) corresponded to the loss of -HCN from these two ions.
respectively. A fragment at $m/e$ 157 ($M-45$) was attributed to the loss of $-\text{COOH}$ from the parent ion and one at $m/e$ 130 ($M-72$) to the loss of both $-\text{COOH}$ and $-\text{HCN}$. A prominent fragment at $m/e$ 162 ($M-40$) was attributed to the loss of $-\text{CH}_2\text{CN}$, an unusual fragmentation, but one already found to be characteristic of 8-cyano-5,6,7,8-tetrahydroquinoline (p. 80).

(e) The chromatographic pattern of drug-related products in bile

The 0-6 h bile of three female bile-duct cannulated rats administered tiquinamide HCl orally at 10 mg/kg contained 5-13% (mean 8%) of the administered dose. Chromatography revealed the presence of four significant components (Fig. IV.11). Three of these had Rf values similar to products seen in urine: RT1, RT4 and RT6 (respectively 0.00, 1.01, and 1.40 relative to THQ COOH). The fourth was a polar band (Rf 0.07 relative to THQ COOH) not seen to be a significant component in urine. Each of the four components represented a similar proportion of the total, excreted products. Thus no individual band accounted for more than approximately 3% of the administered dose.
DISCUSSION

The major end-products of tiquinamide metabolism isolated from rat urine resulted from four separate Phase I processes: desulphuration of the thiocarboxamide group to the corresponding nitrile analogue, \( \omega \)-oxidation of the 3-methyl group, hydroxylation at the 5-position i.e. at the position adjacent to the point of fusion of the saturated and unsaturated rings, and aromatization of the 5,6,7,8-tetrahydroquinoline ring system (Fig. IV.12).

In view of the known instability of the thioamide group of tiquinamide, there remains an element of doubt as to whether the nitriles detected were formed by metabolic or chemical desulphuration or by a mixture of the two processes. Studies on the stability of tiquinamide under the conditions of urine collection showed that although extensive degradation of the thioamide group would have taken place, at least 33% of the thioamide-containing compounds voided would have been detected in the metabolic pattern. Hence for each nitrile compound, there would have been a thioamide analogue. Whilst some of the nitriles and thioamide analogues might have behaved similarly in this t.l.c. system, it is probable that some additional components which subsequently degraded would have been observed. The fact that the major components in the t.l.c. pattern were each homogeneous and did not change their chromatographic behaviour in this system at any stage between their initial observation and their eventual identification constitutes some evidence in favour of the identified nitrile compounds being true metabolites and against the view that substantial proportions of thioamides were voided in urine and were subsequently chemically degraded. It must be added that desulphuration of the thioamide group of tiquinamide was an expected route of metabolism of the compound predictable from the literature concerning the metabolism of other thioamides. For example, antigastrin (Gibson and Hunter, 1969) and 2,6-dichlorothiobenzamide (Griffiths et al., 1966) were both found to undergo extensive metabolic desulphurization and in both cases the workers involved specifically excluded the possibility of chemical desulphuration.

These considerations taken together lead to the conclusion that the nitrile compounds isolated probably represent metabolites of tiquinamide and that although thioamide analogues may have been
excreted and subsequently degraded, they did not form a major proportion of the drug-related products voided by the rat. In all other respects the metabolites of tiquinamide were apparently stable, as indicated by their constant proportions after 7 days at ambient temperature. Thus there was no evidence that any reaction other than the desulphuration could have occurred either by chemical or by microbial action.

Of a total of 40.2% of the dose excreted in rat urine in the first 24 h, almost half i.e. 16.8% represented metabolites formed by methyl group oxidation, the majority of these (15.9%) being 3-carboxylic acids. Hence this total slightly exceeded the sum of the amounts of the 5-hydroxylated metabolite RT5B (8-cyano-5-hydroxy-5,6,7,8-tetrahydro-3-methylquinoline) and its glucuronide conjugate, RT1, which together amounted to 11.3% of the dose in the 0-24 h period. However, the relative amounts of products in urine may not have totally reflected the comparative importance of each of these routes for the metabolism of tiquinamide in the rat. Although the biliary excreted material did not obviously contain a preponderance of products of one route or the other, it was possible that the material unexcreted at 24 h did so.

The products of 3-methyl oxidation were almost entirely 3-carboxylic acids, which by analogy with nicotinic acid would have had carboxyl groups of pKₐ around 2.0 (Wade, 1977). They would therefore have been extensively ionized in urine and thus probably rapidly excreted by the urinary route. Although the excretion kinetics of the 3-carboxylic acid products of tiquinamide have not been studied directly, those of a related compound 5,6,7,8-tetrahydro-3-methylquinoline-8-carboxylic acid, have been investigated. The majority (76%) of an oral dose was excreted in the urine, mostly during the first 24 h (D. M. Pierce, unpublished observation). It is reasonable to presume that the 3-carboxylic acids would have been equally rapidly excreted.

The basic metabolite, 5-hydroxy, 5,6,7,8-tetrahydro-3-methylquinoline on the other hand would have had a pKₐ similar to that of the unchanged drug (5.2) and therefore could have been unionized to a relatively high extent in urine and thus susceptible to renal reabsorption. Thus there was a greater chance that the unexcreted material contained a significant proportion of 5-hydroxylated
The basic compound than that substantial amounts of 3-carboxylic acids remained to be excreted. The implications of such considerations will be discussed more fully in subsequent chapters.

The fourth significant Phase I reaction in the metabolism of tiquinamide in the rat was an aromatization of the tetrahydroquinoline ring, resulting in about 8.5% of the dose being eliminated as 8-cyano-quinoline-3-carboxylic acid.

Conjugation of the 5-hydroxylated metabolite with glucuronic acid was a major route of further metabolism. The proportion of conjugated material excreted in the 0-24 h urine represented 60% of total 5-hydroxylated products.

Consideration on the mechanisms of reactions involved in tiquinamide metabolism in the rat

Desulphuration: The mechanism of metabolic desulphuration of thioamides has not been investigated in detail. The formation of nitrile products of the desulphuration of both antigastrin (Gibson and Hunter, 1969) and 2,6-dichlorothiobenzamide (Griffiths et al., 1966) implied an oxidative removal of H_S rather than the chemically more obvious hydrolysis which would have resulted in the corresponding amides viz the hydrolysis of thioacetamide (Rees, Rowland and Varcoe, 1966). Hence the nature of the reaction seemed to be similar to that found by Scheline, Smith and Williams (1961) to be involved in the desulphuration of 1-phenyl-2-thiourea. It is possible that the metabolic desulphuration of tiquinamide occurred by a similar mechanism. Although the presence of amides could not be totally excluded, the overriding predominance of nitriles ruled out the hydrolytic route as the major desulphuration pathway. However, the possibility also exists that desulphuration of tiquinamide involved an initial sulphoxidation in a manner analogous to that observed for ethionamide (Johnston, Kane and Kibby, 1967), followed by degradation of the sulphonamide product to the nitrile. Attempts to synthesize tiquinamide sulphonamide have resulted in the formation of its nitrile derivative, suggesting this possible degradation (Curran, A. C. W., personal communication). (Fig. IV.13).

3-methyl oxidation: The oxidation of the 3-methyl group of tiquinamide was analogous to other examples of the oxidation of methyl substituents to aromatic rings. The reaction is the simplest form of _oxidation of an alkyl side chain. The mechanism of such
reactions has been discussed by Gillette (1963). Earlier work had established that the alkyl side chains of barbiturates, for example, were oxidized to alcohols and subsequently to carboxylic acids and that the microsomal mono-oxygenase was implicated (Cooper and Brodie, 1955 and 1957). Subsequently, Gillette (1959) studied the mechanism of the reaction more closely, using p-nitro-toluene as a substrate. He established that the oxidation of this compound to p-nitrobenzyl alcohol was carried out by liver microsomal mono-oxygenase. However, in order for further metabolism of the alcohol to ensue, it was necessary to add the soluble fraction of liver homogenate to the incubation mixture. The alcohol was found to be oxidized to p-nitrobenzaldehyde initially in a reaction catalysed by a soluble liver alcohol dehydrogenase and to p-nitrobenzoic acid finally, catalysed by soluble liver aldehyde dehydrogenase. The oxidation of xylene to toluic acid by rabbit lung and liver was observed to proceed via similar stages (Carlone and Fouts, 1974). It is believed that the 3-methyl oxidation of tiquinamide occurred by this same mechanism (Fig. IV.14).

5-hydroxylation: The 5-hydroxylation of tiquinamide represented allylic hydroxylation occurring in a fused saturated and aromatic ring system at a saturated carbon atom α to the double bond. Hydroxylation in the saturated ring rather than in the aromatic ring was consistent with the general rule that in fused alicyclic and aromatic ring systems, the preferred site of hydroxylation is in the alicyclic ring (Parke, 1968c). The reaction was very similar to the 4-hydroxylation which constitutes a major pathway in the metabolism of the 1,2,3,4-tetrahydroisoquinoline compound debrisoquine (Allen et al., 1975; Allen, Brown and Marten, 1976). Other compounds known to undergo this reaction include fluorene (Grantham, 1963; Chen and Lin, 1969), β-ionone (Ide and Toki, 1970), hexobarbital (Gerber et al., 1971), tetralin (Elliott and Hanam, 1968) and indane (Billings, Sullivan and McMahon, 1970). The mechanism of the reaction has been found usually to be catalysed by a microsomal mono-oxygenase requiring NADPH and O₂ e.g. fluorene (Chen and Lin, 1969), hexobarbital (Cooper and Brodie, 1955) and indane (Billings, Sullivan and McMahon, 1970). The precise details of the mechanism have been investigated more closely, for example, for fluorene. Spectral investigation of the interaction of fluorene with cytochrome P450 from the livers of phenobarbital-treated rats revealed that
the first stage of fluorene oxidation involved the formation of a carbanion which complexed with reduced cytochrome P450. This carbanion was subsequently found to further react with molecular oxygen to form a hydroperoxide, which it is believed then gave rise to fluorenol. (Ullrich and Schnabel, 1973) (Fig. IV.15).

In hydroxylation reactions of this type involving replacement of a single hydrogen atom at a methylene group, the hydroxylated product frequently possesses an asymmetric carbon atom, and the potential for optical activity therefore exists if the enzyme catalysing the oxidation forms one diastereoisomer in favour of the other. Thus, for example, the hydroxylation of indane resulted in predominantly (S)(+)-indanol (Billings, Sullivan and McMahon, 1970). Tiquinamide metabolite RT5B (5-hydroxy,8-cyano-5,6,7,8-tetrahydro-3-methylquinoline) possesses an optically active centre, but no attempt has been made to separate diastereoisomers.

Aromatization of the 5,6,7,8-tetrahydroquinoline ring system: Aromatization of the tetrahydroquinoline ring, resulting in 8-cyanoquinoline-3-carboxylic acid represents a fairly uncommon reaction in foreign compound metabolism. There are a few compounds in the literature for which aromatization of alicyclic rings has been reported and at least two different mechanisms have been described. (Fig. IV.16). Thus cyclohexane carboxylic acid was found to be aromatized in a reaction catalyzed by a mitochondrial enzyme of mammalian liver and kidney. The reaction involved the formation of an acyl CoA intermediate which was subsequently aromatized and conjugated with glycine to form hippuric acid (Babior and Bloch, 1966). Another aromatization reaction, that of quinic acid, is apparently carried out not by mammalian liver enzymes, but by gastrointestinal micro-organisms (Adamson et al., 1970a; Cotran, Kendrick and Kass, 1960; Asatoor, 1965). The aromatization of shikimic acid observed first by Asatoor (1965) was found to involve both metabolism by gut bacteria and subsequently by mammalian liver. Contrary to earlier beliefs, the compound was not aromatized directly by the gut bacteria, but rather was reduced by these micro-organisms to cyclohexane carboxylic acid (Brewster, Jones and Parke, 1977a). The subsequent aromatization of this acid was found to take place in isolated perfused rat liver, presumably by the mechanism discussed above (Brewster, Jones and Parke, 1977b). In addition to aromatization
of these alicyclic carboxylic acids, aromatization of tetrahydroquinolines has also been described. The metabolism of 5,6,7,8-tetrahydroquinolines has not previously been studied, but 1,2,3,4-tetrahydroquinoline is known to undergo extensive aromatization (25%) in the dog, resulting in 2-quinolinol. It has been speculated that this reaction might proceed in two stages, since the aromatization of 8-hydroxy,1-methyl-1,2,3,4-tetrahydroquinoline-7-carboxylic acid involved an initial partial dehydrogenation, an isolated intermediate being 1,4-dihydro-4,8-dihydroxy-1-methylquinoline-7-carboxylic acid (Williams; 1959g). Other aromatization reactions which are known to occur are those concerned with the biosynthesis of certain steroid hormones. Thus, for example, 19-hydroxytestosterone undergoes aromatization during the formation of oestriol (Samuels, 1960).

The aromatization of tiquinamide metabolites is unlikely to proceed via a mechanism analogous to that of cyclohexane carboxylic acid because the stereochemistry in 8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid (RT4) does not fulfil the requirements for this reaction. Reactions of this type require the presence of a carboxylic acid moiety substituted in the saturated ring, such that the first dehydrogenation of an acyl CoA derivative of this acid occurs α,β- to the position of the carboxyl group (Babior and Bloch, 1966). Aromatization of metabolite RT4 of tiquinamide by gut micro-organisms is possible, but it would necessitate that the acid and/or its conjugates were excreted in bile in order to expose the unsaturated acid to micro-organisms in the gastrointestinal tract. The low extent of biliary excretion made it unlikely that such a mechanism could have accounted for the excretion in urine of as much as 8.5% of the dose as the aromatized acid. Of the possible mechanisms for the aromatization of 8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid, the most attractive one resembles that proposed for the 1,2,3,4-tetrahydroquinolines. An interesting feature of this mechanism is that it apparently involves hydroxylation in the alicyclic ring at a position α-to the point of fusion with the aromatic ring. Although no metabolite containing both 5-hydroxyl and 3-carboxylic groups has been identified for tiquinamide, it is not impossible that such a compound could exist as a minor metabolite, representing an intermediate in the formation of the fully aromatized
carboxylic acid. Alternatively the aromatization involved in the formation of tiquinamide metabolite RT3 might occur by a mechanism related to one by which aromatization of steroids is effected.
Solvent system: Chloroform/methanol/acetic acid (18:2:1)

**Before treatment with β-glucuronidase/sulphatase**

- O = origin
- A = 5,6,7,8-tetrahydro-quinoline-3-carboxylic acid
- B = Wy-24117
- C = Tiquinamide
- D = 8-cyano-5,6,7,8-tetrahydro-3-methylquinoline
- SF = solvent front

Wy-24117 = 5,6,7,8-tetrahydro-3-methylquinoline-8-carboxamide

**After treatment with molluscan β-glucuronidase/sulphatase**

Fig. IV.1: Chromatography of radioactivity in 0–24h urine of female rats administered 10 mg/kg $^{14}$C-tiquinamide.HCl. orally
Fig IV.2:
Autoradiograph showing the chromatographic pattern of radioactivity in 0-24h urine of female rats administered 10mg/kg. $^{14}$C-tiquinamide.HCl orally
FIG. 1: U.V. Spectra of 5',6',7',8'-tetrahydroquinohtoline and of the major basic ether.

Absorbance

Wavelength (nm)

Neutral ethanol 268

Bassett ethanol 270

Acetlated ethanol

5',6',7',8'-tetrahydroquinohtoline
Table: Absorbance of 3,6',7'-terephthaloglutonolate-3-carboxylic acid and of RT4

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>RT4</th>
<th>5',6',7'-terephthaloglutonolate 3-carboxylic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>260</td>
<td></td>
<td></td>
</tr>
<tr>
<td>270</td>
<td></td>
<td></td>
</tr>
<tr>
<td>280</td>
<td></td>
<td></td>
</tr>
<tr>
<td>290</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Diagram: UV spectra of 3,6',7'-terephthaloglutonolate-3-carboxylic acid and of RT4
Fig. IV.5: UV spectra of some quinoline derivatives and of RT3, an acidic, ether-soluble product of quinonemide isolated from rat urine.

Absorbance

Wavelength (nm)

- Quinolone-3-carboxylic acid
- 8-cyano-quinolone-3-carboxylic acid
- 8-cyano-quinolone
- Quinolone
- RT3
FIG. 16: IR spectra of urinary end products of triquinamide: RT3 and RT5A
Fig. IV.7:
N.m.r. spectra of tiquinamide urinary end products
RT5A and RT5B
Fig. IV.8:
N.m.r. spectrum of tiquinamide urinary end product RT4
Fig. IV.9:
Mass spectra of urinary end products of tiquinamide: RT5A and RT5B
Fig. IV.10:
Mass spectra of urinary end products of tiquinamide: RT3 and RT4
FIG. IV.1 Chromatography of radioactivity in 0-6 h bile of 10 mg/Kg C-14 nitrogenamide HCl orally

10 mg/Kg C-14 nitrogenamide HCl orally
anesthetized female rats administered

Solvent system: Chloroform/methanol/acetic acid (18:2:1)

SP = solvent front
A = 5,6',7',8'-tetrahydronaphthalene-3-carboxylate acid
0 = origin

SP = solvent front
Fig. IV.12: The metabolism of tigunimide in the Rat

Values in parentheses represent percentages of dose excreted as each RT

X = CN/CSNH$_2$

RT$_5$B

RT$_3$ (8.5%)

RT$_4$ (7.4%)

RT$_5$A (0.9%)

Tigunimide
Oxidative desulphuration

Phenyl thiourea $\rightarrow$ Phenyl cyanamide

Degradation of unstable sulphoxide

Thioamide $\rightarrow$ Sulphoxide $\rightarrow$ Nitrile.
Fig. IV.14. Mechanism of methyl group oxidation

- p-nitrotoluene
- p-nitrobenzyl alcohol
- p-nitrobenzaldehyde
- p-nitrobenzoic acid
FIG. 12.15: Mechanism of aliphatic hydroxylation

9-Fluorene 9-fluorenone

9-Fluorenol

Hydroperoxide

Fluorene

NADPH

NADP

Hydroperoxide

Fluorene-9-fluorenone

Fluorene

Fluorene

NADPH

NADP

Microsomal

Mono-oxygenase

H, O2
<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Rat (1)</th>
<th>Rat (2)</th>
<th>Rat (3)</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT1</td>
<td>7.1</td>
<td>6.3</td>
<td>7.0</td>
<td>6.8 ± 0.3</td>
</tr>
<tr>
<td>RT2</td>
<td>3.9</td>
<td>3.4</td>
<td>4.0</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>RT3</td>
<td>8.3</td>
<td>7.6</td>
<td>9.6</td>
<td>8.5 ± 0.6</td>
</tr>
<tr>
<td>RT4</td>
<td>7.5</td>
<td>6.2</td>
<td>8.4</td>
<td>7.3 ± 0.6</td>
</tr>
<tr>
<td>RT5A</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9 ± 0.0</td>
</tr>
<tr>
<td>RT5B</td>
<td>5.9</td>
<td>3.4</td>
<td>4.1</td>
<td>4.5 ± 0.7</td>
</tr>
<tr>
<td>RT6</td>
<td>1.5</td>
<td>1.1</td>
<td>1.2</td>
<td>1.3 ± 0.1</td>
</tr>
</tbody>
</table>

Total % dose in these bands: 35.1 28.9 35.2 33.1
Total % dose in urine: 43.0 35.8 41.9 40.2

Table IV.1: Percentages of the administered dose present in each of the major radioactive components separated by t.l.c. of 0-24 h urine of female rats administered 10 mg/kg C-tiquamidine orally

<table>
<thead>
<tr>
<th>Solvent</th>
<th>RT6 Rf</th>
<th>Wy-24146 Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.76</td>
<td>0.75</td>
</tr>
<tr>
<td>2</td>
<td>0.54</td>
<td>0.52</td>
</tr>
<tr>
<td>5</td>
<td>0.60</td>
<td>0.59</td>
</tr>
<tr>
<td>6</td>
<td>0.55</td>
<td>0.55</td>
</tr>
<tr>
<td>10</td>
<td>0.72</td>
<td>0.73</td>
</tr>
<tr>
<td>12</td>
<td>0.38</td>
<td>0.47</td>
</tr>
<tr>
<td>16</td>
<td>0.49</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Table IV.2: Comparative chromatography of RT6 with authentic Wy-24146 (8-cyano-5,6,7,8-tetrahydro-3-methylquinoline)
<table>
<thead>
<tr>
<th>Solvent No.</th>
<th>RT3</th>
<th>RT4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rf</td>
<td>Rf THQ COOH</td>
</tr>
<tr>
<td>1</td>
<td>0.63</td>
<td>0.95</td>
</tr>
<tr>
<td>2</td>
<td>0.26</td>
<td>1.85</td>
</tr>
<tr>
<td>3</td>
<td>0.45</td>
<td>4.50</td>
</tr>
<tr>
<td>11</td>
<td>0.80</td>
<td>1.03</td>
</tr>
<tr>
<td>12</td>
<td>0.46</td>
<td>1.12</td>
</tr>
<tr>
<td>14</td>
<td>0.23</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Rf values are expressed in absolute terms and relative to THQ COOH
ND = Not Determined

Table IV.3: Comparative chromatography of tiquinamide urinary end-products RT3 and RT4 with 5,6,7,8-tetrahydroquinoline-3-carboxylic acid (THQ COOH)
CHAPTER V: METABOLISM OF Tiquinamide in the Patas Monkey
SUMMARY

A study of the nature of end-products in 0-24 h urine of patas monkeys revealed the presence of at least two of the biotransformation pathways seen previously in the rat. ω-Oxidation was the major route of metabolism, occurring more extensively than in the latter species and more completely, giving rise to 8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid but not apparently to the intermediate 8-cyano-5,6,7,8-tetrahydro-3-hydroxymethyl quinoline. Allylic hydroxylation at the 5-position gave rise to 5-hydroxy,8-cyano-5,6,7,8-tetrahydro-3-methylquinoline. The subsequent extent of conjugation of this compound, mainly with glucuronic acid, was greater than in the rat. There was no evidence of aromatization of the tetrahydroquinoline ring in this species. Desulphuration probably occurred to some extent enzymically, but there was evidence of unstable sulphur-containing metabolites. The most predominant of these was a compound which apparently degraded to 8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid, and may, therefore, have been the corresponding thioamide. Possible reasons for the species difference in metabolism between the patas monkey and the rat are discussed.
INTRODUCTION

Once the major pathways of tiquinamide metabolism had been established in the rat, it became of interest to establish whether the same pathways applied to other species. The patas monkey was particularly relevant because it was a candidate species for toxicity studies. Thus the methods developed for the study of the metabolism of tiquinamide in the rat were subsequently applied to an investigation of metabolism in the patas monkey.
(a) The chromatographic pattern of drug-related products in urine

Two patas monkeys (♂ 288, weighing 5.0 kg and ♀ 360, weighing 5.6 kg) each received 10 mg/kg \(^{14}\)C-tiquinamide HCl as a solution in saline. Urine was collected at the following times after dosing: 0-2, 2-5, 5-12 and 12-24 h. In order to minimize chemical degradation, collections over the first 12 h were made in vessels surrounded by solid CO\(_2\). Subsequently, samples collected over the 0-24 h period were pooled for each animal. The chromatographic pattern of radioactivity in each 0-2 h and 0-24 h pooled urine sample was determined by t.l.c. in Solvent 1. After location of radioactivity on thin-layer plates by scanning and autoradiography, the clearly definable bands were designated MT1-MT4 in order of increasing mobility, which was calculated relative to THQ COOH as for the rat metabolites (Chapter IV, p.73).

The distribution of radioactivity among the bands on the plate was quantitated by scraping 0.5 cm strips into scintillation vials, followed by elution and assay by liquid scintillation counting as described previously (Chapter II).

Two of the mobile components, MT3 and MT4, separated by chromatography in Solvent 1 were scraped off, eluted with methanol, and subjected to further thin-layer chromatography. MT3 was run in Solvents 2, 3 and 11 and MT4 in Solvents 6, 8, 10 and 11.

(b) The stability of the thioamide group under the conditions of urine collection and determination of the chromatographic pattern

As discussed previously in connection with the rat (Chapter IV, p. 74) it was necessary to establish what proportion of any thioamides initially present would have degraded to nitriles under the conditions of determination of the chromatographic pattern.

Solutions of \(^{14}\)C-tiquinamide HCl in control monkey urine (5 ml) at 100 µg/ml were prepared so as to mimic the concentration in urine if all metabolites were thioamides. Triplicate portions were stored in flasks surrounded by dry ice for 0-12 h but allowed to return to room temperature over the period 12-24 h. These represented conditions similar to those pertaining during the collection of urine used to determine the chromatographic pattern of urinary end-products. At the end of 24 h period, unchanged tiquinamide was
separated from any products of degradation by thin-layer chromatography, as previously described for the rat.

(c) Hydrolysis of conjugated material.

Portions (5 ml) of the 0-24 h urine of a single patas monkey administered $^{14}$C-tiquinamide HCl were incubated at 37°C for 18 h with molluscan $\beta$-glucuronidase/sulphatase (Helix pomatia, Type H1 obtained from Sigma Chemical Co., Poole, Dorset) at 20,000 units/ml in pH 4.5 0.1M acetate buffer. Control samples containing acetate buffer but no enzyme were incubated concurrently under identical conditions.

Additionally, a component, MT1, which remained on the origin after chromatography in chloroform/methanol/acetic acid (18:2:1) was eluted from the t.l.c. plate and portions were treated at 37°C for 18 h with either molluscan $\beta$-glucuronidase/sulphatase or with bacterial $\beta$-glucuronidase (E.Coli Type I, obtained from Sigma Chemical Co.) at 20,000 units/ml urine in pH 7.4 0.1M phosphate buffer saturated with chloroform.

In each case, incubation mixtures were centrifuged to remove proteins and the products of hydrolysis recovered from the supernatants by reduction to dryness and dissolution in methanol. Characterization of aglycones was carried out by comparative t.l.c. in chloroform/methanol/acetic acid (18:2:1) against the unconjugated MT4.

(d) Isolation of drug-related products from urine

A single patas monkey, weighing 5.1 kg, received a 250 mg/kg oral dose of $^{14}$C-tiquinamide HCl as a suspension in 0.5% carboxymethylcellulose/Tween 80 at 5 ml/kg. Urine was collected over a 0-24 h period. Isolation of unconjugated drug-related products was carried out as described in Scheme V.4.

The procedure was a simplified version of that described for the isolation of urinary end-products in the rat (Chapter IV p. 75). The acidic compound MT3 was isolated from a diethyl ether extract prepared under acidic conditions (pH 2). The amount of MT4 present was enhanced initially by treatment with molluscan $\beta$-glucuronidase/sulphatase to hydrolyse conjugated material. The ether extraction step under basic conditions employed for isolation of the comparable fraction of products in the rat was omitted and separation effected entirely by t.l.c.
Scheme V.1: The isolation of some end-products of tiquinamide metabolism in the patas monkey

Urine (1) Adjusted to pH 2
(2) Extracted with diethyl ether (2 x 3 vol)

<table>
<thead>
<tr>
<th>Aqueous</th>
<th>Organic phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Incubated with β-glucuronidase/sulphatase at 37°C for 18 h (20,000 units/ml urine in pH 4.5 0.1M acetate buffer).</td>
<td></td>
</tr>
<tr>
<td>(2) Protein removed by centrifugation, pellet washed with H2O, supernatant and washings pooled.</td>
<td></td>
</tr>
<tr>
<td>(3) Prep. t.l.c. in solvent 1 on 1 mm silica plates.</td>
<td></td>
</tr>
<tr>
<td>(4) Eluted with MeOH/H2O (1:1).</td>
<td></td>
</tr>
<tr>
<td>(5) T.l.c. in solvent 5.</td>
<td></td>
</tr>
<tr>
<td>(6) Eluted with MeOH.</td>
<td></td>
</tr>
<tr>
<td>(7) T.l.c. in solvent 13.</td>
<td></td>
</tr>
<tr>
<td>(8) Eluted with MeOH.</td>
<td></td>
</tr>
</tbody>
</table>

| MT4 |

| (1) Reduced to dryness and dissolved in 20 ml 1/10 NaOH |
| (2) Treated with decolourizing charcoal and centrifuged. |
| (3) Supematant reacidified (pH 2) and extracted with diethyl ether (2 x 5 vol). |
| (4) Prep. t.l.c. in solvent 9 on 1 mm silica plates. |
| (5) Eluted with methanol. |

| MT3 |
Characterization of urinary end-products

Preparation of MT3 methyl ester: MT3 methyl ester was made by treatment of the free acid with diazomethane in ethereal solution at ambient temperature for 30 min. Excess reagent was removed by rotary evaporation.

U.v. spectrophotometry: The u.v. spectrum of MT4 was determined on a Perkin-Elmer double-beam spectrophotometer. That of MT3 was determined using a Pye-Unicam SP 700 double-beam spectrophotometer. Compounds were prepared as solutions in 95% ethanol (circa 10 ug/ml).

I.r. spectrophotometry: I.r. spectra were determined using a Perkin-Elmer 521 spectrophotometer. MT3 methyl ester was prepared as a solution in chloroform. MT4 was incorporated in a KBr disc.

N.m.r. spectrophotometry: The n.m.r. spectrum of MT3 methyl ester was determined using a Varian EM360 spectrometer. The compound was prepared as a solution in deuterated chloroform. Tetramethylsilane was present as an internal standard.

Gas chromatography: Gas chromatography of MT3 methyl ester, as a solution in chloroform, was performed on a Pye 104 gas chromatograph. The column was of glass, 2 m x 3 mm i.d. and containing 10% SE30 on AW Chromasorb W. The column temperature was 200°C. The carrier gas was nitrogen with a flow rate of 40 ml/min.

Mass spectrometry: The electron impact mass spectrum of MT3 methyl ester was determined on an AEI MS12 mass spectrometer interfaced to the Pye 104 gas chromatograph. The electron impact mass spectrum of MT4 was prepared on an AEI MS50 mass spectrometer.
RESULTS

(a) The chromatographic pattern of drug-related products in urine

The 0-24 h urine collection from two patas monkeys, ♀ 288 and 360, contained respectively 86.9% and 90.1% of the administered dose. Chromatography of this fraction in chloroform/methanol/acetic acid (18:2:1) revealed the presence of 4 radioactive bands (MT1-MT4) (Fig. V.1).

Percentages of the administered dose found to be present in each of components MT1-MT4 are expressed in Table V.1. By far the largest single component was MT3, which represented 46.6% and 37.8% of the administered dose in monkeys ♀ 288 and ♂ 360 respectively. MT1 constituted a lesser component (15.8% and 18.6% respectively). MT2 and MT4 were minor components. The 0-2 h collection contained 55.6% (♀ 288) and 43.5% (♂ 360) of the administered dose. Chromatography of this fraction in solvent 1 revealed the presence of five radioactive bands (Fig. V.1). These had the same Rf values as those observed in the pooled 0-24 h urine pattern, with the exception that a major additional band, designated MTU, contained a similar proportion of the total radioactivity to component MT3. This component was unstable during storage at -20°C and subsequent thawing, apparently degrading in favour of component MT3.

Following isolation of MT3, the major radioactive component separated from monkey urine by t.l.c. in chloroform/methanol/acetic acid (18:2:1), this component was shown to be essentially homogeneous by further t.l.c. in three different solvent systems.

Similarly, band MT4 was shown by further t.l.c. in four solvent systems to comprise predominantly one component.

(b) The stability of the thioamide group under the conditions of urine collection and determination of the chromatographic pattern

\(^{14}\text{C}-\text{tiquinamide HCl}\) degraded to its nitrile derivative at 100 \text{ug/ml} under the conditions of collection and storage of urine from patas monkeys dosed with \(^{14}\text{C}-\text{tiquinamide HCl}\) for the purpose of determination of the chromatographic pattern of drug-related products. In urine stored in flasks surrounded by dry ice for 12 h and then allowed to return to room temperature over the following 12 h, the extent of degradation to the nitrile was 56.3 ± 1.3%. No other significant degradation product was observed.
(c) Hydrolysis of conjugated material

Following treatment of the 0-24 h urine sample with molluscan \( \beta \)-glucuronidase/sulphatase and t.l.c. in chloroform/methanol/acetic acid (18:2:1), the only significant radioactive components present were MT3 and MT4 (Fig. V.2), (relative mobilities 1.06 and 1.30 respectively, relative to THQ COOH). MT1 was virtually absent. MT4 was apparently significantly enhanced by comparison with its presence in urine treated with pH 4.5 0.1M acetate buffer alone. Hence this observation suggested that MT4 comprised glucuronidase and/or sulphate conjugates of MT4.

Treatment of the polar component MT1 in isolation with bacterial \( \beta \)-glucuronidase followed by t.l.c. showed that hydrolysis was incomplete, but that a band found at the expected location of MT4 (Rf 1.31 relative to THQ COOH) was a significant product (Fig. V.3). Since the bacterial \( \beta \)-glucuronidase preparation was essentially free of aryl sulphatase, it follows that MT1 contained the glucuronide conjugate of MT4 (5-hydroxy-8-cyano-5,6,7,8-tetrahydro-3-methylquinoline). The unhydrolysed fraction may have contained a different conjugate e.g. sulphate or may simply have represented incomplete hydrolysis of the glucuronide.

(d) Characterization of urinary end-products

MT3: The evidence for the structure of MT3 comprises principally the u.v., i.r., n.m.r. and mass spectra of this compound and/or its methyl ester.

Initial evidence of the acidic nature of this compound came from the fact that it could be extracted into diethyl ether under acidic but not basic conditions. Furthermore, it showed chromatographic properties similar, though not identical, to those of the acidic reference compound THQ COOH (Table V.2). The formation of the methyl ester derivative confirmed its acidic character.

The presence of a u.v. absorption maximum at 272 nm was indicative of the pyridine portion of the 5,6,7,8-tetrahydroquinoline ring (Fig. V.4), though the small (3-4 nm) shift to longer wavelength suggested aromatic substitution by an electron withdrawing group and the total spectrum was more closely identical with that of THQ COOH and with the acidic rat metabolite RT4 than with that of unchanged tiquinamide. The absorption maximum was, however, close enough to that of 5,6,7,8-tetrahydroquinoline to suggest that the second ring...
remained saturated, if intact. The absence of any significant effect of alkalinization on the u.v. spectrum of MT3 suggested the absence of aromatic hydroxyl substitution. The i.r. spectrum of the methyl ester of MT3 (Fig. V.5) contained a band at 1720 cm\(^{-1}\) consistent with the presence of an ester carboxyl group. A strong band at 2240 cm\(^{-1}\) could only be attributed to a nitrile group.

In the n.m.r. spectrum of MT3 methyl ester, (Fig. V.6) broad, ill-defined signals at \(\delta 1.5-2.5\) ppm and at \(\delta 2.8-3.1\) ppm were characteristic of the cyclohexane ring protons in a tetrahydroquinoline system. The multiplet at \(\delta 4.2-4.5\) ppm indicated the presence of only one proton at position 8 of this system. The absence of a strong singlet at about \(\delta 2.2\) ppm showed the lack of a 3-methyl substituent. However, the pair of doublets with coupling constant 2Hz in the low field region was characteristic of a pyridine ring substituted at the 3-position as in tiquinamide. Moreover the shift of these signals to \(\delta 8.2\) and 9.15 ppm from the position of \(\delta 7.5\) and 8.2 ppm found with tiquinamide was indicative of the presence of an electron withdrawing group at position 3. A three-proton singlet at \(\delta 3.95\) ppm was characteristic of a single O-methyl group.

On the basis of the u.v., i.r. and n.m.r. spectra, the structure assigned to MT3 was that of 8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid i.e. a structure identical to that of the rat metabolite RT4.

The mass spectrum of MT3 methyl ester (Fig. V.8), obtained after g.l.c. (Fig. V.7), was consistent with this structure, having a parent ion at \(m/e 216\). The next most abundant ions at \(m/e 185\) (M-31) and \(m/e 157\) (M-59) corresponded respectively to loss of \(-OCH_3\) and \(-C=OCH_3\) from the molecular ion. A fragment at \(m/e 130\) (M-86) resulted from the loss of both ester and nitrile groups. A fragment at \(m/e 176\) (M-40) was attributed to the loss of \(-CH_2-CN\) which had proved to be an unusual characteristic feature of the mass spectra of nitrile metabolites of tiquinamide (Chapter IV. p. 80).

MT4: The characterization of MT4 is based on its u.v., i.r. and mass spectra. The presence of a u.v. absorption maximum at 269 nm with a shoulder at 277 nm (Fig. V.4) was characteristic of the pyridine ring of the 5,6,7,8-tetrahydroquinoline system. It also suggested that the cyclohexane ring remained saturated, if
intact. The lack of any effect of change of pH on the u.v. spectrum was indicative of an absence of hydroxyl substitution of the pyridine ring.

The i.r. spectrum of MT4 (Fig. V.5) contained a weak band at 2230 cm⁻¹ which was assigned to a nitrile group. Broad signals at 3000-3600 cm⁻¹ were indicative of the presence of a hydroxyl group.

On the basis of the u.v. and i.r. spectra, the structure tentatively assigned to MT4 was that of a hydroxylated derivative of 3-methyl, 8-cyano-5,6,7,8-tetrahydroquinoline. Since aromatic hydroxylation had been ruled out on the basis of the u.v. spectra, alternative structures were 8-cyano-5,6,7,8-tetrahydro-3-hydroxy-methylquinoline (cf RT5A in rat) or an isomer hydroxylated in the saturated ring.

The mass spectrum of MT4 (Fig. V.8) was consistent with such a hydroxylated structure, having a parent ion at m/e 188. Furthermore, it was found to be very similar to the mass spectrum of component RT5B (Fig. IV.9) isolated from rat urine and identified as 5-hydroxy, 8-cyano-5,6,7,8-tetrahydro-3-methylquinoline. Moreover, it was quite different from that of RT5A, the 3-hydroxy-methyl isomer (Fig. IV.9). Whereas the most prominent ion in the mass spectrum of RT5A was the parent ion at m/e 188, in that both MT4 and RT5B was a fragment at m/e 132. Other fragments shared by MT4 and RT5B but not RT5A were those at m/e 173, 170, 169 and 168. Furthermore, fragments at m/e 159 and m/e 148 in the mass spectrum of RT5A were absent from those of both MT4 and RT5B. Hence the mass spectral evidence definitely eliminated the possibility that MT4 could be the 3-hydroxymethyl compound. The extreme similarity of the mass spectrum of MT4 to that of the rat metabolite RT5B made it most likely that these compounds were identical i.e. that MT4 was 5-hydroxy, 8-cyano-5,6,7,8-tetrahydro-3-methylquinoline.
DISCUSSION

The metabolism of tiquinamide in the patas monkey showed some similarities and some differences from that in the rat. The major end-products isolated from monkey urine resulted from three Phase I processes: desulphuration of the thiocarboxamide group to the corresponding nitrile analogue, oxidation of the 3-methyl group and hydroxylation at the 5-position. All these processes had also been observed to occur in the rat. Unlike the rat, however, the monkey did not aromatize the 5,6,7,8-tetrahydroquinoline ring system (Fig. V.9).

Whereas no evidence could be found to support the existence of a substantial proportion of thioamide-containing metabolites in rat urine (Chapter IV. p.85), there seems every likelihood that such compounds were excreted in the urine of the patas monkey, but were chemically desulphurized before they could be identified. Studies on the stability of $^{14}$C-tiquinamide HCl added to control monkey urine and stored under the conditions of collection and storage of urine from tiquinamide-dosed animals revealed that, although substantial degradation would have taken place, at least 45% of thioamide compounds voided in the 0-24 h urine would have been detected. Hence major thioamide metabolites would have been detected in the chromatographic pattern though it would have been anticipated that they would degrade during subsequent work-up. Hence the observation in the 0-2 h urine of monkeys of a major radioactive component, which subsequently degraded to MT3 (8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid) suggested that this unstable metabolite was such a thioamide i.e. 5,6,7,8-tetrahydroquinoline-8-thiocarboxamide-3-carboxylic acid. That this component was not apparent in the chromatographic pattern of the 0-24 h urine was in all probability due to its degradation during the processes of thawing, pooling of samples collected over shorter periods, and re-freezing and re-thawing which took place before the 0-24 h pattern was determined. It must be concluded therefore that although some metabolic desulphuration may have taken place, some of the desulphuration of tiquinamide-related products in the patas monkey occurred by chemical degradation. Hence the nitrile compounds isolated from urine of monkeys dosed with $^{14}$C-tiquinamide HCl arose, at least partially, as degradation products of thioamide-
containing metabolites. Less extensive metabolic desulphuration in the monkey than in the rat was consistent with the more rapid elimination of metabolites in the monkey. Metabolism at other sites in the molecule apparently occurred more rapidly than did desulphuration, resulting in products containing highly polar groups which were readily excreted in urine before metabolic desulphuration could take place.

The predominant biotransformation step in the monkey was the ω-oxidation of the 3-methyl group of tiquinamide to the corresponding 3-carboxylic acid. This resulted in the recovery from 0-24 h urine of 38-47% of the dose in the form of MT3 (8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid). As discussed above, this compound may have been formed partially as a true metabolite and partially as a degradation product of its thioamide analogue. The rate of the oxidation of the 3-methyl group was extremely rapid in this species, as judged by the fact that such a high proportion of the dose was excreted as carboxylic acid metabolites and that there was no evidence for excretion of the intermediate 3-hydroxymethyl derivative seen in the rat. Furthermore, the 3-carboxylic acid metabolites constituted the major portion of the 50% of the dose which was eliminated within the first 2 h after drug administration. Thus the formation of 3-carboxylic acids was both faster and more extensive than in the rat, which excreted only 15% of the dose in urine in this form and less than 3% in bile. An alternative explanation for the lower amounts of these acids in rat urine was that they were extensively further metabolized. Possible routes of further metabolism included conjugation with glucuronic acid, as for nicotinic acid (Reddi and Kodicek, 1953) or with amino-acids such as glycine, as for 6-chloropicolinic acid (Ramsey et al., 1974).

However, these products would themselves have been rapidly excreted by virtue of their high polarity. Although such compounds may have been present in 0-24 h urine they could not have been present in high enough amounts to account for the difference between the amounts of 3-carboxylic acids excreted in rat and monkey. De-carboxylation was another possible route of further metabolism, but this has only been seen as a minor reaction e.g. 7% for nicotinic acid (Leifer et al., 1951). Thus it was apparent that the
\( \omega \)-oxidation pathway to the 3-carboxylic acid in the monkey proceeded to an extent at least double that in the rat.

The other identified route of metabolism of tiquinamide in the monkey was hydroxylation at the 5-position i.e. benzylic hydroxylation at a position \( \alpha \) to the point of fusion of the saturated and unsaturated rings in the tetrahydroquinoline system. This route could have been responsible for up to 20% of the dose excreted in the 0-24 h urine, assuming that all the conjugated material in band MT1 was comprised of glucuronides or sulphates of 5-hydroxylated metabolites. Thus, at the most, the extent of this process represented one-quarter to a half that of the \( \omega \)-oxidation step. There could be more certainty about the relative importance of these two routes in the monkey than in the rat because virtually all of the dose (circa 90%) was excreted in the former species in the 0-24 h urine and therefore no speculation about the nature of subsequently excreted products was necessary to evaluate the maximum proportions of the dose which could have been excreted by each route.

The extent of conjugation of the 5-hydroxylated product was greater than in the rat. Thus free 5-hydroxylated metabolites represented only 11-13% of the total 5-hydroxylated compounds, compared to 40% in the rat (Chapter IV, p. 87).

Some possible reasons for the species difference in metabolism between the rat and patas monkey:

Two major competing pathways of oxidative metabolism of tiquinamide in the rat and patas monkey are \( \omega \)-oxidation and allylic-hydroxylation at the 5-position. As discussed in Chapter IV, the enzymes probably involved in these biotransformation pathways are those outlined below:

**\( \omega \)-Oxidation:**

\[
\begin{align*}
R \text{CH}_3 \xrightarrow{\text{microsomal mono-oxygenase}} R \text{CH}_2 \text{OH} \xrightarrow{\text{alcohol dehydrogenase}} R \text{CHO} \xrightarrow{\text{aldehyde dehydrogenase}} R \text{COOH}
\end{align*}
\]

**Allylic-hydroxylation:**

\[
\begin{align*}
\text{HC} \xrightarrow{\text{microsomal mono-oxygenase}} \text{CHOH}
\end{align*}
\]
Whilst examples of \( \omega \)-oxidation of aromatic methyl substituents by the rat are known and several compounds have been found to be oxidized completely to the corresponding carboxylic acids e.g. banol (Strother, 1972), 5-methyl-4-(2-hydroxy-3-t-butyl-aminoproxy), coumarin (Hayashi et al., 1975) and 3-chloro-4-methyl-aniline (Peoples and Westberg, 1975), there is evidence that the rat performs this oxidation less well than primates so that an alternative route of metabolism may often be favoured. Thus, for example, diazoxide was metabolized to the extent of up to 70% in the monkey and 54-60% in man by oxidation of an aromatic methyl substituent to hydroxymethyl and carboxylic acid derivatives, but was excreted without extensive metabolism in the rat (Pruitt, Faraj and Dayton, 1974). Similarly, mepirazole was extensively metabolized by methyl group oxidation to the carboxylic acid derivative in man, but the only significant route of metabolism in the rat involved aromatic hydroxylation of the pyrazole ring (Kodama and Takabatake, 1972). For a number of compounds, this \( \omega \)-oxidation proceeds only as far as the hydroxymethyl compound in the rat, whereas primates oxidize completely to the corresponding carboxylic acid. Thus for tolbutamide, the hydroxymethyl oxidation product was the major metabolite in the rat (80%) and the carboxylic acid only relatively minor (4%), whereas in man as much as 33% of the dose was excreted as the acid (Thomas and Ikeda, 1966). Similarly, for 2-isopropylaminomethyl-6-methyl-7-nitro-1,2,3,4-tetrahydroquinoline, the 6-carboxylic acid metabolite was a major product in man, but the rat formed only the 6-hydroxymethyl derivative (Kaye and Woolhouse, 1972). For the sulphonamide compound, tosifen, no comparable data in primates is available, but it is known that the rat performs \( \omega \)-oxidation as far as the hydroxymethyl derivative (54-57%), but further oxidation to the carboxylic acid is less extensive (7-19%) (Lin et al., 1978).

Part of the reason for the poorer oxidation of aromatic methyl substituents to the corresponding carboxylic acids by the rat may be that the activity of liver alcohol dehydrogenase in the rat is substantially lower than in primates. It has long been known that the oxidation of the hydroxymethyl compound to the aldehyde which occurs as an intermediate step in the \( \omega \)-oxidation of methyl groups to carboxylic acids is catalysed by a soluble
dehydrogenase in the liver (Gillette, 1959). More recently it has been demonstrated that this reaction is carried out, in fact, by the liver alcohol dehydrogenase which catalyses ethanol oxidation (Von Wartburg and Schürch, 1971). Furthermore, these authors showed that, for the oxidation of $\beta$-pyridyl carbinol, human liver possesses 2.8 times the alcohol dehydrogenase activity of rat liver and monkey liver 2.3 times that of rat liver. With ethanol as substrate, they found activities in man and monkey of 1.6 and 1.7 times that in the rat, slightly lower than the ratio of $\approx$ 2.3 between man and rat observed by Krebs and Perkins, 1970. Alcohol dehydrogenase, rather than aldehyde dehydrogenase, was the rate-limiting step in the oxidation of $\beta$-pyridyl carbinol to nicotinic acid.

In addition to the retarding effect of the lower alcohol dehydrogenase activity in the rat on the side-chain oxidation, there may well be an additional factor, namely the specificity of the microsomal mono-oxygenases in this species, tending to promote 5-hydroxylation rather than $\omega$-oxidation. There is some evidence from work on the kinetics of hexobarbital metabolism to support the view that this reaction occurs more rapidly in rats than in primates. Hydroxylation in the cyclohexane ring at a position $\alpha$- to the double bond constitutes the rate-limiting step in the elimination of this compound and is an analogous reaction to the 5-hydroxylation of tiquinamide. The most recent estimate of the half-life of hexobarbital in the rat suggest that it may be as short as 12 min (Holcomb, Gerber and Bush, 1974), whereas in the rhesus monkey it has been found to be much longer i.e. nearly 2 h (Davis, Brown and Strike, 1971). Hence the mono-oxygenase catalysing this reaction may possess a greater activity in the rat than in the monkey. It is envisaged, in contrast, that the mono-oxygenase catalysing the $\omega$-oxidation has a greater activity in the monkey than in the rat. This explanation assumes the more recent view, for which there is increasing evidence, that there are several different microsomal mono-oxygenases with diverse activities. This view is supported, for example, by evidence that enzymes catalysing the hydroxylation of pentobarbital, the N-demethylation of ethylmorphine and the 16$\alpha$-hydroxylation of testosterone have been differentiated from testosterone-7-$\alpha$- and 6-$\beta$-hydroxylase by their differing stabilities to storage at -15°C (Levin et al., 1969).
Similarly, the 7-α, 6-β- and 16-α-hydroxylations of testosterone have been distinguished from one another (Kuntzman et al., 1968). Substantive evidence has also come from the finding of multiple forms of cytochrome P-450 in liver microsomes (e.g. Coon et al., 1977; Johnson and Muller-Eberhard, 1977) which have varying substrate specificities. It is not difficult to envisage that such polymorphism among microsomal mono-oxygenase could account for many of the known species differences among routes and rates of drug oxidation, including possibly the observed species difference in the oxidative metabolism of tiquinamide in rats and monkeys.

Why the monkeys did not form the fully aromatized metabolite, 8-cyano-quinoline-3-carboxylic acid, as did the rat, was unclear. Species differences in the extent of aromatization have been reported for cyclohexane carboxylic acid (Babior and Bloch, 1966) and for quinic acid (Adamson et al., 1970a). However, as has previously been discussed, (Chapter IV. p. 89) these reactions occur by different mechanisms and it is quite possible that the aromatization which apparently occurs during the metabolism of tiquinamide in the rat does so by yet another mechanism. Hence the reason for the absence of the aromatization reaction in the patas monkey necessarily remained obscure in the absence of a known mechanism for this reaction.
**Fig. V.1: Chromatography of radioactivity in 0-2h. urine of patas monkeys administered 10 mg/kg $^{14}$C-tiquinamide HCl orally**

Solvent system: Chloroform/methanol/acetic acid (18:2:1)

- **O** = Origin
- **A** = 5,6,7,8-tetrahydroquinoline-3-carboxylic acid
- **SF** = solvent front

**Chromatography of radioactivity in 0-24h urine of patas monkeys administered 10 mg/kg $^{14}$C-tiquinamide HCl orally**
Acetate buffer

Solvent system:
Chloroform/methanol/acetic acid
(18:2:1)

O = Origin
A = 5,6,7,8-tetrahydroquinoline-3-carboxylic acid
SF = solvent front

Fig. V.2: Chromatography of radioactivity in 0-24h.
urine of a patas monkey administered
10 mg/kg. $^{14}$C-tiquinamide HCl. orally
after treatment with molluscan
$\beta$-glucuronidase/sulphatase or acetate buffer
Fig V.3:
Chromatography of products of hydrolysis
of MT1 by bacterial $\beta$-glucuronidase

Solvent system:
Chloroform/methanol/acetic acid (18:2:1)

$O =$ Origin
$A =$ rat metabolite RT5B
$SF =$ solvent front
Absorbance

Neutral ethanol 272
Basic ethanol 272
Acidic ethanol 272

MT3

Wavelength (nm)

Neutral ethanol 269 277

MT4

Wavelength (nm)

Fig. V.4: U.v. spectra of urinary end products of tiquinamide: MT3 and MT4
Fig. V5: I.r. spectra of urinary end products of tiquinamide: MT3 methyl ester and MT4 methyl ester.
Fig.V.6: N.m.r. spectrum of the methyl ester of tiquinamidé, urinary end product MT3
Fig. V.7: Gas chromatography of MT3 methyl ester

Gas chromatograph: Perkin-Elmer F11

Column: 2 m x 3 mm (i.d.) glass, containing 10% SE 30 on AW Chromosorb W

Column temperature: 20°C

Nitrogen flow rate: 40 ml/min
Fig.V.8: Mass spectra of urinary end products of tiquinamide: MT3 methyl ester and MT4
FIG. 9: The metabolism of tiguanamide in the rhesus monkey

Values in parentheses represent percentages of dose excreted as each metabolite in 0-24 h urine

MT1 (16-19%) (38-47%)

MT2 (2-3%)

X = CN/CSSNH2

Other conjugates possibly including some
<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>♂ 360</td>
</tr>
<tr>
<td>MT1</td>
<td>18.6</td>
</tr>
<tr>
<td>MT2</td>
<td>8.0</td>
</tr>
<tr>
<td>MT3</td>
<td>37.8</td>
</tr>
<tr>
<td>MT4</td>
<td>2.7</td>
</tr>
<tr>
<td>Total % in these bands</td>
<td>67.1</td>
</tr>
<tr>
<td>Total % dose in urine</td>
<td>90.1</td>
</tr>
</tbody>
</table>

Table V.1: Percentages of the administered dose present in each of the major radioactive components in 0-24 h urine of patas monkeys administered 10 mg/kg ^14C-tiquinamide orally.

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Rf MT3</th>
<th>Rf THQ COOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.65</td>
<td>0.63</td>
</tr>
<tr>
<td>2</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>3</td>
<td>0.15</td>
<td>0.04</td>
</tr>
<tr>
<td>11</td>
<td>0.82</td>
<td>0.74</td>
</tr>
<tr>
<td>12</td>
<td>0.38</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Table V.2: Comparative t.l.c. of MT3 with 5,6,7,8-tetrahydroquinoline-3-carboxylic acid.
CHAPTER VI: METABOLISM OF TIQUINAMIDE IN MAN
SUMMARY
A study of the nature of end-products in 0-24 h urine of male volunteers suggested that there were pathways identical to those in the rat. ω-Oxidation resulted in the formation of 8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid and, after aromatization, in 8-cyano-quinoline-3-carboxylic acid. Allylic hydroxylation at the 5-position gave rise to 5-hydroxy,8-cyano-5,6,7,8-tetrahydro-3-methylquinoline, which was extensively conjugated at least partially with glucuronic acid. Characterization of products isolated from urine suggested that they were nitriles, but no distinction could be made between chemical and enzymic degradation of the thioamide group. Therefore the extent of metabolic desulphuration in man was not known. With respect to the degree of conjugation of the 5-hydroxylated compound and the extent of ω-oxidation to carboxylic acids, man resembled the patas monkey more closely than the rat. Thus there were similarities with both animal species studied.

The comparative metabolism of tiquinamide in rat, patas monkey and man is discussed.
INTRODUCTION

Studies of the metabolism of tiquinamide in rat and monkey had defined the major route of biotransformation in these species and had indicated some similarities and some differences between them. It was essential to define the metabolic pattern in man in order to relate biological activities and disposition in man to those in the rat and monkey.

Concentrations of drug-related products in urine after a single 50 mg dose were too low to make isolation of sufficiently large quantities for physico-chemical characterization a practical proposition. However, it seemed likely that the establishment of the chromatographic pattern of urinary end-products in man would indicate how closely the metabolism in this species resembled that in the rat or monkey. Thus it seemed a useful approach to define the qualitative and quantitative 0-24 h urinary pattern of radioactive products following t.l.c. in solvent 1 and to supplement this, where possible, by additional comparative t.l.c. in other solvent systems to confirm the identities of products with compounds isolated from rat urine.
EXPERIMENTAL

(a) Chromatographic pattern of drug-related products in urine

Two male volunteers, DM and ML, were each administered a 50 mg dose of $^{14}$C-tiquinamide (50 uCi) orally by capsule as previously described (Chapter II. p. 42). Urine was collected at 3-hourly intervals for the first 12 h, and subsequently at 24 h. Portions of a pooled 0-24 h urine sample (20 ml) were reduced to dryness and residues dissolved in methanol (2 ml). Aliquots were subjected to t.l.c. in solvent 1 on 0.25 mm silica plates with pre-adsorbent zone. Radioactivity was located by scanning.

The clearly definable components were designated HT1-HT4 in order of increasing mobility, which was determined relative to THQ-COOH.

The distribution of radioactivity on the plate was quantitated by scraping 0.5 cm strips into scintillation vials followed by elution with methanol/water (1:1, 2 ml) and liquid scintillation counting.

(b) Comparative t.l.c. of ether-soluble acidic compounds with those in rat urine

The acidic, ether soluble components HT3 and HT4 were isolated from human urine by extraction into diethyl ether at pH2 followed by thin-layer chromatography in solvent 1 on 0.25 mm silica plates with pre-adsorbent zone. Radioactive bands were located by scanning, scraped off and eluted with methanol. RT3 and RT4 were isolated in a similar manner from rat urine.

The chromatographic properties of HT3 and RT3 and of HT4 and RT4 were compared in solvents 1, 2, 3*, 11*, 12 and 14. (Chapter II. p. 38)

(c) Hydrolysis of conjugated material

The residue of a 20 ml portion of 0-24 h human urine after diethyl ether extraction under basic (pH 9) and acidic (pH 2) conditions was incubated for 18 h at 37°C with bacterial $\beta$-glucuronidase (E. Coli Type I Sigma Chemical Co., Poole, Dorset) at 18,000 units/ml in pH 7.4 0.1M phosphate buffer saturated with chloroform. Protein precipitation was performed by addition of methanol, followed by centrifugation. Supernatants were reduced by dryness, dissolved in water (5 ml) and subjected to extraction with diethyl ether (2 x 4 vol) under basic (pH 9) or acidic (pH 2) conditions.
Chromatography of the basic, ether-soluble material was performed in solvents 4, 5, 7 and 10 (Chapter II. p. 38). Location of radioactivity was by scanning and comparison was made with previous traces of RT5B isolated from rat urine and subjected to t.l.c. in these same solvent systems.
RESULTS

(a) Chromatographic pattern of drug-related products in urine

The 0-24 h urine of two male volunteers administered a single 50 mg dose of \(^{14}\)C-tiquinamide HCl orally contained 61% and 88% of the dose. Chromatography of this fraction in solvent 1 revealed the presence of four principal radioactive bands, designated HT1-HT4, which corresponded to bands RT1-RT4 in the rat (Fig. IV.1). HT1, HT3 and HT4 were clearly evident as individual components, whereas HT2 was a more diffuse band, possibly containing several components (Fig. VI.1).

In Table VI.1 are expressed the percentages of the administered dose found in each of these radioactive components. The most prominent bands were HT1 (17-27%) and HT4 (17-25%). The component HT3 was present in somewhat lower amounts (7-10%). Band HT2 contained 11-20%, but was a diffuse, ill-defined area.

(b) Comparative t.l.c. of ether-soluble acidic compounds with those in rat urine

A diethyl ether extract of untreated human urine made under acidic conditions (pH 2) contained only components HT3 and HT4.

HT3 was found to have very similar chromatographic properties in six solvent systems (Table VI.2) to rat metabolite RT3, identified as 8-cyano-quinoline-3-carboxylic acid (Chapter IV. p.82).

Similarly, HT4 had similar chromatographic properties in six solvent systems (Table VI.2) to those of rat metabolite RT4, identified as 8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid (Chapter IV. p.83).

(c) Hydrolysis of conjugated material

Treatment of the polar component HT1 with bacterial \(\beta\)-glucuronidase resulted in partial hydrolysis of this material. Extraction of the incubation mixture with diethyl ether following basification (pH 9) resulted in 24% extraction of radioactivity into the ether phase. Chromatography of the extracted radioactivity in three solvent systems revealed essentially a single component with similar chromatographic properties to those of rat metabolite RT5B, previously identified as 5-hydroxy,8-cyano-5,6,7,8-tetrahydro-3-methylquinoline (Table VI.3). A further 9% of total radioactivity in the hydrolysate was extracted into diethyl ether under acid conditions (pH 2) but was not characterized.
DISCUSSION

Limited studies on the metabolism of tiquinamide in man have revealed that the processes involved (Fig. VI.2) were probably qualitatively similar to those observed in the rat and monkey. The major pathways involved were $\omega$-oxidation of the 3-methyl group and 5-hydroxylation, as in both rat and monkey. In addition, there was apparently a significant degree of aromatization of the 3-carboxylic acid metabolite as observed in the rat but not in the monkey. The extent of metabolic desulphuration of the thioamide group in man could not be estimated on the basis of these studies. The urinary end-products characterized were observed to be nitriles on the basis of a comparison of their chromatographic properties with those of corresponding compounds found in rat urine and identified by physico-chemical analysis. However, it is quite possible that any thioamides present in urine would have undergone chemical degradation to the nitrile during the prolonged storage at $-20^\circ C$ and subsequent thawing.

The indications from these results were that the metabolism of tiquinamide in man was not identical in every detail either with that in the rat or with that in the monkey, but shared some features with both these species (Table VI.4). Man excreted in 24 h similar amounts (7-10%) of the fully aromatic 8-cyano-quinoline-3-carboxylic acid to the rat (9%), whereas the monkey did not form this metabolite. However, in most other aspects, man resembled the monkey to a greater degree. Excretion of the partially saturated acid 8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid was 17-25% in man i.e. approximately three times that in the rat (7%) but about half that in the monkey (38-47%). Excretion in 0-24 h urine of total acidic metabolites was 23-34% i.e. 1.5 to 2 times that in the rat (16%) and approximately three-quarters that in the monkey (38-47%). At 17-27% in man, the total material remaining on the origin and believed to represent primarily conjugates of 5-hydroxy,8-cyano-5,6,7,8-tetrahydro-3-methyl-quinoline, was greater substantially than in the 0-24 h rat urine (7%), and possibly slightly greater than in monkey urine (16-19%). In that there was no evidence of free 5-hydroxy,8-cyano-5,6,7,8-tetrahydro-3-methyl-quinoline, man resembled the monkey in this respect more than the rat.
It is fairly well established that in general monkeys and other sub-human primates show greater similarities to man in terms of their drug-metabolizing activities than do other species (Williams, 1974; Smith, 1967; Smith and Williams, 1974), although the different species of primate may vary among themselves and it is not always easy to predict in advance which will represent the best model for man. The rhesus monkey has been found to be most generally useful. Thus Smith and Caldwell (1977) found that for 17/23 drugs, metabolism in the rhesus monkey was similar to that in man. The patas monkey, like the rhesus, is an Old World monkey and therefore perhaps similarly closely related to man in evolutionary terms. Though it has been less widely investigated, it might be anticipated that this species also would represent a good model for man. In the present studies on the comparative metabolism of ti Quinnamide, this was found to be generally true in that the extent of \( \omega \)-oxidation and of 5-hydroxylation were of similar orders in patas monkey and man. However, man behaved quite differently from the monkey, but identically to the rat, in carrying out the aromatization reaction which resulted in the formation of 8-cyano-quinoline-3-carboxylic acid. Species differences in the aromatization of quinic acid (Adamson et al., 1970a) and of cyclohexane carboxylic acid (Babior and Bloch, 1966) are known, but it is possible that the species difference in ti Quinnamide aromatization has quite a different explanation, since it probably occurs through a different mechanism (Chapter IV. p. 89).
Solvent system: Chloroform/methanol/acetic acid (18:2:1)

**Subject ML**

O = Origin  
A = 5,6,7,8-tetrahydro-quinoline-3-carboxylic acid  
SF = solvent front

**Subject DM**

**Fig.VI.1:** Chromatography of radioactivity in 0-24h. urine of male volunteers administered 50mg¹⁴C-tiquinamide HCl orally.
Fig. VI.2: The metabolism of tioguaninamide in man

Values in parentheses represent percentages of dose excreted as each metabolite in 0-24h urine

HT1 (17-27%) possibly including other

HT2

X=CN/CSNH2

HT3 (7-9%) gluconide

HT4 (17-25%)
Table VI.1: Percentages of the administered dose present in each of the major radioactive components in 0-24 h urine of male volunteers administered 50 mg $^{14}$C-tiquinamide orally.

<table>
<thead>
<tr>
<th></th>
<th>% Dose</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ML</td>
<td>DM</td>
</tr>
<tr>
<td>HT1</td>
<td>17.2</td>
<td>27.1</td>
</tr>
<tr>
<td>HT2</td>
<td>(11.2)</td>
<td>(19.9)</td>
</tr>
<tr>
<td>HT3</td>
<td>6.8</td>
<td>9.7</td>
</tr>
<tr>
<td>HT4</td>
<td>16.6</td>
<td>25.4</td>
</tr>
<tr>
<td>Total % dose in the bands</td>
<td>51.8</td>
<td>82.1</td>
</tr>
<tr>
<td>Total % dose in 0-24 h urine</td>
<td>60.8</td>
<td>87.5</td>
</tr>
</tbody>
</table>

Figures in parentheses denote total percentage of dose within a diffuse area designated HT2.
<table>
<thead>
<tr>
<th>Solvent</th>
<th>Rf THQ COOH</th>
<th>Relative Rf values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HT3</td>
</tr>
<tr>
<td>1</td>
<td>0.52</td>
<td>1.14</td>
</tr>
<tr>
<td>2</td>
<td>0.09</td>
<td>3.34</td>
</tr>
<tr>
<td>3</td>
<td>0.05</td>
<td>3.24</td>
</tr>
<tr>
<td>11</td>
<td>0.60</td>
<td>1.22</td>
</tr>
<tr>
<td>12</td>
<td>0.24</td>
<td>1.27</td>
</tr>
<tr>
<td>14</td>
<td>0.17</td>
<td>1.29</td>
</tr>
</tbody>
</table>

Table VI.2: Comparative chromatographic properties of acidic end-products of tiquinamide in man and rat

Rf values for acidic end-products of tiquinamide are expressed relative to THQ COOH. Absolute Rf values for this reference compound are quoted for each solvent system.

<table>
<thead>
<tr>
<th>Rf</th>
<th>Solvent</th>
<th>Hydrolysed HT1</th>
<th>Rat metabolite RT5B</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.07</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.65</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.32</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.35</td>
<td>0.35</td>
<td></td>
</tr>
</tbody>
</table>

Table VI.3: Comparative chromatographic properties of the major product of hydrolysis of HT1 with those of rat urinary end-product RT5B

Results are absolute Rf values.
<table>
<thead>
<tr>
<th>Metabolite Description</th>
<th>Female Mean ± SEM</th>
<th>Rat 360</th>
<th>Monkey 368 ML</th>
<th>Man DM</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>8-cyano-quinoline-3-carboxylic acid</td>
<td>8.5 ± 0.6</td>
<td>ND</td>
<td>ND</td>
<td>6.8</td>
<td>9.0</td>
</tr>
<tr>
<td>8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid</td>
<td>7.4 ± 0.6</td>
<td>37.8</td>
<td>46.6</td>
<td>16.6</td>
<td>23.6</td>
</tr>
<tr>
<td>8-cyano-5,6,7,8-tetrahydro-3-hydroxy-methylquinoline</td>
<td>0.9 ± 0.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5-hydroxy-8-cyano-5,6,7,8-tetrahydro-3-methylquinoline</td>
<td>4.5 ± 1.3</td>
<td>2.7</td>
<td>1.9</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>8-cyano-5,6,7,8-tetrahydro-3-methylquinoline</td>
<td>0.7 ± 0.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Conjugates including glucuronide of 5-hydroxy-8-cyano-5,6,7,8-tetrahydro-3-methyl-quinoline</td>
<td>6.8 ± 0.3</td>
<td>18.6</td>
<td>15.8</td>
<td>17.2</td>
<td>25.3</td>
</tr>
<tr>
<td>Unidentified metabolites</td>
<td>12.1 ± 0.6</td>
<td>31.0</td>
<td>22.6</td>
<td>20.2</td>
<td>30.4</td>
</tr>
<tr>
<td>Total</td>
<td>40.2 ± 2.2</td>
<td>90.1</td>
<td>86.9</td>
<td>60.8</td>
<td>87.5</td>
</tr>
</tbody>
</table>

Table VI.4: Percentages of the dose of \(^{14}\)C-tiquinamide HCl excreted as individual metabolites in 0-24 h urine of rats, patas monkeys and human volunteers.

* Amounts of these nitrile metabolites may include their thioamide analogues.

ND - not detectable.
CHAPTER VII: PLASMA KINETICS OF TIQUINAMIDE AND ITS METABOLITES IN RAT, PATAS MONKEY AND MAN
SUMMARY

Study of the plasma kinetics of tiquinamide and its metabolites in the rat and patas monkey revealed that the drug was quickly absorbed and rapidly eliminated in both species, but that there was a very pronounced species difference in rates of elimination of metabolites. Total drug-related products were very rapidly eliminated in the patas monkey but very slowly eliminated in the rat. Although unchanged drug was not assayed in human plasma, the kinetics of a toluene-soluble basic fraction which was assayed had been found in animal studies to be representative of those of unchanged tiquinamide. The kinetics of this fraction in plasma of human volunteers indicated once again both rapid absorption and elimination. The total metabolite fraction in human plasma was mostly eliminated quickly, as in the monkey, but there was some evidence of a slower elimination phase akin to that in the rat.

Kinetics of total metabolites in whole blood were similar to those in plasma and showed no evidence of a significant association of drug or metabolites with red cells.

Binding to plasma proteins was found to be moderate in all three species, with no significant species differences.

A fraction of drug-related products found in rat plasma 25 h after dosing was found apparently to contain a single component of different chromatographic properties from that of any major product isolated from 0-24 h urine. Preliminary characterization suggested that it was of polar nature, and possibly a conjugate. Some possible structures are discussed.

The species difference in elimination of metabolites is discussed in the light of these findings and of knowledge of the identities of major metabolites and their physico-chemical properties.
INTRODUCTION

The plasma concentration-time profile of a drug and its metabolites provides information about the extent and rates of its absorption, and elimination from the body which represent fundamental parameters of the compound’s behaviour in a mammalian system. The relevance of such information to the process of drug development and optimal clinical use has been discussed previously (Chapter I. p. 32).

Experiments were carried out in animals and man in order to provide such fundamental pharmacokinetic information for tiquinamide as might facilitate decision-making during its development. Thus, for example, the time at which peak plasma concentrations occurred in man would be indicative of the intrinsic rate of absorption of tiquinamide. Determination of the rate of drug elimination in volunteers would be expected to aid the making of an informed decision about the frequency of dosing likely to offer maximum therapeutic benefit. Similarly, knowledge of the rate of elimination of total drug-related substances would show whether long-lived metabolites with a potentially increased risk of toxicity were likely to be a troublesome feature of this compound. Comparison of these parameters in man with those in the rat and patas monkey would establish whether the kinetic behaviour of the drug in these three species was sufficiently similar for chronic toxicity studies performed in the given animal species to be predictive of potential safety of tiquinamide on repeated administration to man.
EXPERIMENTAL

(a) Plasma kinetics

Rats: Four male and four female rats received 10 mg/kg $^{14}$C-tiquinamide HCl orally as previously described (Chapter II p. 42). Blood was collected at various times after dosing (Fig. VII.1), over periods of 0-264 h in male rats and 0-292 h in females.

Radioactivity in aliquots of whole blood were determined by scintillation counting after preparation of samples by a modification of the Mahin and Lofberg perchloric acid digestion procedure (Chapter II. p. 43). Plasma was separated from red cells by centrifugation. The toluene-soluble fraction of radioactivity in plasma, containing unchanged drug, was determined after an in-vial extraction, by liquid scintillation counting (Chapter II. p. 44). Radioactivity in the residue was assayed by liquid scintillation counting and concentrations of total radioactivity calculated.

The specificity of the toluene extraction procedure was determined by chromatography of an extract. Plasma was collected as above from a group of 10 female rats at 1-2 h after administration of $^{14}$C-tiquinamide HCl. It was made alkaline by addition of pH 9 1M bicarbonate buffer and extracted with toluene (2 x 3 vols).

Stability of unchanged tiquinamide during the extraction procedure was ensured by loading the plasma to a concentration of 100 µg/ml with unchanged tiquinamide HCl. The extract was subjected to thin-layer chromatography in solvents 5, 6 and 19.

In an additional experiment, to determine the kinetics of unchanged drug, groups of 4 female rats were killed at various times over a period of 0-8 h after dosing (Fig. VII.4) and exsanguinated from the inferior vena cava whilst under ether anaesthesia. Plasma was separated from red cells by centrifugation. A toluene extract of plasma was made as described above. Unchanged drug was separated from other components in toluene extracts by thin-layer chromatography in Solvent 5 (Chapter II. p. 38). Radioactive areas on plates were located by autoradiography. Bands corresponding to unchanged drug were scraped off, eluted with methanol/water (1:1) and quantitated by liquid scintillation counting. Absolute concentrations in plasma were calculated with reference to standard
amounts of $^{14}$C-tiquinamide HCl added to control plasma and taken through the entire procedure. Kinetic analysis was performed as described previously (Chapter II. p. 47).

**Patas monkeys:** Two male and four female patas monkeys received a 10 mg/kg oral dose of $^{14}$C-tiquinamide HCl. Blood was collected from the femoral vein at various times over a 0-48 h period after dosing (Fig. VII.5). Total radioactivity in plasma and whole blood and toluene-soluble radioactivity in plasma were determined as for rats. For the determination of the specificity in the patas monkey of the toluene extraction procedures, an experiment was performed in which a single animal was exsanguinated whilst under pentothal anaesthesia. A toluene extract of plasma, prepared as previously described was subjected to thin-layer chromatography in solvents 5 and 6.

In another experiment, three female patas monkeys received a 10 mg/kg oral dose of the drug and subsequently 10-15 ml portions of blood were removed from the femoral vein at various times up to 4 h after dosing (Fig. VII.7). Monkeys were each administered 60 ml distilled water after the third blood sample had been taken, i.e. approximately 1.5 h after dosing, in order to compensate for the extensive fluid loss resulting from the removal of such large blood samples. Concentrations of total radioactivity were determined by liquid scintillation counting. Toluene extraction was performed and determination of unchanged drug carried out by the procedure described previously. Kinetic analysis was performed as already described (Chapter II. p. 47).

**Man:** Two male volunteers each received a dose initially of 20 mg containing 5 μCi $^{14}$C-tiquinamide HCl and a week later a subsequent dose of 50 mg containing 50 μCi of radio-labelled drug. On each occasion, blood was collected from the cephalic vein at various times after dosing (Fig. VII.8) up to 48 h in the first experiment and 96 h in the second.

Toluene-soluble and total radioactivity in plasma were determined by liquid scintillation counting as previously described. Kinetic analysis was carried out according to the previously described method (Chapter II. p. 47).

(b) **Plasma protein-binding**

The extent of protein-binding of tiquinamide added at various concentrations to plasma of rat, patas monkey and man was determined by equilibrium dialysis. The dialysis was performed in cylindrical
perspex cells, each comprising two halves separated by a dialysis membrane (Spectrapor Grade 2, MSE Ltd., Sussex). Into one half-cell was introduced 250 μl 0.1M pH 7.4 phosphate buffer and into the other an identical volume of a solution of 14C-tiquinamide HCl at concentrations of 100 ng/ml-5 μg/ml (Fig. VII.10) in the same phosphate buffer or in plasma. Triplicate samples at each concentration were prepared. Cells were mounted in a clamp, immersed in a water-bath at 37°C and shaken gently backwards and forwards for 2-3 h, a sufficiently long period for equilibration to be achieved. At the end of the equilibration period, radioactivity in 200 μl aliquots taken from each half of the dialysis cell was determined by liquid scintillation counting. The percentage of drug bound to protein at each drug concentration could be determined as follows:

\[
\text{Percentage protein binding} = \frac{(\text{Plasma radioactivity} - \text{Buffer radioactivity}) \times 100}{\text{Plasma radioactivity}}
\]

(where all concentrations of radioactivity are expressed as dpm/200 μl).

(c) Characterization of drug-related products in 25 h rat plasma

Three female rats (circa 300 g) were administered 14C-tiquinamide HCl at 10 mg/kg orally and after 25 h were exsanguinated from the inferior venae cavae whilst under ether anaesthesia. Plasma was separated from red cells by centrifugation. Removal of protein from plasma was performed by ethanol precipitation. The chromatographic pattern of radioactivity in the ethanolic supernatant of plasma was compared with that in urine by t.l.c. in Solvent 1 on 0.25 mm silica plates with pre-adsorbent zone.

Further chromatography of products in plasma was carried out in solvents 6, 8, 12, 15 and 18. Tiquinamide and the related compounds Wy-24146 (8-cyano-5,6,7,8-tetrahydro-3-methylquinoline), Wy-24117 5,6,7,8-tetrahydro-3-methylquinoline-8-carboxamide were run for comparative purposes.

Aliquots of the ethanolic supernatant (equivalent to 1.5 ml of plasma) were reduced to dryness and taken up in 1.5 ml portions of β-glucuronidase/sulphatase (Helix pomatia Type H1, Sigma Chemical Co. Ltd., 20,000 units/ml in pH 4.5 0.1M acetate buffer) or in acetate buffer alone. The nature of the products in the incubation mixture were examined, after ethanolic precipitation of proteins, by t.l.c. in solvent systems 1 and 15.
For determination of a u.v. spectrum, material was isolated at 25 h after dosing from the plasma of 15 female rats (180-200 g) administered $^{14}$C-tiquinamide HCl at 100 mg/kg. Plasma proteins were precipitated with ethanol and the ethanolic supernatant subjected to a single t.l.c. step in solvent 12. The radioactive component was eluted with ethanol and the u.v. spectrum determined in ethanolic solution at concentrations of 12 and 59 $\mu$g equivalent $^{14}$C-tiquinamide HCl/ml.

(d) Partition coefficients of tiquinamide and related compounds between toluene and pH 7.4 phosphate buffer

Partition coefficients between toluene and pH 7.4 phosphate buffer were determined for the following compounds: tiquinamide, 8-cyano-5,6,7,8-tetrahydro-3-methylquinoline, 5-hydroxy-8-cyano-5,6,7,8-tetrahydro-3-methylquinoline, 5,6,7,8-tetrahydro-3-methylquinoline and 5,6,7,8-tetrahydroquinoline-3-carboxylic acid. These were model compounds containing structural features representative of the known tiquinamide metabolites. To duplicate 5 ml portions of a solution (25 $\mu$g/ml) of each compound in pH 7.3 0.1M K$^+$ phosphate buffer in glass centrifuge tubes were added 5 ml toluene ("Pronalys", May and Baker). Tubes were shaken gently for 30 min on a rotary shaker and toluene phases decanted. Concentrations of each compound remaining in the aqueous phase were determined spectrophotometrically with reference to unextracted 5 $\mu$g/ml solutions. Absorbance was determined at 275 nm instead of the more usual 270 nm (max for the 5,6,7,8-tetrahydroquinoline ring) in order to avoid interference from an impurity back-extracted from toluene and having an absorption maximum at 268 nm.
RESULTS
(a) Plasma kinetics

Rats: After oral administration of a 10 mg/kg dose of $^{14}$C-tiquinamide HCl to rats, total radioactivity in plasma increased slowly, only reaching its maximum concentration at 8-12 h after drug administration (Fig. VII.1). Peak values were 8.3-9.2 µg equivalent $^{14}$C-tiquinamide HCl/ml in males and 9.9-11.5 µg equivalent in females. Subsequent elimination of radioactivity took place very slowly and apparently monoexponentially, with mean half-lives of 101 h and 85 h in males and females respectively (Table VII.1), so that concentrations were still as high as 1.1 µg equivalent/ml eleven days after dosing. Elimination was significantly slower in males than in females ($p = 0.02-0.05$, as judged by Student's t-test).

A toluene-soluble fraction of radioactivity extracted into alkaline plasma (pH 9) represented approximately 60% of total radioactivity at 0.5 h after dosing, this toluene-soluble fraction achieved maximum concentrations of 2.1-3.4 µg equivalent/ml in male rats and 2.1-3.9 µg equivalent/ml in female rats by 0.5-1 h after administration (Fig. VII.2). Thereafter, the concentration declined rapidly to 0.1-0.2 µg equivalent/ml in males and 0.2-0.5 µg equivalent/ml in females by 12 h after dosing. Elimination was apparently monoexponential, with mean half-lives of 2.8 h and 3.7 h in males and females respectively (Table VII.1). Chromatography of the toluene-soluble fraction of alkaline plasma collected at 1-2 h after dosing revealed the presence of four significant components (Fig. VII.3). Only 12% of radioactivity in the toluene-extract of rat plasma was unchanged tiquinamide. The most prominent component, representing 44% of applied radioactivity was the nitrile derivative Wy-24146 (8-cyano-5,6,7,8-tetrahydro-3-methylquinoline). Two unknown metabolites containing 28% and 6% of extracted radioactivity were also present.

When an additional experiment was performed in order to measure concentrations of unchanged drug in groups of 4 rats exsanguinated at various times after dosing, the toluene-soluble radioactivity achieved a maximum concentration of 4.3 µg equiv/ml at 0.5 h after dosing, a similar order to that seen previously (Fig. VII.4). The elimination half-life of this fraction, 2.0 h, was a little shorter.
but still in the same range as that observed in the earlier experiment. Unchanged drug reached a maximum concentration of 2.7 µg equiv $^{14}$C-tiquinamide HCl/ml at 0.5 h after dosing. Thereafter the concentration declined rapidly, so that the concentration was only 3 ng/ml at 12 h after dosing. After an initial rapid disposition phase lasting 1 h, elimination of tiquinamide from plasma proceeded monoexponentially with a half-life of 1.6 h, a similar order to that of toluene-soluble radioactivity.

The time-course of radioactivity in whole blood of male and female rats following oral administration of $^{14}$C-tiquinamide HCl was similar to that in plasma, although absolute concentrations were somewhat lower. There was no evidence of association of drug-related products with red cells.

Patas monkey: After a 10 mg/kg oral dose of $^{14}$C-tiquinamide HCl to patas monkeys, total radioactivity achieved its maximum concentrations at 0.5 h after dosing in two male and two female animals (Fig. VII.5). Values were 6-14 µg equivalent $^{14}$C-tiquinamide HCl/ml, with no observed difference between males and females. In a third female, the peak concentration (7.8 µg equivalent/ml) was at 1.5 h after dosing and in a fourth, the maximum value (4.8 µg equivalent/ml) occurred as late as 3 h after drug administration. In these two animals, the slower absorption apparently limited the rate of elimination which took place more slowly than in the other animals. In those animals in which absorption was rapid, most of the radioactivity (95%) was eliminated over a period of 0-6 h with a half-life of approximately 0.7 h (Table VII.2). The second phase of elimination was slower, having a half-life of 44 h, but accounted for a very small proportion of the radioactivity in plasma (5%).

A toluene-soluble fraction of radioactivity extracted from alkalinated plasma (pH 9) represented only 15-20% of total radioactivity at 0.5 h after dosing. Times of maxima were somewhat variable, as for total radioactivity. In two male and two female monkeys, peak concentrations of 1-2 µg equivalent $^{14}$C-tiquinamide HCl/ml were observed at 0.5 h after drug administration (Fig. VII.5). In the other two females, however, maximum concentrations of 1.4 µg equivalent/ml and 0.5 µg equivalent/ml were not seen until respectively 1.5 h and 3 h after dosing. Once again, there was evidence of
slower, absorption rate-limited elimination in these animals. In the rapid absorbers, elimination occurred rapidly and monoexponentially, with a half-life of approximately 0.7 h (Table VII.2). Chromatography of a toluene-extract revealed the presence of four components (Fig. VII.6). Unchanged tiquinamide represented 40% of total radioactivity in this fraction. A further 20% was present as the nitrile derivative Wy-24146 (8-cyano-5,6,7,8-tetrahydro-3-methylquinoline). Two further unknown metabolites accounted for 8% and 11%.

Plasma concentrations of unchanged tiquinamide in three female monkeys achieved maximum values of 1.1-2.4 μg equivalent \(^{14}\text{C}-\) tiquinamide HCl/ml at 0.5 h after drug administration (Fig. VII.7). They subsequently declined fairly rapidly, such that at 1.5 h after dosing, values were 0.2-0.6 μg equivalent/ml. A secondary peak was observed at 2 h after drug administration. This was particularly marked in one animal in which the observed concentration at this time was as high as 1.6 μg equivalent/ml i.e. two-thirds of the highest value seen. This secondary peak followed administration at 1.5 h after dosing, of a 60 ml volume of water to compensate the animal for the fluid loss as a result of the relatively high volume of blood taken up to this time. Since the rate of gastric emptying is dependent on the volume of gastric contents (Hunt and McDonald, 1954), it is possible that the sudden increase in this volume would have stimulated rapid emptying of any residual drug in the stomach and thus promoted more rapid absorption of this material than would normally have taken place. Subsequently, plasma concentrations again declined rapidly such that they were in the range 0.1-0.3 μg equivalent/ml by 3 h after dosing. The half-life of elimination of unchanged drug from plasma in one monkey was 0.7 h. In the other two animals, the presence of the secondary peak made it difficult to determine meaningful elimination half-lives. However, with the exception of this irregularity, the general shape of the time-course was similar to that previously determined for the toluene-soluble, basic fraction of radioactivity.

The time-course of radioactivity in whole blood of male and female patas monkeys following oral administration of 10 mg/kg \(^{14}\text{C}-\) tiquinamide HCl was similar to that in plasma. There was,
however, substantial association of radioactivity (27.7-56.0% (Table VII.3) with red cells at 0.5 h after dosing, although the degree of association decreased markedly during the period of rapid elimination. Thus by 7.5 h after dosing, only 7-18% of radioactivity was associated with red cells.

Man: Following oral administration to two male volunteers of capsules containing 20 mg $^{14}C$-tiquinamide HCl, the highest concentration of total radioactivity, 0.4 µg equivalent $^{14}C$-tiquinamide HCl/ml occurred at 1 h in subject DM and 2 h in subject ML (Fig. VII.8). Thereafter the concentrations declined at first relatively rapidly to 0.2 µg equivalent/ml by 12 h after dosing and then more slowly to 0.1 µg equivalent/ml by 48 h. After the 50 mg dose, the maximum concentration, 1.5 µg equivalent/ml in subject DM and 1.1 µg equivalent/ml in subject ML occurred at respectively 1 and 3 h after drug administration. Hereafter, the concentration of radioactivity in both subjects was reduced to 0.6 µg equivalent/ml at 12 h and 0.3 µg equivalent/ml at 96 h after dosing. Areas under the plasma concentration-time curve were proportional to the dose (Table VII.4), indicating an absence of dose-dependent kinetics. The decline of total radioactivity in plasma appeared to take place bi-exponentially. In a first, more rapid, phase of elimination, lasting for about the first 12 h, 68.6 ± 0.6% of the dose was eliminated with a half-life of 3.7 ± 0.5 h. The slower phase of elimination was more poorly defined. Estimates of its half-life after the 50 mg dose were of the order of 140 h (Table VII.5).

The toluene-soluble, basic fraction represented approximately 60% of total plasma radioactivity at 0.5 h. Maximum values following the 20 mg dose were 0.2 µg/ml, at 0.5 h after dosing, and following the 50 mg dose were 0.4 µg/ml in subject ML and 0.5 µg/ml in subject DM, occurring at 1 h after drug administration (Fig. VII.9). This toluene-soluble fraction declined monoexponentially with a half-life of 2 h (Table VII.5). As observed for total radioactivity, the areas under the plasma concentration-time curves were proportional to the dose (Table VII.4). The only difference in the kinetics of the two doses was the slightly later time of peak concentration after the 50 mg dose.

(b) Plasma protein binding

In Fig. VII.10 are presented data describing the binding of tiquinamide to rat, monkey and human plasma over a concentration
range of 0.1-5.0 µg/ml, which spanned the range of concentrations observed in plasma of these species in vivo.

In the rat, tiquinamide was bound to plasma proteins to the extent of 50-60% throughout this concentration range. In the monkey, the degree of binding was somewhat higher at low plasma concentrations e.g. 74% of 0.1 µg/ml, but at concentrations above 0.5 µg/ml was of a similar order, approximately 50%. In man, the degree of protein binding was in the range 52-66% throughout the concentration range.

(c) Characterization of drug-related products in 25 h rat plasma

Plasma collected from rats 25 h after administration of 10 mg/kg [14C]-tiquinamide HCl apparently contained a single component with a low Rf value (0.11 relative to 5,6,7,8-tetrahydroquinoline-3-carboxylic acid). This material did not correspond to any of the major components in 0-25 h urine (Fig. VII.11). It had different chromatographic properties from tiquinamide and from the reference compounds Wy-24146 (8-cyano-5,6,7,8-tetrahydro-3-methylquinoline), Wy-24117 (5,6,7,8-tetrahydro-3-methylquinoline-8-carboxamide) and 5,6,7,8-tetrahydroquinoline-3-carboxylic acid. Only in the polar solvent systems 12 and 15 did it move very far away from the origin (Table VII.6).

Treatment with molluscan β-glucuronidase/sulphatase did not affect its chromatographic behaviour.

The u.v. spectrum of the material isolated from 25 h plasma was poorly defined. However, there was evidence of a peak at 267-270 nm, approximating to the characteristic wavelengths of maximum absorption of the pyridine portion of the 5,6,7,8-tetrahydro-3-methylquinoline ring system.

(d) Partition coefficients of tiquinamide and related compounds between toluene and pH 7.4 phosphate buffer

Results of partition coefficient determinations (Table VII.7) on tiquinamide and several compounds serving as models for metabolites revealed that the unchanged drug and its nitrile derivative, as well as 5,6,7,8-tetrahydro-3-methylquinoline, were all readily extracted into toluene. The hydroxylated derivative, 5-hydroxy-5,6,7,8-tetrahydro-3-methylquinoline was much less readily extracted. As expected, the acidic derivative 5,6,7,8-tetrahydroquinoline-3-carboxylic acid remained almost entirely in the aqueous phase at pH 7.4.
DISCUSSION

The generally rapid achievement of maximum plasma concentrations of unchanged tiquinamide and/or toluene-soluble radioactivity in the species studied implied that tiquinamide was normally quickly absorbed. In view of the absence of extensive gastric absorption in earlier experiments (Chapter III p. 60), it was probable that the rate of gastric emptying was the most important determinant of the absorption rate and that slow or delayed gastric emptying accounted for the slower absorption in some monkeys. Once in the small intestine, tiquinamide (pKₐ 5.2) would have been completely unionized and, being relatively lipophilic would, on the basis of the pH-partition hypothesis, have been expected to be rapidly absorbed (Chapter I p.19).

The comparative pharmacokinetics in both rats and monkeys of unchanged tiquinamide and toluene-soluble radioactivity demonstrated that the kinetics of the latter fraction were a good model for those of the unchanged drug, although it contained other components, including the nitrile derivative (Chapter II. p. 44) and thus its absolute concentrations were higher than those of tiquinamide.

Whilst the absolute bioavailability of tiquinamide could not be deduced without comparable data obtained after intravenous administration, there was some evidence that bioavailability was only moderate, in that quite high concentrations of metabolites were present in plasma even by 0.5 h after dosing. Thus in rats unchanged drug represented 40% of total radioactivity in plasma at this time. In monkeys, the proportion of unchanged drug was even lower at around 15%. Although concentrations of unchanged drug in human plasma were not determined, it is possible that bioavailability in man was closer to the rat than to the monkey, since concentrations of toluene-soluble radioactivity, which contained unchanged drug, represented 60% of total radioactivity at 0.5 h after dosing, a similar proportion to that in the rat but considerably greater than that in the patas monkey, only 15-20%.

Although there was a tendency to more rapid elimination in the patas monkey than in rat or man of tiquinamide and/or toluene-soluble radioactivity, the two to four times greater rate in the monkey was relatively unremarkable, in that elimination was rapid.
in each of the species investigated. However, there was clear evidence of a species difference in the elimination of total radioactivity, which occurred with a half-life at least one-hundred-fold greater in the rat than that of the major phase in the monkey, with man occupying an intermediate position closer to the monkey than the rat.

The weak plasma-protein binding of tiquinamide was unlikely to have influenced its disposition. Martin (1965) has suggested that in this respect plasma-protein binding will have no effect unless the association constant for the drug-protein complex exceeds $1 \times 10^4$. Such a drug would be 80-90% bound at concentrations $<0.1 \text{ mM}$, compared with only 50-60% for tiquinamide.

There was no evidence of binding to red cells. Thus this process was not a significant factor in the disposition of tiquinamide.

The material present in plasma 25 h after drug administration was apparently a single component not seen as a major product in 0-25 h urine. It did, however, have a similar Rf value to an unidentified polar product in bile. Its u.v. spectrum was indicative of the pyridine portion of the tetrahydroquinoline ring system, but its chromatographic properties suggested that it was somewhat more polar than either tiquinamide-related bases or their corresponding 3-carboxylic acid derivatives. Possible polar metabolites included pyridinium compounds substituted on the nitrogen atom of the tetrahydroquinoline ring and various conjugates. There was a precedent for the formation of pyridinium compounds by methylation of the pyridine nitrogen atom in the N-methylation of nicotinic acid, which is subsequently excreted as N-methylnicotinamide (Reddi and Kodicek, 1953).

The most likely conjugates were those of the basic metabolites. As discussed previously (Chapter V p.122), the 3-carboxylic acids and resulting conjugates would have been rapidly excreted in urine.

Glucuronide conjugates were excluded because the chromatographic behaviour of the compound was not altered by treatment with molluscan $\beta$-glucuronidase/sulphatase. However, a sulphate conjugate remained a possibility because the sulphatase component of the enzyme preparation was an aryl sulphatase which may not have hydrolysed sulphates of alcoholic hydroxyl groups such as present in 5-hydroxylated or 3-hydroxymethyl metabolites of tiquinamide. Alternative
possibilities included conjugates of the tetrahydroquinoline nitrogen atom with a sugar. An N-riboside has been identified for 2-hydroxy-nicotinic acid (Schwartz et al., 1973) and a conjugate with an unidentified pentose, probably ribose, for pyrazole (Clay, Watkins and Murphy, 1977). Similarly, a N-glucoside of 3-(4-pyrimidinyl)-5-(4-pyridyl)-1,2,4-triazole is known (Duggan et al., 1974). However, such a conjugate of a tiquinamide metabolite, by analogy, with these compounds, would only be formed after an initial 2-hydroxylation, allowing the subsequent possibility of enol-keto tautomerism so that the tetrahydroquinoline nitrogen acquired partially the character of a secondary nitrogen atom. Since there was no evidence for 2-hydroxylated metabolites of tiquinamides this was perhaps the least likely possibility.

Whatever the identity of this metabolite, in view of its polar nature it seemed unlikely that it was, itself, poorly eliminated. However, it could have represented the product of further metabolism of a compound in the basic metabolite fraction, the disposition of which controlled the overall rate of elimination.

Considerations on the consequences for elimination of tiquinamide metabolites

The rates at which drugs and their metabolites are eliminated from the body are influenced predominantly by the ease with which they are excreted in urine and bile. They are also affected by the extent to which the compounds are taken up into tissues and by the affinity of any binding to tissue constituents such as proteins, nucleic acids or pigments. Recycling of compounds by gastric secretion and intestinal reabsorption and by entero-hepatic circulation tend to slow elimination. As discussed previously (Chapter I, p. 20), these processes vary in their significance from compound to compound as a function of the physico-chemical and structural properties of the molecule.

As discussed in Chapter I (p. 20), lipophilic bases of low \( pK_a \) which are largely unionized at physiological pH's have a tendency to be readily taken up into tissues and to be poorly excreted in urine, the latter as a result of extensive renal reabsorption. In contrast, polar compounds in the ionized state are rapidly excreted in urine without significant tissue uptake because they are relatively water-soluble and will not easily traverse lipoidal membranes.
The identified metabolites of tiquinamide fell into two groups, one basic and more lipophilic, the other acidic and polar. The ease with which the various metabolites were excreted in urine would thus have been largely influenced by these properties.

The first group of basic metabolites comprised principally 5-hydroxy, 8-cyano-5,6,7,8-tetrahydro-3-methylquinoline (rat RT5B and monkey MT4) and 8-cyano-5,6,7,8-tetrahydro-3-hydroxymethylquinoline (rat RT5A), but also contained small amounts of toluene-soluble metabolites which included unchanged tiquinamide and its nitrile derivative. These compounds shared the pKa of tetrahydroquinoline nitrogen i.e. 5.2 and therefore would have been extensively unionized at pH of plasma and urine. The lipophilicity of tiquinamide was of a similar order to other weak bases such as aniline (pKa 5.0) and aminopyrine (pKa 5.0) which are known to readily traverse biological membranes (Mayer, Maickel and Brodie, 1959; Brodie, Kurz and Schanker, 1960). Thus partition coefficients for the drug between heptane, benzene or chloroform and pH 7.4 buffer were respectively 0.17, 5.5 and 17.3 compared with published values of 0.55, 0.90 and 17 for aniline and 0.15, 0.40 and 73 for aminopyrine. Similar partition coefficients for the metabolites are not available. However, results of an experiment to investigate the effects of various functional groups on the partition coefficient of 5,6,7,8-tetrahydro-3-methylquinoline between pH 7.0-0.1M phosphate buffer and toluene showed that 8-nitrile substitution increased the lipophilicity, partition coefficients of respectively 8.6 and 16.9 being recorded, whereas the thioamide was apparently somewhat less lipophilic, with a corresponding partition coefficient of 5.8. 5-Hydroxylation apparently made this molecule considerably less lipophilic, reducing the partition coefficient to 0.56. However, comparisons with the data of Mayer, Maickel and Brodie (1959) using the comparable solvent benzene suggested that the lipophilicity of the metabolite 5-hydroxy, 8-cyano-5,6,7,8-tetrahydro-3-methylquinoline would still have been sufficiently high to permeate biological membranes fairly rapidly, especially given that the presence of the 8-nitrile group would have increased the lipophilicity compared to the 5-hydroxylated base. For example, in their experiments amidopyrine, with a partition coefficient of only 0.40 between benzene and pH 7.4 buffer, achieved a ratio of 1 between plasma and cerebrospinal...
fluid within 10 min. As a consequence of the tendency of the basic metabolites of tiquinamide to be unionized in plasma and urine and as a result of their relatively high lipophilicity it is possible that they would have been subject to a significant degree of renal reabsorption as well as to extensive uptake into tissues. Thus they would not have been expected to be rapidly excreted in urine.

The second group, the acidic metabolites, contained two types of compound. Firstly, there were the 3-carboxylic acids 8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid (rat RT4, monkey MT7, human HT4) and 8-cyano-quinoline-3-carboxylic acid (rat RT3 and human HT3), as well, possibly, as some thioamide analogues of these. Secondly, there were the polar conjugated metabolites (rat RT1, monkey MT1 and human HT1) which comprised wholly or partly the glucuronide conjugate of 5-hydroxy-8-cyano-5,6,7,8-tetrahydro-3-methylquinoline. In contrast to the basic metabolites all these acidic compounds would have been extensively ionized both in plasma and urine. By analogy with nicotinic acid, the 3-carboxylic acids would have possessed carboxyl groups of pKa around 2.0 (Wade, 1977) and hence would have been virtually completely ionized in plasma and urine. Furthermore, the partition coefficient of 5,6,7,8-tetrahydroquinoline-3-carboxylic acid into toluene at pH 7.4 was very low (0.04), suggesting that acids related to this structure would permeate lipoidal membranes very poorly. These properties would have promoted rapid urinary elimination as well as predisposing against uptake into tissues. Support for this view comes from the literature concerning the elimination kinetics of related carboxylic acids. Thus nicotinic acid had a half-life of 1 h in rats (Fumagalli, 1971). Similarly, following oral administration of 5-chloropicolinic acid to rats, the compound was eliminated from plasma with a half-life of 1.1 h (Ramsey et al., 1974).

5-Methyl-pyrazole-3-carboxylic acid had half-lives of 1.0 h, 1.8 h and 1.9 h in rat, dog and man respectively (Smith, Wagner and Gerritsen, 1967). Similarly, glucuronide conjugates are usually very water-soluble molecules of pKa 3-4, fully ionized at urinary pH, and as such do not readily cross biological membranes but are rapidly excreted in urine (Smith and Williams, 1966). There is no reason to suppose that the glucuronide metabolite of tiquinamide would not have been similarly rapidly eliminated in urine.
It follows from the above considerations that the proportions of basic and acidic metabolites formed in the different species may have been a major contributing factor in determining how rapidly eliminated was the total metabolite fraction. In the rat, it was true that the majority of the identified metabolites excreted during the first 24 h were acidic compounds. However, it must be remembered that only 40% of the dose was excreted in rat urine in this period (Chapter IV. p. 78). Since the identified acidic compounds would have been rapidly eliminated, it follows that the 28% of the dose excreted as acidic metabolites comprising 3-carboxylic acids and glucuronides in the first 24 h probably represented the sum total of these compounds formed in the rat. This conclusion is confirmed by the absence of the known 3-carboxylic acids and of the known glucuronide conjugate in the 25 h plasma. By comparison, at least 60% of the dose in the patas monkey and 40-60% in man was excreted either as 3-carboxylic acids or as polar conjugates during the first 24 h. More extensive formation in primates than in rats of 3-carboxylic acids and more complete conjugation of 5-hydroxy, 8-cyano-5,6,7,8-tetrahydro-3-methylquinoline would have contributed to this difference. In contrast, the excretion of free hydroxylated bases in the rat (5% of dose) raised the possibility that, as a result of renal reabsorption of these compounds, greater amounts had been retained in the body.

The existence of conjugated metabolites added the further possibility that entero-hepatic circulation following biliary secretion of these compounds could have contributed to the slow elimination in the rat. However, in view of the slow biliary excretion observed, representing only 5-13% of the dose in 6 h (Chapter IV. p. 84), it was unlikely that this process made a major contribution.

The high polarity of the metabolite found in 25 h plasma would have predisposed it to rapid excretion. It therefore seems unlikely that the formation of this product was itself responsible for the slow elimination. More probably, it was the rate of formation of this product which determined the rate of elimination.
Fig. VII.1: Total radioactivity in rat plasma following oral administration of \( ^{14} \text{C} \) Wy 24081 HCl at 10 mg/kg bodyweight

Total radioactivity in rat plasma following oral administration of \( ^{14} \text{C} \) Wy 24081 HCl at 10 mg/kg bodyweight
Fig.VII.2:
Toluene extractable radioactivity in male rat plasma following oral administration of $^{14}$C Wy 24081 HCl at 10 mg/kg bodyweight

Toluene extractable radioactivity in female rat plasma following oral administration of $^{14}$C Wy 24081 HCl at 10 mg/kg bodyweight
After oral administration of 10 mg/kg $^{14}C$-tiqunamide.HCl,

F1G. V113: Chromatography of toluene-soluble radioactivity in rat plasma at 1-2 h.

Toluene/ethanol (4:1)  Dichloromethane/acetone (5:4)  Chloroform/methanol (95:5)

$B = MY 24146 (6-Cyano-5',7',8'-tertaryclo-3-methylquinoline)$

$A = Tiqunamide$
Fig. VII.: Unchanged tiqunamide in plasma of female rats following 10mg/kg oral administration of 14C-tiqunamide HCl.

Each result represents a single determination in pooled plasma from 4 animals.

Plasma concentration (μg. equiv. 14C-tiqunamide HCl/ml plasma)

Time after dosing (h)
Fig. VII.5: Total and toluene-soluble radioactivity in plasma of patas monkeys administered 10 mg/kg \(^{14}\text{C}-\text{tiquinamide}.\text{HCl}\) orally.
Thin-layer chromatography of toluene-soluble radioactivity in monkey plasma at 1h. after oral administration of \( ^{14}C \)-tiquinamide \( \text{HCl} \) at 10 mg/kg. 

Radioactivity (cpm)

(a) Dichloromethane/acetone (5:4)
(b) Toluene/ethanol (8:2)

Distance from origin (cm)
Fig. VII.7: Unchanged tiquinamide in plasma of patas monkeys administered 10 mg/kg $^{14}$C-tiquinamide HCl.
Fig. VII.8: Total radioactivity in plasma of two volunteers following oral administration by capsule of $^{14}$C Wy 24081 HCl.

- ML : 20 mg
- DM : 50 mg

Total radioactivity (μg equivs. $^{14}$C Wy 24081 HCl/ml plasma) vs. Time after dosing (h)
Fig. VII.9: Toluene-soluble radioactivity in plasma of two male volunteers following oral administration of 20 or 50 mg doses of \(^{14}\text{C\textDash{}tiquinamide HCl}^\text{.}

Toluene-soluble radioactivity (\text{\textmu{g. equiv.} \(^{14}\text{C\textDash{}tiquinamide HCl/ml})}

\begin{align*}
\text{ML} & \text{ 20mg} \\
\text{DM} & \text{ 50mg}
\end{align*}

Time after dosing (h)
Fig.VII.10: Protein binding of $^{14}$C-tiquinamide in plasma of rat, patas monkey and man.

Initial concentration in dialysis cell ($\mu$g $^{14}$C-tiquinamide HCl/ml phosphate buffer)
Comparative chromatography of radioactivity in 25h. plasma with that in 0-24h. urine of female rats administered 10mg/kg $^{14}$C-tiquinamide HCl.
### 10 mg/kg oral administration of 14C-triquinamidate HCl to rats

**Table III.1: Half-lives of toluene-soluble and toal radiactivity in plasma following**

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>41.3</td>
<td>84.7</td>
</tr>
<tr>
<td></td>
<td>5 + 0 80</td>
<td>108</td>
</tr>
<tr>
<td>5 + 0 7</td>
<td>100</td>
<td>7</td>
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<td>5 + 0 2</td>
<td>88.5</td>
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<td>5</td>
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<td>(h)</td>
<td>Animal</td>
<td>Animal</td>
</tr>
<tr>
<td>MALE</td>
<td>FEMALE</td>
<td>FEMALE</td>
</tr>
</tbody>
</table>

(a) Toluene-soluble radiactivity

(b) Total radiactivity
oral administration of 10 mg/kg 14C-tiquinamide HCl to patas monkeys

of total radioactivity eliminated in fast (f) and slow (s) phases following.

Table VII.2: Half-lives of toluene-soluble and total radioactivity in plasma and percentages

<table>
<thead>
<tr>
<th></th>
<th>4.2</th>
<th>39.3</th>
<th>95.8</th>
<th>69</th>
<th>0.69</th>
<th>0.295</th>
<th>0.57</th>
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<tr>
<td>5.8</td>
<td>55.9</td>
<td>94.2</td>
<td>0.71</td>
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<td>294</td>
<td>0.82</td>
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<td>6.3</td>
<td>28.8</td>
<td>93.7</td>
<td>0.67</td>
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<td>297</td>
<td>0.82</td>
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<tr>
<td>4.7</td>
<td>43.9</td>
<td>93.5</td>
<td>0.75</td>
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<td>296</td>
<td>0.76</td>
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<th>Phase</th>
<th>% Elimin.</th>
<th>% Elimin.</th>
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<th>(h)</th>
<th>No</th>
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<tr>
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<td></td>
<td>Total Radioactivity</td>
<td>Toluene-soluble Radioactivity</td>
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</table>

180
<table>
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<tr>
<th>Time after dosing (h)</th>
<th>M294</th>
<th>M295</th>
<th>F296</th>
<th>F297</th>
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<tr>
<td>0.5</td>
<td>27.7</td>
<td>28.4</td>
<td>31.7</td>
<td>56.0</td>
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<tr>
<td>1.0</td>
<td>21.4</td>
<td>22.0</td>
<td>29.0</td>
<td>17.3</td>
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<tr>
<td>2.0</td>
<td>59.2</td>
<td>30.5</td>
<td>37.6</td>
<td>26.8</td>
</tr>
<tr>
<td>3.25</td>
<td>22.9</td>
<td>NS</td>
<td>17.5</td>
<td>15.5</td>
</tr>
<tr>
<td>4.50</td>
<td>21.0</td>
<td>12.4</td>
<td>19.0</td>
<td>31.9</td>
</tr>
<tr>
<td>5.50</td>
<td>19.3</td>
<td>6.5</td>
<td>6.3</td>
<td>11.3</td>
</tr>
<tr>
<td>6.50</td>
<td>10.6</td>
<td>NS</td>
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<td>14.3</td>
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<tr>
<td>7.50</td>
<td>6.8</td>
<td>ND</td>
<td>14.4</td>
<td>17.5</td>
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<tr>
<td>11.75</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>6.7</td>
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<tr>
<td>24.0</td>
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<td>11.3</td>
<td>5.2</td>
<td>11.6</td>
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<td>11.2</td>
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<td>2.2</td>
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<tr>
<td>48.0</td>
<td>8.3</td>
<td>ND</td>
<td>2.2</td>
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</table>

NS = No sample  
ND = No detectable association  
Results are expressed as percentage total radioactivity in whole blood which was associated with red cells

Table VII.3: Association of radioactivity with red cells 0-48 h following 10 mg/kg oral administration of $^{14}$C-Wy 24061 HCl to patas monkeys
Table VII.4: Areas under plasma-concentration-time curves following oral administration by capsule of 20 or 50 mg 14C-triquinamide HCl to two male volunteers

<table>
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<th>Dose (mg)</th>
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<th>2.1</th>
<th>7.5</th>
<th>0.75</th>
<th>0.029</th>
<th>0.035</th>
<th>0.38</th>
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</thead>
<tbody>
<tr>
<td>Area curves expressed as Ig min ml⁻¹</td>
<td>0.56</td>
<td>0.38</td>
<td>0.41</td>
<td>0.042</td>
<td>0.036</td>
<td>0.029</td>
<td>0.035</td>
<td>0.38</td>
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</table>

* Area under curve is expressed as Ig min ml⁻¹
** Area dose is expressed as Ig ml⁻¹

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<tr>
<th>Subject</th>
<th>DM</th>
<th>ML</th>
<th>DM</th>
<th>ML</th>
<th>ML</th>
<th>DM</th>
<th>ML</th>
<th>ML</th>
<th>Toluene-soluble radioactivity</th>
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<tr>
<td>20 mg</td>
<td>50 mg</td>
<td>20 mg</td>
<td>50 mg</td>
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<td>50 mg</td>
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<td>50 mg</td>
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</table>
Table VII.2: Half-lives of colune-soluble and coltate radioactivity in plasma and percentage of

<table>
<thead>
<tr>
<th></th>
<th>0.6</th>
<th>1.7</th>
<th>2.1</th>
<th>5.5</th>
<th>7.6</th>
<th>Mean (h)</th>
<th>SEM</th>
<th>+</th>
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<tr>
<td></td>
<td>31.4</td>
<td>139.6</td>
<td>68.6</td>
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<tr>
<td></td>
<td>120</td>
<td>66.4</td>
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<td>110</td>
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<td></td>
<td>125</td>
<td>69.5</td>
<td>3.8</td>
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<tr>
<td></td>
<td>200</td>
<td>68.9</td>
<td>4.9</td>
<td>1.1</td>
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Subject (h) 

Elimination

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<th>2.5</th>
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<th>Mean (h)</th>
<th>SEM</th>
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<td></td>
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<td>2.0</td>
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<td>69.0</td>
<td>5.0</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>2.0</td>
<td>69.0</td>
<td>5.0</td>
<td></td>
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</table>

Subject (h) 

Total Radioactivity (b)
<table>
<thead>
<tr>
<th></th>
<th>Solvent 6</th>
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<th>Solvent 15</th>
<th>Solvent 18</th>
<th>Solvent 12</th>
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</thead>
<tbody>
<tr>
<td>Tiquinamide</td>
<td>0.37</td>
<td>0.31</td>
<td>0.57</td>
<td>0.56</td>
<td>0.77</td>
</tr>
<tr>
<td>Wy-24146</td>
<td>0.49</td>
<td>0.57</td>
<td>0.78</td>
<td>0.73</td>
<td>0.83</td>
</tr>
<tr>
<td>Wy-24117</td>
<td>0.21</td>
<td>0.11</td>
<td>0.44</td>
<td>0.49</td>
<td>0.65</td>
</tr>
<tr>
<td>THQ COOH</td>
<td>0.10</td>
<td>0.01</td>
<td>0.53</td>
<td>0.01</td>
<td>0.15</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.02</td>
<td>0.07</td>
<td>0.59</td>
<td>0.00</td>
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<tr>
<td>metabolite</td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Wy-24146 = 8-cyano-5,6,7,8-tetrahydro-3-methylquinoline  
Wy-24117 = 5,6,7,8-tetrahydro-3-methylquinoline-8-carboxamide

Table VII.6: Rf values of the radioactive component in 0-25 h plasma of rats administered 10 mg/kg ¹⁴C-tiquinamide HCl, compared with those of tiquinamide and related compounds in a variety of solvent systems.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Partition Coefficient</th>
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<tbody>
<tr>
<td>Tiquinamide</td>
<td>5.8</td>
</tr>
<tr>
<td>Wy-24146</td>
<td>16.9</td>
</tr>
<tr>
<td>3-methyl,5,6,7,8-tetrahydro-3-methylquinoline</td>
<td>8.6</td>
</tr>
<tr>
<td>5-hydroxy,5,6,7,8-tetrahydro-3-methylquinoline</td>
<td>0.56</td>
</tr>
<tr>
<td>5,6,7,8-tetrahydro-quinoline</td>
<td>0.04</td>
</tr>
<tr>
<td>3-carboxylic acid</td>
<td></td>
</tr>
</tbody>
</table>

Table VII.7: Partition coefficients (Toluene/pH 7.4 0.1M potassium phosphate buffer) of tiquinamide and some related compounds
CHAPTER VIII: TISSUE DISTRIBUTION OF TIQUINAMIDE AND ITS METABOLITES IN RAT AND PATAS MONKEY
SUMMARY

Studies of the tissue distribution of tiquinamide-related products in the rat and patas monkey revealed that the major site of accumulation was in the stomach and its contents, and, to a lesser extent, in other regions of the gastrointestinal tract. This observation was a reflection of gastric secretion. Elimination from the stomach took place slowly, at a rate identical to that from plasma, but there was no evidence that the stomach constituted a depot site limiting the overall rate of elimination from the body. However, gastric re-cycling may have contributed to the slow elimination.

In addition, there was evidence of localization in the nasal turbinal region in the rat. Otherwise, there was generally even distribution in a wide range of tissues. There was no evidence of localization in the thyroid, a potential accumulation site for thioamides. Nor was there very marked uptake into the lung, a common site of localization of basic drugs.

The tissue distribution of tiquinamide-related products is discussed with reference to the nature of the major identified metabolites and their anticipated physico-chemical properties. The gastric re-cycling and re-distribution from the tissues of basic metabolites are implicated as factors in the slow elimination from the rat.
INTRODUCTION

The tissue distribution of drugs and their metabolites may be an important factor both with respect to their general disposition and their potential toxicity.

The binding of drugs to tissues can severely limit the rates of their elimination from the body. Thus, for example, binding of aniline mustard to nucleic acids in the kidney resulted in a half-life of 1-2 days for the bound material (Warwick, 1969). Similarly, the poor elimination by the rat of the sulphonamide drug sulphadimethoxine, only 16% in the first 24 h, was attributed to strong tissue binding (Adamson et al., 1970b).

Furthermore the accumulation of high concentrations of compounds at specific sites can result in toxic lesions. Thus the retinopathies caused by phenothiazines and chloroquine may be related to the high concentrations achieved in the uveal tracts of pigmented animals as a result of binding to melanin (Potts, 1962; Bernstein et al., 1963).

Therefore it was of interest to examine the tissue distribution of tiquinamide and its metabolites both as part of the safety evaluation of the drug and in order to assess its significance in relation to the species difference in elimination.
EXPERIMENTAL
(a) Tissue excision studies
Rat: Four male (410-470 g) and four female rats (280-320 g) were fasted overnight before dosing and administered a 10 mg/kg dose of \(^{14}\text C\)-tiquinamide orally by gastric intubation. The animals were killed by ether anaesthesia at 12 h after dosing, the previously determined time of maximum plasma concentrations of total radioactivity. Small portions (50-100 mg) of a comprehensive range of tissues (Fig. VIII.1) were taken in triplicate for digestion by the method of Mahin and Lofberg (Chapter II. p. 43). Total radioactivity was then determined by liquid scintillation counting.

Major organs (heart, brain, lung, liver and kidney) were removed for assay of toluene-soluble radioactivity. Each organ was homogenized in 9 ml distilled water. Duplicate samples (0.5 ml) were basified with pH 9.2 1M bicarbonate buffer (0.5 ml) and subjected to in-vial extraction with toluene-scintillant (Chapter II. p. 44) followed by liquid scintillation counting.

Patas monkey: One male and one female patas monkey, weighing 2.3 and 2.0 kg respectively received a 10 mg/kg oral dose of \(^{14}\text C\)-tiquinamide HCl. At 0.5 h after dosing, when plasma radioactivity was maximal, animals were killed by an intravenous injection of pentothal (250 mg/kg). Samples (80-100 mg) of a range of tissues (Fig. VIII.2) were subjected in triplicate to acid digestion by the method of Mahin and Lofberg. Total radioactivity was determined by liquid scintillation counting.

(b) Whole body autoradiography
Whole body autoradiography was performed by Dr. P. Nicholls and Dr. D. K. Luscombe (Department of Pharmacology, University of Wales Institute of Science and Technology, Cardiff). One male and one female rat were killed under chloroform anaesthesia at each of the following times after administration of a 10 mg/kg oral dose of \(^{14}\text C\)-tiquinamide HCl: 0.5, 12 and 24 h. Cutting of sections and subsequent preparation of autoradiography was described in Chapter II. (p. 47).

(c) The accumulation of drug-related products in rat stomach wall and contents
Groups of five male rats, weighing approximately 200 g each, were fasted overnight before dosing and administered 30 mg/kg
C-tiquinamide HCl by subcutaneous injection as a solution in saline (2.5 ml/kg). Two hours before each rat was due to be killed, its stomach was ligated under halothane anaesthesia, according to the method of Shay, Sun and Gruenstein (1954). Animals were sewn-up and allowed to recover. Gastric juice was collected in the ligated stomach for two hours. At the end of this period, rats were killed by ether anaesthesia, bled from the inferior venae cavae, stomachs removed and contents ejected. Times of sample collection were at various times after dosing as detailed in Fig. VIII.6.

Toluene-soluble and total radioactivity in plasma were measured by the previously described method (Chapter II, p. 44). Gastric contents were centrifuged to remove mucus and solid debris and toluene-soluble and total radioactivity determined similarly. Each stomach was washed in saline and divided longitudinally into two portions. One half was dissolved in SHT solubilizer (NaOH, 80 g; methanol, 300 ml; Triton-X-405, 100 ml; in one litre, made up with water) according to the method of Dent and Johnson (1974) as described previously (Chapter II, p. 44). The other half was frozen in liquid nitrogen, powdered with pestle and mortar and homogenized, with the aid of a Potter homogenizer, in pH 9.3 bicarbonate buffer to yield a 10% homogenate. Aliquots (0.5 ml) of the homogenate were subjected to in-vial extraction with toluene-based scintillant (10 ml) and total and toluene-soluble radioactivity determined by liquid scintillation counting as described previously (Chapter II, p. 45).

(d) The elimination of drug-related products from rat plasma and stomach wall over a 0-21 day period

Female rats, weighing 180-200 g, fasted overnight before dosing, received a 10 mg/kg oral dose of 14C-tiquinamide HCl. At various times up to 21 days after dosing, as detailed in Fig. VIII.7, rats were killed by ether anaesthesia and bled from the inferior venae cavae. Stomachs were removed and washed free of contents. Total radioactivity in plasma was assayed by liquid scintillation counting. Stomachs were digested in SHT solubilizer and total radioactivity determined by liquid scintillation counting.
RESULTS

(a) Tissue excision studies

Rat: Tissue excision studies in rats carried out at 12 h after administration of a 10 mg/kg oral dose of $^{14}$C-tiquinamide HCl revealed that the outstanding feature of the tissue distribution was the accumulation of drug or metabolites by the stomach and its contents. Stomach wall contained about four and six times the plasma concentration in male and female rats respectively (Fig. VIII.1). Stomach contents contained approximately seven times the plasma concentration in males and fifteen times in females. Although the concentrations of radioactivity in stomach and its contents were very high, the total material contained in this organ represented only 3% and 7% of the dose respectively in males and females.

In the skin/fur of male rats but not females, radioactivity was present at approximately twice the concentration in plasma.

In no other tissue sampled did the total radioactivity concentration exceed that in plasma. There was, however, widespread even distribution. In most tissues, radioactivity concentrations were in the range 20-60% of those in plasma. With the exception of the greater accumulation in the stomachs of female and of male animals, there was apparently no other difference in tissue distribution between male and female rats.

The mean tissue concentration (± SEM) calculated from values for all tissues except gastrointestinal and excreting organs were 4.3 ± 0.3 μg/g and 3.6 ± 0.4 μg/g in males and females respectively. Since a 10 mg/kg dose distributed evenly throughout the animal would have resulted in concentrations of 10 μg/g in all tissues, the implication was that approximately 30-40% of the dose was generally distributed throughout the somatic tissues of rats at 12 h after dosing. By comparison, only about 4% was in the plasma, assuming a plasma volume for the rat of 41.5 ml/kg (Spector, 1956).

Toluene-soluble radioactivity represented a low proportion (< 14%) of the total radioactivity in any of the major organs investigated (Table VIII.1).

Patas monkey: Tissue excision studies in monkeys, carried out at 0.5 h after administration of a 10 mg/kg oral dose, revealed high concentrations of radioactivity in stomach wall and kidney,
representing respectively four times and three to four times plasma concentration (Fig. VIII.2). Slightly higher concentrations of radioactivity were achieved in liver than in plasma. No other tissue extensively accumulated radioactivity. As in the rat, most tissues contained 20-60% of the concentration of radioactivity in plasma.

The mean tissue concentration (± SEM) calculated from all tissues except gastrointestinal and excreting organs were 4.8 ± 0.5 µg/g for the male monkey and 6.5 ± 0.5 µg/g for the female. Thus by an analogous argument to that used for the rat, the proportion of the dose distributed throughout the somatic tissues of the monkey at 0.5 h after dosing was of the order of 50-60%. By comparison, approximately 7% of the dose was to be found in plasma, based on the assumption of a plasma volume in the monkey of 45 ml/kg (Spector, 1956).

Bile collected from the gall-bladder contained concentrations five to six times those in plasma.

(b) Whole body autoradiography

Data gathered from whole body autoradiographic studies confirmed and extended the results of the tissue excision studies.

Whole body autoradiography at 0.5 h after dosing showed very high concentrations of radioactivity in the stomach and small intestine. High concentrations were also observed in the liver, kidney, bladder and nasal turbinal region. (Figs. VIII.3, VIII.4). There was general distribution of radioactivity throughout other tissues but no marked accumulation.

At 12 h after dosing, whole body autoradiography confirmed the results of the tissue excision studies, demonstrating the presence of high concentrations of radioactivity only in the stomach and its contents, in the large intestine and in faecal pellets in the rectum. Again, distribution in other tissues was widespread, but at much lower concentration than in the gastro-intestinal tract.

At 24 h after drug administration, the distribution pattern was very similar to that at 12 h, with high concentrations of radioactivity being evident only in the stomach and large intestine. Enlargement of autoradiographs of the stomach revealed that radioactivity had begun to disappear from the gastric lumen by this time, but was concentrated in the gastric mucosa (Fig. VIII.5).
(c) The accumulation of drug-related products in rat stomach wall and contents

The subcutaneous administration of a single 30 mg/kg dose of $^{14}$C-tiquinamide HC1 to male rats resulted in extensive gastric secretion of radioactivity and in uptake by the stomach wall (Fig. VIII.6).

The toluene-soluble, basic fraction containing unchanged tiquinamide achieved its maximum concentrations in plasma, stomach wall and gastric contents at the same time, 3 h after dosing. This observation was indicative of rapid equilibration between plasma and gastric contents. Once the peak value had been achieved, concentrations of this fraction declined rapidly.

Total radioactivity, similarly, achieved maximum concentrations in plasma and stomach wall at 3 h after dosing. Thereafter a flow of drug-related products between plasma and stomach wall was evident, since plasma concentrations began to decline, whereas those in gastric contents continued to rise until 5 h after drug administration, before they too began to fall. Concentrations in stomach wall remained constant for up to 14 h once the peak value had been achieved. There was a marked concentration gradient increasing from plasma to gastric contents, so that at the time of maximum plasma concentration ($47 \mu g$ equiv/ml), the concentration in stomach wall was 2.8 times this value and that in gastric contents was already 5.3 times as much. The maximum concentration in gastric contents, $544 \mu g$ equiv/ml at 5 h, represented 37 times that occurring simultaneously in plasma.

The much more rapid elimination of the toluene-soluble fraction than of total radioactivity meant that radioactivity accumulated in the stomach wall and secreted into gastric contents was not predominantly toluene-soluble. In stomach wall, toluene-soluble basic radioactivity, which would have included unchanged drug, represented only 30% of total radioactivity at 2 h after dosing and only 8% at 4 h. In gastric contents, this fraction represented 24% of total radioactivity at 2 h and only 7% at 4 h after dosing.

(d) The elimination of drug-related products from rat plasma and stomach wall over a 0-21 day period

Following oral doses of 10 mg/kg $^{14}$C-tiquinamide HC1 to groups of female rats, the maximum plasma total radioactivity concentration was observed between 5 h and 12 h after dosing. The highest value
of the mean concentration (± SEM) was at 5 h (11.2 ± 0.8 µg-equivalent 14C-tiquinamide HCl/ml), but a higher standard error in the 12 h value (9.3 ± 1.2 µg equivalent/ml) reflected some inter-animal variation in the time of the peak concentration (Fig. VII.7). Plasma concentration of total radioactivity subsequently declined monoexponentially with a half-life of 89 h.

The concentration of radioactivity in stomach wall achieved its maximum value, 45 µg equivalent/g at 12 h after drug administration, after which it declined monoexponentially with a half-life of 94 h (Fig. VIII.7).
DISCUSSION

A significant feature of the tissue distribution of tiquinamide and its metabolites in rats and monkeys was the extensive accumulation of radioactivity in the stomach and contents and to a lesser extent in the other regions of the gastrointestinal tract. Thus in this respect, whole body autoradiography resembled those following administration of weak bases such as nicotine (Andersson, Hansson and Schmitterlöw, 1965) and mepivicaine (Kristerson, Hoffmann and Hansson, 1965). As for these drugs, such accumulation in the stomach and its contents must have resulted primarily from gastric secretion, since absorption of unchanged drug has been shown to be complete within 0.5-1 h after dosing (Chapter VII, p. 157, 162), but high concentrations of radioactivity persisted in the stomach until 12 h and 24 h after an oral dose, when concentrations of unchanged drug in plasma were negligible. Furthermore, very high concentrations of radioactivity were evident in the stomach and contents after a subcutaneous dose of 

\[ { }^{14}C \text{-tiquinamide HCl} \text{.} \]

Gastric secretion of basic compounds into the acidic medium of the contents is a well-known phenomenon established by Shore, Brodie and Hogben (1957).

Bases are ionized at acidic pH's and unionized under more alkaline conditions. Because of the much lower pH (1-3) in the gastric contents than in plasma (pH 7.4), basic drugs of \( pKa > 4 \) will be very extensively ionized in the gastric contents, but extensively unionized in plasma unless their \( pKa \) values exceed about 8. Therefore, if such a compound is supplied to the stomach in the bloodstream there will always be a large concentration gradient of unionized drug between plasma and gastric contents and if the compound is also lipid-soluble diffusion into gastric contents will be rapid. Once in the gastric contents drug is retained there by ionic trapping until emptied via the pylorus into the small intestine. Back diffusion into plasma is not usually possible because of the generally poor lipid solubility of ionized drugs.

Other examples of basic, lipid-soluble drugs which are readily secreted into gastric contents include aniline and aminopyrine (Shore, Brodie and Hogben, 1957). These compounds were found to be so readily gastrically secreted that the ratio of their concentrations in gastric contents and plasma was limited only by the rate of blood
flow through the gastric epithelium. Tiquinamide has very similar physico-chemical properties to these bases, as discussed previously (Chapter VII. p. 163). It has also been deduced that the hydroxylated basic metabolites would have been sufficiently lipophilic to have traversed lipoidal membranes. It follows that the rapid gastric secretion of tiquinamide and its basic metabolites was to be predicted on the basis of their known physico-chemical properties.

The very even distribution of radioactivity in the majority of tissues except those of the gastrointestinal tract suggested entry by passive diffusion. The range of concentrations, 20-60% of plasma radioactivity, reflected to some degree the vascularity of the tissues concerned, in that the more highly vascular organs such as liver, kidney and lung tended to contain somewhat higher concentrations than did those less well perfused. The higher concentration of radioactivity in plasma than in tissues probably represented the containment within the plasma compartment of acidic metabolites not readily distributed into tissues. The basic, lipid soluble compounds on the other hand may have been present at higher concentrations in tissues than in plasma.

In one respect, the distribution of tiquinamide was atypical of a basic drug in that drug or metabolites were not localized in lung at concentrations in excess of those in plasma. Extensive uptake of basic drugs into the lung is quite common, as indicated in a recent review by Brown (1974). The mechanism of this uptake has been ascribed by some authors to ionic trapping as a result of the lower pH in the pulmonary extravascular space (pH 6.7) compared to that in pulmonary plasma (pH 7.4) (Effros and Chinard, 1959; Waddell, 1973). The failure of tiquinamide or its metabolites to localize in the lung was consistent with this hypothesis in that it reflected the low pKa (5.2) of this drug. The drug would have been virtually completely unionized even at pH 6.7 and thus the difference in pH between lung and plasma would not have significantly affected the distribution of the drug between them.

A further significant feature of the tissue distribution of tiquinamide was the absence of any apparent accumulation of the drug or its 14C-labelled metabolites by the thyroid. This was a
possible consequence of the thioamide character of the drug, in view of the known thyroid accumulation of a number of thioureas and thiouracils active as anti-thyroid agents e.g. thiourea (Maloof and Soodak, 1965) thiouracil (Maloof and Soodak, 1957), propylthiouracil (Marchant et al., 1971) and methimazole (Marchant and Alexander, 1972). In addition, the thioamide anti-tubercular compound ethionamide has been shown to exert an anti-thyroid effect at high doses (Moulding and Fraser, 1970). The fact that tiquinamide was found in practice not to be accumulated in this organ may be a reflection of the extent of its metabolism to desulphurized products and to acidic derivatives not readily distributed into tissues.

The evidence for above average concentrations of radioactivity in the skin/fur of male rats was not strong enough to suggest substantial secretion of drug or metabolites in sweat. The possibility of contamination of the fur with urine could not be excluded. Furthermore, in this respect the results of whole body autoradiography were unreliable since photographic artefacts can result in the appearance of accumulation of radioactivity in skin/fur.

The apparent high concentrations of radioactivity in the bile of monkeys reflected the rapid elimination occurring at the time the animals were killed. The significance of the extent of biliary excretion will be discussed more fully in Chapter IX.

Considerations on the significance of the tissue distribution of tiquinamide metabolites with respect to their slow elimination in the rat

Although very high concentrations of tiquinamide-related products were achieved in the stomach and its contents, the total amount of material present in this organ at 12 h after dosing represented only 3-7% of the dose in rats. Thus the slow elimination of tiquinamide metabolites in the rat was not the result of slow release from a depot in the stomach. However, it was evident that gastric secretion was extensive and that concentrations of drug-related products in the stomach remained many times those in plasma for a very long period. Thus it was possible that gastric re-cycling, involving re-absorption of gastrically secreted material was a contributory factor.
The total proportion of the dose present in rat plasma at 12 h after dosing was only about 4%. It was therefore unlikely that plasma-protein binding of metabolites played a significant role. It has been calculated that for a compound which is 90% bound by plasma proteins, the amount of drug retained in the plasma will be 38% of total drug in the body (Butler, 1971). Therefore the percentage binding of tiquinamide metabolites in plasma would have been considerably less than 90%. As previously discussed (Chapter VII p.163), Martin (1965) has suggested that plasma protein binding of a compound will not seriously affect its disposition unless it has an affinity constant \( > 10^4 \) under which circumstances more than 85% of total drug concentration in plasma will be in the bound form.

The largest proportion of the material remaining in the rat at 12 h after dosing, representing some 30–40% of the dose, was found to be distributed throughout the tissues. In view of the absence of evidence for either extensive plasma-protein binding or enterohepatic circulation of tiquinamide metabolites, it follows that it must have been the re-distribution and/or further metabolism of the tissue-distributed material which represented the major rate-limiting step in elimination. Possibly some binding to tissue components contributed to the slow re-distribution from tissues.
Tissue distribution of radioactivity in rats administered $10 \text{ mg/kg} \cdot ^{14}\text{C}-\text{tiquinamide.HCl}$ orally

Each result represents the mean $\pm$ SEM of determinations in 5 animals, except that for sciatic nerve, bone marrow and thyroid single determinations on pooled samples were performed.
**Fig. VIII:** Tissue distribution of radioactivity in Red Patas Monkeys 0.5 hrs. following 10 mg/kg oral administration of $^{14}$C Wy 24081 HCl.

- **♀ 296**
- **♂ 294**

Tissue radioactivity (µg equivs. $^{14}$C Wy 24081 HCl gm wet tissue)
Fig. VIII.3: Whole body autoradiography of male rats after a 10mg/kg. oral dose of $^{14}$C-tiquinamide·HCl.
Fig. VIII.4: Whole body autoradiography of female rats after a 10 mg/kg oral dose of $^{14}$C-tiquinamide.HCl.
Fig. VIII.5: **Autoradiography of $^{14}$C-Wy 24081.**

Localisation in the stomach.

**Female rats at 24 hrs.**
- Liver
- Gastric mucosa
- Spleen
- Outer wall of stomach

**Male rats at 24 hrs.**
- Liver
- Gastric mucosa
- Spleen
- Kidney
- Outer wall of stomach
Fig. VIII.6:

Toluene-soluble and total radioactivity in plasma, stomach wall and gastric contents of male rats administered 30 mg/kg $^{14}$C-tiquinamide, HCl subcutaneously.

Radioactivity (μg equiv. $^{14}$C-tiquinamide HCl/ml plasma or g stomach)

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Stomach</th>
<th>Gastric contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>10</td>
<td>0.1</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Time after dosing (h)
Fig.VIII7: Elimination of total radioactivity from plasma and stomach wall of female rats 0-21 days following oral administration of 10mg/kg $^{14}$C-tiquinamide HCl.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Female 1</th>
<th>Female 2</th>
<th>Female 3</th>
<th>Female 4</th>
<th>Male 5</th>
<th>Male 6</th>
<th>Male 7</th>
<th>Male 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.2</td>
<td>0.16</td>
<td>0.23</td>
<td>0.18</td>
<td>0.34</td>
<td>$&lt; 0.16$</td>
<td>$&lt; 0.16$</td>
<td>$&lt; 0.16$</td>
</tr>
<tr>
<td>Lung</td>
<td>$&lt; 0.33$</td>
<td>0.12</td>
<td>0.14</td>
<td>$&lt; 0.13$</td>
<td>0.55</td>
<td>$&lt; 0.20$</td>
<td>$&lt; 0.16$</td>
<td>$&lt; 0.14$</td>
</tr>
<tr>
<td>Brain</td>
<td>0.13</td>
<td>$&lt; 0.16$</td>
<td>0.16</td>
<td>$&lt; 0.14$</td>
<td>0.14</td>
<td>$&lt; 0.15$</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td>Liver</td>
<td>0.13</td>
<td>0.17</td>
<td>0.31</td>
<td>0.25</td>
<td>0.69</td>
<td>0.19</td>
<td>0.14</td>
<td>0.38</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.24</td>
<td>0.22</td>
<td>0.25</td>
<td>0.22</td>
<td>1.52</td>
<td>$&lt; 0.14$</td>
<td>0.15</td>
<td>0.39</td>
</tr>
</tbody>
</table>

All results are expressed as μg equivalents tiquinamide/g of tissue.

Table VIII.1: Toluene-soluble radioactivity present in male and female rat tissues after oral administration of $^{14}$C-tiquinamide HCl at 10 mg/kg body weight.
CHAPTER IX: EXCRETION OF Tiquinamide AND ITS METABOLITES IN RAT, PATAS MONKEY AND MAN
SUMMARY

The extent of urinary and faecal excretion and the kinetics of urinary excretion of drug-related products in rat, patas monkey and man were studied. The rate of biliary excretion of drug-related products in the anaesthetized rat was also investigated.

Results showed that radioactivity was rapidly and completely excreted in the patas monkey almost entirely in the urine. In rats, a fraction of metabolites was rapidly excreted, but the majority was slowly eliminated at a rate equivalent to that of elimination from plasma. In man, the majority of radioactivity was rapidly eliminated in urine. Faecal excretion occurred to a significant extent only in the rat. Biliary secretion in this species occurred to an extent commensurate with that of faecal excretion with no indication of entero-hepatic circulation.

The species difference in the rate of urinary excretion is discussed with respect to the species difference in metabolism.
INTRODUCTION

The main reason for investigating the rate of excretion of a potential drug substance and its metabolites is to ensure that they are relatively quickly eliminated from the body and thus to avoid the risk of residues of potentially toxic substances remaining in the tissues for long periods after termination of a course of treatment. In addition, it is necessary to know the rate of elimination of a drug in order to determine the most satisfactory frequency of dosing. Furthermore, one important criterion by which to judge whether animal species are suitable for chronic toxicity studies for evaluation of the potential safety of a given drug in man is whether it is excreted at similar rates and by the same routes in these animals as in man. The relative importance for the compound of different routes of excretion needs to be known also so that the implications for drug excretion of changes in the relevant physiological processes resulting from disease, pregnancy etc. can be assessed.

Investigations of the plasma kinetics and metabolism of tiquinamide had indicated that metabolism constituted the major route of elimination of the drug. Thus the excretion of unchanged tiquinamide was quantitatively of relatively little significance. However, the rate of excretion of its metabolites was of interest because any drug-related substance which remains in the body for a long period constitutes a potential hazard. Plasma kinetic studies (Chapter VII) had revealed that tiquinamide metabolites were very slowly eliminated in the rat, but very quickly eliminated in the patas monkey and that man showed a greater similarity to the monkey in this respect but did have a significant slow phase of elimination. Studies of the rates of excretion of drug-related substances from the body were expected to confirm the existence of such a species difference. In combination with the information already obtained about the nature of the excreted metabolites, the results of such studies might also have cast some light on the reasons for the slow elimination in the rat.
EXPERIMENTAL

(a) Excretion kinetics in rats

Urine, faeces and expired air: Four male and four female rats housed individually in all-glass metabolism cages as previously described (Chapter II p.43) each received a 10 mg/kg oral dose of \(^{14}\text{C}-\text{tiquinamide HCl}\) (8.82 \(\mu\text{Ci}/\text{mg}\)). Urine and faeces were collected at various intervals, as indicated in Fig. IX.1 over a period up to 7 days after drug administration. Total and toluene-soluble radioactivity in urine were assayed by liquid scintillation counting as previously described (Chapter II p.44). Total radioactivity in faeces was assayed by liquid scintillation counting after perchloric acid digestion (Chapter II p.44). The kinetics of urinary excretion of radioactivity were analysed by the sigma-minus method of Martin (1967), as previously described (Chapter II p.47).

In a further experiment, two female rats were individually housed in all-glass metabolism cages equipped for the collection of expired air (Chapter II p.43). \(^{14}\text{C}-\text{Tiquinamide HCl}\) was administered as above. Expired air was collected by absorption into 33\% ethanol for a period of 24 h and urine and faeces for 7 days at intervals outlined in Table IX.1. At the end of this period, the rats were killed by ether anaesthesia and residual radioactivity in carcasses assessed by liquid scintillation counting after alkaline digestion in SHT solubilizer (Chapter II p.44).

Bile: Biliary excretion was studied in three bile-duct cannulated rats maintained under halothane anaesthesia and administered \(^{14}\text{C}-\text{tiquinamide HCl}\) (2-4 \(\mu\text{Ci}/\text{mg}\)) at 10 mg/kg orally. Bile was collected over hourly intervals for periods of 6 h. Radioactivity in bile was assayed by liquid scintillation counting.

(b) Excretion kinetics in patas monkeys

Urine and faeces: Two male and two female patas monkeys were administered \(^{14}\text{C}-\text{tiquinamide HCl}\) (2.21 \(\mu\text{Ci}/\text{mg}\)) at 10 mg/kg orally. Urine and faeces were collected until 7 days after dosing at intervals specified in Fig. IX.3. Total and toluene-soluble radioactivity in urine were assayed by liquid scintillation counting. Total radioactivity in faeces was assayed by liquid scintillation counting after perchloric acid digestion (Chapter II p.44). The kinetics of urinary excretion of radioactivity were analysed by the sigma-minus method of Martin (1967) (Chapter II p.47).
(c) Excretion kinetics in man

Urine and faeces: Two male volunteers (ML and DK) received $^{14}$C-tiquinamide HCl at a dose of 20 mg (containing 5 μCi) orally by capsule. Urine and faeces were collected for 5 days at intervals as in Fig. IX.4. Seven days after the first dose, the same volunteers received a second dose of 50 mg (containing 50 μCi) in an identical fashion. Urine and faeces were again collected for 5 days at intervals as in Fig. IX.4. Total and toluene-soluble radioactivity in urine was assayed by liquid scintillation counting. Total radioactivity in faeces was assayed by liquid scintillation counting after perchloric acid digestion (Chapter II p. 44).
RESULTS

(a) Excretion kinetics in rats

Urine, faeces and expired air: After a single administration of a 10 mg/kg dose to 4 animals of each sex, tiquinamide and its metabolites were excreted primarily in the urine of rats, a mean of 57% of total radioactivity being eliminated by this route in males and 50% in females in 7 days after dosing. Faecal excretion was evident, but was less pronounced than elimination by the urinary route, representing 15% of the dose in males and 22% in females (Fig. IX.1). The total recoveries of radioactivity in urine and faeces of these animals over a 0-7 day period were lower than might have been expected, comprising 72% of the dose in both sexes. In a further experiment, in 2 additional female rats, to examine the reasons for this low recovery, the total percentages of the dose accounted for were 84.4% and 89.9% (Table IX.1). Urine and faeces contained 56-58% and 13-20% respectively i.e. similar amounts to those observed in the first experiment. Elimination of radioactivity in expired air was low (1.9% and 3.0%). When these animals were killed 7 days after drug administration, however, substantial proportions of the dose (12.4% and 10.8%) were found to be still resident in the carcasses. This observation provided an adequate explanation for the somewhat low recoveries in excreta observed previously.

There was a trend towards somewhat more extensive elimination of radioactivity in urine of male rats compared with females. However, the difference was fairly small, so that when the mean at 0-7 days for male animals (56.9 ± 2.7%; n = 4) was compared by Student's t-test with that for all females (52.1 ± 1.6%; n = 6), the difference was not significant at the 5% level (t = 1.641, p = 0.1-0.2). Conversely, faecal excretion was apparently slightly greater at 0-7 days in females than in males (20.4 ± 1.7% and 15.0 ± 1.8% respectively but again the difference was not statistically significant (t = 2.114, p = 0.05-0.1).

Negligible amounts of unchanged tiquinamide were excreted in urine. Only 2.5% of the dose was recovered from basified urine as toluene-soluble radioactivity, which would have included unchanged drug.
Analysis of the kinetics of urinary excretion of total radioactivity by the sigma-minus method of Martin (1967) required allowance for the fact that excretion was incomplete at 7 days. It could be deduced from the results of total balance studies in two female rats (Table IX.1) that 85% of the dose should ultimately be recovered from urine and faeces. The proportion of radioactivity eliminated by each route in the post-7 day period were deduced from the mean daily faecal/urinary elimination ratios (post-48 h), which were 0.5 ± 0.1 and 1.3 ± 0.1 in male and female animals respectively. The sigma-minus analysis revealed two exponential phases. In the first, more rapid phase of elimination having half-lives of about 4 h in males and 3 h in females, approximately 43% and 38% of the dose respectively were excreted (Table IX.2). In the second, slower phase of half-life 122 h and 98 h respectively, 22% and 18% of the dose were eliminated.

Bile: Excretion of radioactivity in bile following 10 mg/kg oral administration of $^{14}$C-tiquinamide to three halothane-anaesthetized, bile duct-cannulated female rats totalled 5-13% in a 6 h period (Fig. IX.2).

(b) Excretion kinetics in patas monkeys

Urine and faeces: Excretion of radioactivity by 2 male and 2 female patas monkeys, administered $^{14}$C-tiquinamide HCl by the oral route, occurred principally in the urine. The proportion excreted by this route in a period 0-7 days after drug administration amounted to 70-95% of the dose. Faecal excretion was low, representing only 3-7% of the dose (Fig. IX.3). The total recovery of radioactivity, 77-100% of the dose, represented a very satisfactory recovery from monkeys, since the unclean habits of these animals e.g. rubbing excreta into fur tend to lead to lower recoveries than would be acceptable in rats. The sex of the monkey did not significantly affect the amount of the dose excreted in urine or faeces.

Not more than 1.5-3.0% of the dose was present in urine as unchanged drug, as represented by the basic toluene-soluble fraction of radioactivity.

Urinary excretion of tiquinamide metabolites was extremely rapid. Analysis of excretion kinetics of total radioactivity in urine by the sigma-minus method of Martin (1967) revealed two
The majority of radioactivity, 68-93% of the dose, was excreted in the first very rapid phase of half-life 1 h. Only 2-3% of the dose was eliminated in the slower phase of half-life 31-50 h (Table IX.2).

(c) **Excretion kinetics in man**

**Urine and faeces**: After oral administration of ¹⁴C-tiquinamide HCl to two male volunteers, excretion of radioactivity occurred mainly in the urine. Following the 20 mg dose, 69-77% of the dose was excreted in urine in 4 days and after a 50 mg dose 66-92% (Fig. IX.4). Faecal excretion of radioactivity was low. After the 20 mg (5 μCi) dose the amounts of radioactivity detected in faeces were largely below the level of sensitivity of the assay, 2-3% of the dose in up to 4 days. After the 50 mg (50 μCi) dose, 6-8% of the radioactivity was eliminated by the faecal route in 5 days. The total recovery of radioactivity from urine and faeces was 71-100%.

Excretion of unchanged tiquinamide in urine was low. Only 2% of the administered dose was excreted in the basic, toluene-soluble fraction of urinary radioactivity.

Sigma-minus analysis could not be performed satisfactorily, because recoveries of radioactivity were not sufficiently complete nor the collection period sufficiently long to make possible the certain conclusion that the slower phase of elimination had ceased by the termination of the experiment. However, it could be safely concluded that the majority of radioactivity was excreted rapidly, since 66-73% of the dose appeared in the 0-24 h urine after the 20 mg dose and 61-88% after the 50 mg dose.
DISCUSSION

It was clear from these studies that there was a marked species difference in the excretion of tiquinamide and its metabolites with regard to both the rate of urinary elimination of radioactivity and the importance of the faecal route. An initial rapid phase of urinary excretion was observed in all species. However, whereas this constituted the most important phase in monkeys and man (approximately 80% and 70% of dose respectively), it was of lesser importance in the rat (circa 40%). A slower urinary excretion phase was more important in the rat (about 20%) than in monkey (3%). This phase could not be properly delineated in man, but at least 3-4% of the dose was excreted more slowly in the post 24 h period. In addition, faecal elimination in the rat (20%) was of the order of 3-4 times that in either of the other species (5% and 7% approx. respectively in monkey and man). In accordance with the slow elimination of the compound in the rat about 10% of the dose remained in the carcass 7 days after dosing.

The findings of the excretion kinetic studies generally confirmed those of plasma kinetic studies in demonstrating much slower elimination of tiquinamide metabolites in the rat than in the other species. However, the results also demonstrated that even in the rat there was an initial rapid phase of urinary excretion which was not evident from the results of plasma kinetic studies. This observation implied that metabolites eliminated in this initial phase were so readily excreted that their rate of formation was the rate-limiting factor in their elimination and they, therefore, never achieved high concentrations in plasma. By the time the peak concentration of radioactivity in plasma had been achieved at 8-12 h after dosing (Chapter VII p.157) the rapid phase of urinary elimination was virtually complete. Thus the slow elimination from plasma observed after the maximum concentration of radioactivity had been achieved corresponded to the slower phase of urinary excretion.

Closer analysis of the kinetics of urinary excretion and comparison with those of elimination from plasma revealed that in the rat and monkey the half-life of the rapid phase of urinary excretion of total radioactivity was of very similar order to that of elimination from plasma of the basic, toluene-soluble fraction
containing unchanged drug, i.e. approximately 3-4 h in the rat and 1 h in the monkey (see Chapter VII p. 157). Whilst the rapid phase of elimination in human urine could not be accurately delineated, inspection of sigma-minus plots revealed that the half-life for this phase would have been of the order of 3 h, again not very different from the half-life for elimination from plasma of the basic, toluene-soluble fraction. Since the proportion of toluene-soluble basic compounds excreted in urine was very low, this observation implied that it was the rate of metabolism of these compounds which controlled the rate of the fast phase of urinary elimination. Thus it seemed clear that there was a group of metabolites which, once formed, were very quickly excreted in urine. In view of the considerations discussed previously in Chapters VII and VIII, it was most likely that it comprised 3-carboxylic acids and glucuronides. Comparisons of the proportion of the dose found as such acidic metabolites with the proportions excreted in the rapid phase of urinary excretion seemed to generally bear out this conclusion. Thus in the female rat, a total of 23% of the dose was found as acidic metabolites, compared with 37% excreted in the fast phase. In the monkey, these values were respectively 62% and 80% and in man 58% and 70%.

In contrast, the half-life of the slow-phase of urinary elimination greatly exceeded that of the toluene-soluble basic fraction. Material eliminated in this phase may have constituted the products of further metabolism of the basic group of metabolites which included 5-hydroxy, 8-cyano-5,6,7,8-tetrahydro-3-methyl quinoline and 8-cyano-5,6,7,8-tetrahydro-3-hydroxymethyl quinoline. It has been suggested in Chapters VII and VIII that these compounds may have been poorly eliminated as a result of extensive tissue uptake and gastric re-cycling. There existed the additional possibility that, being extensively unionized in urine as a result of the low pKa of the tetrahydroquinoline nitrogen (pKa 5.2), they may also have been subject to renal reabsorption, which would have limited their direct urinary excretion.

The excretion of 5-13% of a 10 mg/kg dose of 14C-tiquinamide HCl in bile within 6 h of dosing the compound to female rats was broadly consistent with the observed extent of excretion of drug-related products in faeces (9% in 24 h). It did not suggest a significant entero-hepatic circulation. If such a process had constituted a
major factor in the slow elimination of tiquinamide metabolites, the hypothesis demanded that when toluene-soluble basic metabolites disappeared from plasma with a half-life of 3 h, the 70% of the dose which did not appear in the urine as acidic metabolites should have been predominantly excreted in the bile. Thus something of the order of 50% of the dose should have appeared in bile within a 6 h period. Since the observed biliary excretion was much lower and consistent with the known extent of faecal excretion, there was no evidence that tiquinamide metabolites were substantially re-absorbed from bile. It followed that entero-hepatic circulation of metabolites could not have significantly contributed to the slow elimination in this species.

The rate of biliary secretion was not measured directly in species other than the rat. However, on the basis of concentrations of radioactivity found in gall-bladder bile of monkeys killed at 0.5 h after dosing for tissue excision studies (Chapter VIII), it could be calculated that a maximum of 4-7% of the dose would have been excreted in bile. These calculations assumed that concentrations in bile at this time, when the rate of excretion in this species was at its most rapid, were maintained over the period of 6 h during which most of the drug-related products were in fact excreted in urine. This estimate was consistent with the appearance of around 5% of the dose in monkey faeces. In man, the recovery of only 7% of the dose in faeces was presumed to reflect a similarly low extent of biliary secretion. The greater extent of biliary excretion in the rat than in the other two species was consistent with the lower molecular weight threshold for biliary secretion in this species. The threshold value for biliary secretion of organic anions in the rat has been found to be of the order of 325 ± 50, whereas the value in rhesus monkey and man is believed to be similar to the value found for the rabbit i.e. 475 ± 50 (Millburn, Smith and Williams, 1967; Aziz et al., 1971; Smith, 1973). As discussed in Chapter I (p. 22), on this basis tiquinamide itself had too low a molecular weight (205) to be extensively biliary excreted in any of these species. However, conjugated metabolites such as, for example, the glucuronide of 5-hydroxy-8-cyano-5,6,7,8-tetrahydro-3-methylquinoline (MW 363) would have been sufficiently large to be excreted to a significant extent in the bile of the rat but not of monkey or man.
Fig. IX.1 Cumulative urinary and faecal excretion of radioactivity following 10 mg/kg oral administration of tiqunamide HCl to rats.

Cumulative % dose excreted

Time after dosing (h)
Biliary excretion of radioactivity following oral administration of 10mg/kg $^{14}$C-tiquinamide HCl to anaesthetized rats.

Each result represents the mean ± SEM of determinations in 3 animals.
Fig.IX.3: Cumulative urinary & faecal excretion of radioactivity by male & female monkeys following oral administration of 10 mg/kg $^{14}$C Wy 24081 HCl.
Fig IX.4: Cumulative excretion of radioactivity by two male volunteers following oral administration by capsule of $^{14}$C Wy 24081 HCl.
<table>
<thead>
<tr>
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<th>Percentage recovery of administered dose</th>
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<tr>
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<td>Rat 9</td>
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<tr>
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<td>Carcass</td>
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<td>Urine</td>
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<td>Faeces</td>
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<tr>
<td>Total</td>
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Table IX:1: Recovery of radioactivity from urine, faeces, expired air and carcasses of female rats 0-7 days following oral administration of 10 mg/kg $^{14}$C-tiquinamide HCl
A solution of 14C-tryptophan in HCl

Table IX.2: Half-lives and percentages of administered dose excreted in fast (f) and slow (s) phases of urinary excretion of radioactivity in rats and rhesus monkeys following an oral dose of 14C-tryptophan in HCl

<table>
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<th>Gender</th>
<th>2.8</th>
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<th>81.2</th>
<th>45.0</th>
<th>6.7</th>
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<td></td>
<td></td>
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<td>1.0</td>
<td>74.4</td>
<td>50.2</td>
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<tr>
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<td>1.0</td>
<td>92.8</td>
<td>31.1</td>
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<td></td>
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</table>

(b) Monkey

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<th>45.0</th>
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(a) Rat
CHAPTER X: DISCUSSION
DISCUSSION

At the outset of the present work, the objective was to define the metabolism and disposition of tiquinamide in the species of interest and to apply this knowledge to the development of the drug as a gastric anti-secretory agent. During the course of the work a species difference in elimination of metabolites was discovered and it became pertinent to understand this difference in terms of the known routes of metabolism of the compound.

The absorption of tiquinamide

Investigations of the plasma kinetics of tiquinamide have revealed that the drug is rapidly absorbed, as might be predicted of a weakly basic, lipophilic compound. Whilst results of studies to determine the extent of gastric absorption have upheld the preliminary case that tiquinamide could be absorbed from the stomach under conditions of elevated gastric pH and inhibited gastric emptying, they have also suggested that absorption from this site would not be predominant under normal physiological conditions. This finding is consistent with others suggesting that the small intestine may be the more important site of gastrointestinal absorption even for compounds which can potentially be absorbed from the stomach, for example the weak acids sulphaethidole and barbital (Crouthamel et al., 1971). It is the relative rates of absorption from the two sites and the rate of gastric absorption relative to that of gastric emptying which will determine how significant is the total extent of gastric absorption in conscious animals with normal gastric emptying. The vastly greater surface area of the small intestine than that of the stomach tends to favour absorption from the former site and it would seem that this factor can be more important than considerations about the degree of ionization which might ostensibly favour the latter site.

The metabolism of tiquinamide

Study of the metabolism of tiquinamide in rat, patas monkey and man has defined four major routes of Phase I biotransformation and one significant conjugation pathway. The 3-methyl substituent underwent \( \omega \)-oxidation to give rise to 8-cyano-5,6,7,8-tetrahydro-3-hydroxymethylquinoline and 8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid. The reaction pathway was analogous to that for p-nitrotoluene worked out by Gillette (1959). On this basis it was
likely that the oxidation to the intermediate alcohol was carried out by a microsomal mono-oxygenase. Further oxidation would have been performed by a soluble alcohol dehydrogenase, giving rise to an undetected aldehyde intermediate which would have been oxidized by a soluble aldehyde dehydrogenase to the carboxylic acid.

The tetrahydroquinoline ring was hydroxylated at an allylic position, yielding 5-hydroxy, 8-cyano-5,6,7,8-tetrahydro-3-methylquinoline, which subsequently underwent glucuronide conjugation. The hydroxylation reaction resembled that undergone by hexobarbital and known to be catalysed by a microsomal mono-oxygenase (Cooper and Brodie, 1955 and 1957).

Desulphuration of tiquinamide and its metabolites occurred chemically during their isolation and thus it was impossible to ascertain the precise degree to which this reaction occurred in vivo. However, it is almost certain that it represented an additional route of metabolism consistent with the behaviour of other thioamide compounds such as 2,6-dichlorothiobenzamide (Griffiths et al., 1966). It was not clear whether the metabolic desulphuration involved a reductive loss of hydrogen sulphide similar to that proposed for 1-phenyl-2-thiourea (Smith and Williams, 1961) or a sulphotiation analogous to that of ethionamide (Johnston, Kane and Kibby, 1967) with subsequent degradation of the unstable tiquinamide sulphoxide to the corresponding nitrile.

Aromatization of the tetrahydroquinoline ring was also observed to take place, resulting in the fully aromatized 8-cyano-quinoline-3-carboxylic acid. The mechanism of this reaction was unclear.

Differences between the three species studied were observed with respect to both the routes and rates of metabolism. Thus $\omega$-oxidation represented the major route of metabolism in the patas monkey and man, accounting respectively for 38-47% and 23-34% of the dose in the 0-24 h urine in the form of 3-carboxylic acids. Among products recovered from rat urine, however, only 15.8% of the dose could be unequivocally attributed to the products of $\omega$-oxidation. Although only detected in relatively small amounts (circa 1% of the dose), the intermediate compound 8-cyano-5,6,7,8-tetrahydro-3-hydroxy-methylquinoline was present in this species but not in patas monkey or man. The more complete oxidation through to the carboxylic acids in the two primate species studied than in the rat was attributable to the greater activity of liver alcohol dehydrogenase in these species (Krebs and Perkins, 1970; Von Warburg and Schürch, 1971).
The total 5-hydroxylated products excreted in 0-24 h urine of patas monkeys and man, respectively 18-21% and 17-27% of the dose, were greater than that excreted over the same period in the rat (11.3%). However, it has been suggested (Chapter IV p. 86) that these values may not have truly represented the total extent of formation of 5-hydroxylated metabolites. The 0-24 h urinary excretion accounted for the majority of the dose in patas monkey and man, but for only 40% of the dose in the rat and it was more likely on physico-chemical grounds that the basic metabolites, which included 5-hydroxy,8-cyano-tetrahydro-3-methylquinoline, were poorly eliminated than that this was true of the 3-carboxylic acids. The extent of conjugation with glucuronic acid was lower in the rat than in the other two species. Thus 40% of total 5-hydroxylated products excreted in 0-24 h of this species was unconjugated, compared to only 10% in the patas monkey and negligible amounts in man.

With respect to the aromatization of the tetrahydroquinoline ring, the rat showed greater similarity to man than did the patas monkey. In the former two species the proportions of the dose detected as 8-cyano-quinoline-3-carboxylic acid were 8.5% and 7-10% respectively, whereas in the latter species the aromatization reaction was not observed.

There was evidence that desulphuration occurred to a greater extent in the rat than in the patas monkey, despite the uncertainties regarding the occurrence of this reaction in vivo. Thus in the rat there was nothing to suggest that thioamides were excreted and subsequently degraded chemically in spite of estimates that at least one-third of any thioamide-metabolites excreted in this species would have been detectable in the chromatographic pattern of urinary products. In the patas monkey, however, a metabolite which formed a substantial proportion of the 50% of the dose excreted in urine in the 0-2 h period was found to subsequently degrade to 8-cyano-5,6,7,8-tetrahydroquinoline-3-carboxylic acid. Circumstantial evidence suggested that it was the thioamide analogue of this acid. The excretion of this compound in the patas monkey but not the rat probably reflected the more rapid ω-oxidation in the former species. Thus it is envisaged that 3-carboxylic acid formation proceeded more readily than desulphuration and because these acids were so readily excreted in urine a mixture of sulphur-containing and
desulphurated products appeared. In the rat, however, desulphuration evidently occurred more rapidly than \( \omega \)-oxidation, so that the two carboxylic acid metabolites excreted were both nitriles. No evidence was obtained concerning the extent of metabolic desulphuration in man. The relationship between the metabolism and disposition of tiquinamide

The factors which affect the disposition of molecules have been discussed fully in Chapters I, VII, VIII and IX. Briefly, the most important factor is perhaps the relative lipid/water solubilities of the ionization state most prevalent at \( \text{pH} \)’s pertaining to plasma, tissues and urine, for example. Water-soluble, acidic molecules extensively ionized under these physiological conditions tend to be poorly taken up into tissues and rapidly excreted in urine. Conversely, lipophilic bases which are largely unionized are more likely to suffer widespread tissue distribution and retarded elimination resulting from renal reabsorption and recycling processes such as gastric recycling.

Entero-hepatic circulation can substantially delay excretion of molecules which are relatively lipophilic themselves but which form polar conjugates of high molecular weight which are extensively excreted in bile but subsequently hydrolysed by the action of bacteria in the large intestine and re-absorbed. Factors which promote the extensive localization of molecules in particular tissues thereby retard their elimination. Thus, very lipid-soluble molecules which accumulate in fat depots will be poorly eliminated, as will molecules predisposed to high affinity binding either to plasma proteins or to proteins, nucleic acids, pigments or other macromolecules in tissues.

Study of the metabolism of tiquinamide has revealed why the majority of the products in patas monkey and man were rapidly excreted in urine and why this was not so in the rat. Two important routes of metabolism in all species studied involved \( \omega \)-oxidation to 3-carboxylic acids and 5-hydroxylation with subsequent glucuronide conjugation. In the patas monkey and in man, the predominance of these reactions resulted in the majority of the dose being excreted in an acidic fraction comprising 3-carboxylic acids and glucuronides. Because such compounds are poorly taken up into tissues, are fully ionized at physiological \( \text{pH} \)’s, and are very water-soluble, they were rapidly excreted in urine. This constituted virtually the only
route of excretion of these compounds because even the glucuronide conjugate resulting from tiqinamide was of too low a molecular weight to exceed the relatively high molecular weight threshold for biliary excretion believed to exist in primates. In the rat, the lower extent of \( \omega \)-oxidation and the lower proportion of 5-hydroxylated products which were subsequently conjugated resulted in a smaller proportion of total metabolites than in the other two species being present in the acidic fraction. Consequently, in the rat the phase of rapid urinary excretion was of lower significance than in the patas monkey or man.

What is less clear is the reason for the slow elimination of the alternative products in the rat. The only known metabolites suspected of being slowly eliminated were in the basic fraction containing unchanged drug, its nitrile derivative, and the 5-hydroxy- and 3-hydroxymethyl analogues. It is suggested that this fraction was more pronounced in the rat than in the other two species because oxidation to the carboxylic acids was less extensive and the degree of conjugation of the 5-hydroxylated metabolite was lower. These basic compounds could have been poorly excreted in urine because their low \( pK_a \) values and relatively high lipophilicity predisposed them to renal reabsorption. In addition, these same properties would have promoted uptake into tissues and gastric secretion of these compounds. Tissue distribution studies in the rat have revealed a widespread even distribution of drug-related products without evidence of a depot tissue from which metabolites were slowly leached out. It seems likely that the material distributed in the tissues included the basic metabolite fraction. Gastric secretion resulted in the stomach and its contents being the major site of accumulation and it is probable that the basic metabolite fraction was involved. Although drug-related products were found to be eliminated from this site at a slow rate identical to that from plasma, at no time were the concentrations in this organ high enough to constitute a depot controlling the rate of elimination. It was more likely that continued gastric re-cycling following re-absorption of these compounds from the small intestine contributed to their slow elimination.

A metabolite different from any of the major urinary end-products was found circulating in rat plasma at a time when the rapid phase of urinary elimination had ceased. This was a polar product of
similar chromatographic properties to a metabolite seen to the extent of 2-3% of the dose in 0-6 h bile. It was the predominant component in plasma at 25 h, but in view of its high polarity, it was unlikely that it was itself slowly eliminated. Possibly it represented the product of further metabolism of a compound in the basic fraction of tiquinamide metabolites.

The extent of biliary secretion of tiquinamide metabolites in the rat was approximately consistent with the amounts of drug-related material appearing in the faeces. Thus there was no evidence that entero-hepatic circulation contributed substantially to the slow elimination in the rat. Whilst faecal excretion in the rat was a relatively minor route of excretion, it occurred more extensively than in the patas monkey or man. This was believed to reflect more extensive biliary secretion in the rat as a result of the lower molecular weight threshold for biliary secretion in this species than in primates (Millburn, Smith and Williams, 1967; Aziz et al., 1971; Smith, 1973).

In conclusion, it can be said that the greater extent of formation of 3-carboxylic acids by $\omega$-oxidation and the greater extent of conjugation of 5-hydroxylated products in the monkey and man compared to the rat promoted more rapid elimination in these species. Conversely the greater amounts of unconjugated basic metabolites in the rat may have hindered the elimination of drug-related material as a result of renal reabsorption, tissue uptake and gastric re-cycling. 

The biological significance of the metabolism and disposition of tiquinamide

Oral bioavailability: The limited amount of data available on the oral bioavailability of tiquinamide suggested that it was of a moderate order and probably somewhat less than that of cimetidine. Although rapidly absorbed, tiquinamide was found to be rapidly and extensively metabolized. A substantial proportion of metabolism probably occurred during the first-pass, since the toluene-soluble fraction of basic compounds, which included the unchanged drug, in man represented maximally 60% of total radioactivity in plasma, at the time of peak plasma concentration, around 0.5 h after dosing. It was possible that the true bioavailability was even less than this value, since it is known, at least in rats and monkeys, that this
fraction contained other metabolites apart from unchanged tiquinamide. By comparison, cimetidine undergoes a relatively low degree of metabolism, being excreted predominantly unchanged in urine (Taylor, Creswell and Bartlett, 1978) and has an oral bioavailability as high as 72% (Griffiths, Lee and Taylor, 1977). It seems, however, that the lower bioavailability of tiquinamide is more than offset by its higher intrinsic potency, since, as discussed previously (Chapter I p. 15), 75 mg tiquinamide elicited 60-70% inhibition of acid secretion in man, whereas this order of inhibition was only achieved after a 200 mg dose of cimetidine (Burland et al., 1975).

Site of gastrointestinal absorption: Investigations into the predominant site of absorption of tiquinamide did not demonstrate substantial gastric absorption of the drug in normal conscious animals. However, there was an indication that the compound had a potential for gastric absorption if the pH conditions promoted the presence of the unionized drug. Administration of a loading dose as a buffered tablet or in combination with an antacid might conceivably promote gastric absorption which would be repeated after subsequent doses once gastric secretion came under effective control by the drug. Gastric absorption of tiquinamide might improve on the availability at its presumed site of action in the gastric mucosa by delivering the drug there directly and thus avoiding initial first-pass metabolism.

The relationship between plasma concentration of drug and its anti-secretory effect: Although a clear relationship between plasma concentrations of the drug and the intensity and duration of its pharmacological effect has not been established, there is circumstantial evidence to suggest that the drug rather than its metabolites is primarily responsible for its anti-secretory activity. Experiments to examine the time-course of inhibition of acid secretion by tiquinamide in pylorus-ligated rats after a 30 mg/kg subcutaneous dose had shown that the effect on gastric secretory volume was maximal (80-90% inhibition) until approximately 7 h after dosing, and remained statistically significant (p < 0.01) until 13 h after dosing. The effect on acid concentration persisted a little longer, until 24 h after drug administration (Beattie et al., 1979a).

Simultaneous studies on the time-course of the drug and metabolites demonstrated that the decline of the toluene-soluble fraction of
radioactivity in plasma and stomach wall occurred rapidly over the same time-scale as the decline of anti-secretory activity (Chapter VIII. Fig. VIII.6. By contrast, total metabolites in plasma declined slowly and, more significantly, those in the stomach wall remained constant at least until 14 h after dosing. It was thus likely that the primary anti-secretory agent was a constituent of the toluene-soluble basic fraction. It has been established (Chapter II. p. 45) that this fraction contains the unchanged drug and has a similar time-course in plasma (Chapter VII. p. 152).

The significance of the plasma half-life of tiquinamide for the control of nocturnal acid secretion: It was postulated in Chapter I (p. 33) that an ideal plasma half-life for a gastric anti-secretory compound would be determined by the need to maintain effective control of nocturnal acid secretion. Thus a half-life of approximately 8 h, equivalent to the average period of sleep, would be useful. In this respect, the half-life of tiquinamide, around 2 h in man, was somewhat short but was no worse than that of cimetidine (Griffiths, Lee and Taylor, 1977; Eprland et al., 1975).

The pharmacological properties of tiquinamide metabolites: The major identified metabolites of tiquinamide were found difficult to synthesize and thus their pharmacological properties have not been evaluated. However, the anti-secretory properties of one metabolite, the nitrile derivative of tiquinamide (8-cyano-5,6,7,8-tetrahydro-3-methyl quinoline) have been studied. This compound was found to be only weakly active (Beattie et al., 1977).

Furthermore, studies of structure-activity relationships amongst a number of related compounds have suggested that although the thioamide group may be substituted, for example by an alkyl group, its presence is very important to the manifestation of anti-secretory activity by this class of substituted 5,6,7,8-tetrahydroquinoline compounds (Beattie et al., 1977). This requirement for the thioamide group and an additional one for a heterocyclic nitrogen in close proximity were consistent with the findings of other workers with respect to some substituted thioacetamide compounds active as anti-secretory agents (Malen, Danree and Pascaud, 1971; Lee, Phillips and Sause, 1972).
Thus it seems unlikely that metabolites of tiquinamide identified as nitriles contributed significantly to the anti-secretory effects. In view of the possibility that metabolites of tiquinamide existed which retained an intact thioamide group, some contribution by these compounds to the pharmacological activity cannot be excluded. Thioamide containing 3-carboxylic acids would perhaps be less useful as anti-secretory agents than the parent drug since their physicochemical properties would not promote uptake into the acidic environment of the stomach, but would rather lead to rapid urinary elimination. However, thioamide-containing 5-hydroxylated or 3-hydroxymethyl compounds may have had significant anti-secretory activity in their own right. Taking all these considerations into account, it would appear that unchanged tiquinamide was the largest contributor to the anti-secretory activity, but that thioamide-containing metabolites, when present, may have played a part.

Whilst the cardiovascular properties of the majority of the known metabolites could not be evaluated because of the difficulties associated with their synthesis, there was indirect evidence to implicate the unchanged drug in the tiquinamide-induced tachycardia. Firstly, the nitrile derivative of tiquinamide had no significant effect on blood pressure or heart rate (Waterfall, 1978). This nitrile was a known metabolite of tiquinamide and one which was likely to represent an intermediate between the drug and the 5-hydroxylated compounds and 3-carboxylic acids which represented the major products of further metabolism. Therefore the absence of nitrile-induced tachycardia directly implicated the thioamide group. Methyl substitution of the thioamide group in tiquinamide and two related compounds was found to abolish the tachycardia, confirming the link between the cardiovascular properties of these compounds and the stereochemistry around the thioamide group. Furthermore the unchanged drug was found to cause relaxation of vascular smooth muscle in vitro in the isolated, perfused rat mesentery preparation. This finding suggested a possible mechanism for the tachycardia involving a reflex response to the lowering of blood pressure, mediated by the baro-receptors.

A possible role for the 3-carboxylic acid metabolites of tiquinamide in its cardiovascular properties was considered because nicotinic acid is a peripheral vaso-dilator, though fairly weak
(Goodman and Gilman, 1975) and because the structurally related compounds fusaric acid and picolinic acid are known to decrease blood pressure and induce tachycardia (Antonaccio, Cote and Cavaliere, 1976). However, this possibility had to be excluded because Wy-23995 (5,6,7,8-tetrahydroquinoline-8-thiocarboxamide) was a more potent inducer of tachycardia in the rat than was tiquinamide. This compound could not form 3-carboxylic acid metabolites because it contained no aromatic methyl substituent. On the same grounds, 3-hydroxymethyl metabolites could not have been implicitly involved.

Thus the available evidence suggested that the unchanged drug could cause the tachycardia without the need for a prior metabolic step. Thiocarbamide-containing metabolites may, however, have contributed to this effect when present.

The acute toxicity of tiquinamide metabolites: The desulphuration of tiquinamide in vivo had potential implications for the acute toxicity of the compound because it raised the question of whether hydrogen sulphide was generated in tissues as a by-product. Hydrogen sulphide is a very toxic gas, inducing symptoms ranging from headache, fatigue and respiratory tract irritation at lower concentrations to death by respiratory failure at higher levels (Milby, 1962). It has an LD$_{50}$ value in rats as low as 0.3-0.5 mg/kg when administered intravenously. Furthermore, its generation in tissues as a result of metabolic desulphuration had been implicated in the high acute toxicity of mono-substituted aryl thioureas such as phenylthiourea (LD$_{50}$ 5 mg/kg in rats; Scheline, Smith and Williams, 1951). However, tiquinamide was found to have a remarkably low acute toxicity in animals. LD$_{50}$ values in rats and mice were as high as 1634 mg/kg and 400 mg/kg respectively after oral administration (West-Watson, 1970). Thus if hydrogen sulphide was liberated in tissues, the rate of generation was not sufficiently fast to exceed the rate of its subsequent elimination. Alternatively, the desulphuration may have proceeded by an oxidative route, via the sulphoxide, with subsequent degradation to the nitrile, as discussed previously (Chapter IV, p. 87).

The low acute toxicity of tiquinamide also implied that the nitrile metabolites were innocuous. This was consistent with their stability to further metabolic alteration. In this respect they
resembled aryl cyanides rather than alkyl cyanides which have a high acute toxicity as a result of rapid hydrolysis to the corresponding amides with consequent release of hydrogen cyanide (Williams, 1959h).

**The choice of rat and patas monkey as species for chronic toxicity studies:** The results of the comparison of metabolism and disposition of tiquinamide in rat and patas monkey with those in man confirmed the usefulness of both these species in evaluating the potential long-term toxicity of the drug. Whilst the patas monkey was overall a better model for man, the absence of the fully aromatized 8-cyanoquinoline-3-carboxylic acid in this species meant that the toxicological properties of this compound would have been overlooked if the patas monkey alone had been used to evaluate the long term toxicity of tiquinamide. The additional use of the rat, which, like man, did form this metabolite provided full compensation for the omission.

The extremely slow elimination of some metabolites in the rat apparently had no serious toxicological consequences. The most significant abnormality observed after repeated daily administration of tiquinamide at doses up to 400 mg/kg for 26 weeks was an effect on serum cholesterol concentrations, which were elevated by up to 60% in a non-dose related fashion (Paterson, 1974). However, there was a similar trend to elevated serum cholesterol levels in patas monkeys. Although values for this parameter in the monkey did not fall outside the normal range for this species (Paterson, 1975), the percentage elevations over control values (38% in males and 48% in females after doses up to 250 mg/kg daily for 24 weeks) were not markedly lower than those in the rat (Paterson, 1975). Thus the species difference in elimination did not result in any clear species difference in toxicity.
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A mixed isotope method for the study of gastric absorption of drugs

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The most widely used method for studying gastric absorption of drugs is that devised by Shanker, Shore & others (1957), which involves the measurement of the decline in concentration of drug in the pylorus-ligated stomach. In the absence of adsorption to the stomach wall, this provides a means of studying the potential for gastric absorption of a drug in relation to its lipophilicity and pKa and for comparing this potential with that of other drugs. However, the method is subject to certain limitations. Firstly, it does not permit evaluation of the significance of gastric absorption in the presence of normal gastric emptying. Secondly, the presence of an anaesthetic as an accessory to the pylorus ligation may itself influence the course of gastric absorption by altering gastric motility and blood flow to the stomach. Hence if the significance of gastric absorption of a particular drug under normal physiological conditions is to be properly evaluated, the measurement of the rate of this process should be performed in animals with a functioning pylorus and an allowance made for disappearance of drug from the stomach by gastric emptying.

Several workers have described the use of non-absorbable markers in absorption studies to make an allowance for loss of drug by gastric emptying and also for dilution of stomach contents by gastric secretion. For example, Cooke & Hunt (1970) used the marker phenol red when studying the gastric absorption of acetylsalicylic acid in man. The method relies on the principle that changes in drug concentration due to gastric emptying and dilution of stomach contents parallel similar changes in the concentration of the marker. Any additional decline in drug concentration in gastric contents must then result from gastric absorption. In previously reported studies, the non-absorbable marker was unlabelled and was determined spectrophotometrically by an assay separate from that used to measure concentrations of drug in stomach contents. The low sensitivities of the assay methods necessitated the use of relatively high concentrations of non-absorbable markers. Under these conditions, the method is subject to the criticism that the marker itself may influence the absorption of the drug (Beerman, Groschinsky-Grind & Rosen, 1976).

The present communication describes a modification which simplifies the procedure when radiolabelled drug is available. The ratio of concentration of a 14C-labelled drug to that of a 3H-labelled marker is used to make automatic compensation for effects of volume changes and gastric emptying without the need for separate assays. The ratio of 14C/3H in gastric contents remains constant, despite gastric secretion and gastric emptying, unless drug is absorbed from the stomach. In this case, the 14C/3H ratio declines at a rate equal to the rate of gastric absorption of the drug. Furthermore, simultaneous determination of the gastric emptying rate allows calculation of the total percentage of the dose absorbed from the stomach.

The drug chosen for study of gastric absorption by this method was 14C-salicylic acid, since Shanker & others (1957) had shown it to be well-absorbed from the pylorus-ligated stomach of the rat, and not significantly adsorbed to stomach wall. The marker was [3H]inulin, an inert substance negligibly absorbed from the gastrointestinal tract (Miller & Schedl, 1970), and not subject to significant exchange of tritium with water under physiological conditions (Marlow & Sheppard, 1970). The animals used were one male and two female patas monkeys (Shamrock Farms Ltd., Brighton, Sussex), 3-0-3-5 kg, fasted overnight. The dose (0.5 mg kg⁻¹) of salicylic acid was administered by oral intubation such that each animal received 5 ml kg⁻¹ of a solution containing 100 µg ml⁻¹ 14C-salicylic acid and 1 µg ml⁻¹ [3H]inulin (The Radiochemical Centre, Amersham, Bucks) in isotonic saline. Immediately after dosing and subsequently at 10 min intervals for 1 h, aliquots of approximately 1 ml of gastric contents were aspirated and after the hour the entire stomach contents were aspirated. At the end of the period of serial sampling, stomach contents were aspirated so that the amount of remaining [3H]inulin could be assayed. Because of the possibility of incomplete recovery of stomach contents in the final gastric wash, the efficiency of this recovery was checked by introduction of [14C]polyethylene glycol (PEG 4000) (14C-PEG) (The Radiochemical Centre).

14C-PEG (8-15 µCi) was administered by oral intubation of 20 ml of a 12 µg ml⁻¹ solution in isotonic saline, and was recovered within 3 min of dosing by washing out with 3 x 50 ml isotonic saline. Radioactivity administered as 14C-PEG 4000 was in large excess over that of any remaining 14C-salicylic acid, which thus did not interfere with the determination of the 14C-labelled marker. Mixing of 14C-PEG 4000 and [3H]inulin in stomach contents was assumed to have been achieved by the regular contractions of the stomach and by subsequent washes. Three 50 ml portions of isotonic saline were introduced into the stomach and the gastric contents aspirated after each wash. Recoveries of 14C-PEG 4000, determined in 2 animals only, were 90 and 101%. Consequently no compensation for loss of gastric contents was considered necessary. Samples of gastric contents and the pooled gastric washes were centrifuged to precipitate any solid particles. Tritium and 14C concentrations in suitable aliquots were assayed...
by liquid scintillation counting under conditions in which the two isotopes could be separately determined.

The half-life of gastric absorption was calculated from the slope of the regression line relating log
\[ 14C/3H \] ratio to times after dosing. The gastric emptying half-life was determined from the amount of [\(^3\)H]-inulin not emptied from the stomach in the 60 min after dosing. The calculation assumed monoexponential gastric emptying, as established by Hunt & MacDonald (1954) from studies in man. The method of calculation used the formula:

\[
\text{Gastric emptying } t_{1/2} = \frac{0.301 \times t}{\log_2 (X_0)}
\]

The total percentage of the administered dose absorbed from the stomach was calculated from the rate constants for gastric absorption and gastric emptying, \( k_{as} \) and \( k_{ge} \) respectively, as follows:

\[
\text{Percentage dose} = \frac{k_{as}}{k_{as} + k_{ge}} \times 100\% \text{, where } k = \frac{0.693}{t_{1/2}}
\]

As illustrated in Fig. 1, the ratio of [\(^14\)C]salicylic acid to [\(^3\)H]inulin declined monoexponentially with time (correlation coefficient = \(-0.996\)), indicating that substantial gastric absorption of salicylic acid had taken place. The mean gastric absorption half-life (± s.e.m.) was 12.8 ± 1.8 min. The mean gastric emptying half-life (± s.e.m.) was 37.3 ± 9.0 min, in good agreement with the value of 30 min previously reported by Franklin (1975). The mean percentage of the administered dose absorbed from the stomach (± s.e.m.) was 69.0 ± 6.1%.

Thus, this mixed isotope ratio method provides a simple means of determining the rate of gastric absorption of a drug without recourse to artificial preparations involving surgical ligation of the pylorus. Whilst there are existing methods to compensate for changes in drug concentration resulting solely from gastric secretion and gastric emptying, this method confers the greater convenience of a single assay by comparison with the more usual determination of drug and non-absorbable marker by separate specific assays. Finally, the method incorporates the determination of gastric emptying simultaneous with that of absorption, thus permitting a simple assessment of the total extent to which gastric absorption occurs under conditions of unimpaired gastric emptying.

The authors gratefully acknowledge the skilled technical assistance of Mr S. Meacham in the collection of samples of gastric contents.

January 17, 1977

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given pimozide at doses of 8, 6 and 4 mg daily over successive days. All five improved. As shown in Table 1, pimozide, in contrast to fenfluramine, produced a marked reduction in the manic content of the patient's speech and euphoria; it also improved behaviour without leading to sedation. Subsequently there was a tendency to relapse on the fixed dose of pimozide (4 mg) daily, but increasing the dose in two patients up to 8 mg twice daily was successful in controlling their manic symptoms.

While our findings confirm the earlier report (Pearce, 1973) that fenfluramine improves manic symptoms, we consider that it did so by producing sedation accompanied by a reduction in motor activity, rather than by lowering mood or manic ideation. The biochemical basis for this sedative effect of fenfluramine is uncertain. It is known that fenfluramine releases 5-HT and blocks its reuptake in the CNS (Garattini & Samanin, 1976), and such an action might potentiate the activity of those 5-HT neurones of the median raphe nuclei which are thought to be involved in slow wave sleep (Jouvet, 1972). This interpretation runs counter to the view that mania is due to over-activity of DA pathways and that blockade of DA receptors with methysergide is a specific treatment for mania (Dewhurst, 1969). Unfortunately for this view Coppen, Prange & Whybrow (1969) failed to confirm that methysergide had any anti-manic activity, on the contrary it seemed to make matters worse.

In contrast to the general sedative action of fenfluramine, pimozide appeared to affect the core symptoms of mania more directly. This finding is consistent with the idea that mania results from over-activity of DA pathways.

References


**PHARMACOKINETIC STUDIES ON TIQUNAMIDE, A NOVEL INHIBITOR OF GASTRIC ACID SECRETION**

Tiquinamide (3-methyl-5,6,7,8-tetrahydroquino- line-8-thiocarboxamide, Figure 1) is a new compound having potent effects as an inhibitor of gastric acid secretion in a variety of animal species (Curran, Crossley & Hill, 1976). It inhibits basal acid secretion and has a wide spectrum of activity against chemical stimulants of acid secretion e.g. pentagastrin, histamine and the cholinergically-mediated stimulant, carbachol. Studies in our laboratories (Beattie, personal communication) have shown that it is not a classical anticholinergic drug, as demonstrated by its lack of activity in the guinea-pig isolated ileum preparation at concentrations up to $10^{-4}$ M. It does have weak histamine H2-receptor antagonist activity, but this is probably a relatively minor feature of its pharmacological profile, since it is substantially more potent in inhibiting basal acid-secretion than the established H2-receptor antagonists, metiamide and burimamide. Preliminary studies in four...
human volunteers have indicated approximately 50% inhibition of pentagastrin-induced acid secretion after a single 40 mg oral dose (Lancaster-Smith, personal communication).

The pharmacokinetics and metabolism of \([^{14}\text{C}]\)-tiquinamide have been investigated after administration to two healthy male volunteers. In one study, each subject ingested a gelatin capsule containing 5 \(\mu\)Ci of radio labelled tiquinamide HCl in a total dose of 20 mg. In a second study, the dose was 50 mg containing 50 \(\mu\)Ci of the radio-labelled drug. Blood was sampled from the cephalic vein at frequent intervals up to 48 or 72 h after dosing. Urine was collected over short intervals during the first 24 h after drug administration, and subsequently daily. Faecal samples were collected daily.

Radioactivity in plasma and urine was assayed by liquid scintillation counting. Faecal samples were homogenised and small portions digested by the method of Mahin & Lofberg (1966). Aliquots of plasma and urine were extracted with toluene. Extracted radioactivity contained unchanged tiquinamide, but would also have included some non-polar metabolites. The chromatographic pattern of metabolites was investigated in 0-24 h urine from one subject who had received the 50 mg dose. Urine was applied to a column of XAD-2 Amberlite resin and eluted with methanol. Methanolic extracts were hydrolysed by molluscan \(\beta\)-glucuronidase/sulphatase (Sigma Chemical Co. Ltd) and subjected to silica gel thin-layer chromatography in the following solvents:
(a) diethyl ether–ethanol–ammonia (60:40:10)
(b) dimethylformamide–diethylaniline–ethanol–ethyl acetate (1:1:6:12)
(c) ethyl acetate–methanol–water (65:10:5)
Chromatograms were scanned by a Panax radiochromatogram scanner.

Absorption was very rapid, as indicated by the appearance of the peak concentrations of toluene-soluble radioactivity in plasma at 0.5-1 h after dosing (Table 1). Furthermore the low faecal recovery of radioactivity, together with the high recovery of urine constitute evidence that absorption was extensive (Table 2).

Elimination of toluene-soluble radioactivity, from plasma occurred rapidly in a monoeponential fashion, having a half-life of 2.2 ± 0.2 h. This represented the maximum plasma half-life of unchanged tiquinamide. Total radioactivity was eliminated somewhat more slowly from plasma. Peak concentrations were attained within 1-3 h after dosing, and declined to 25-35% of these maxima by 48 h after administration.

Excretion of radioactivity occurred quickly and almost entirely in the urine. In the first 24 h after drug administration, 61-88% of the dose was eliminated by this route. These figures had risen to 66-92% by 4 days after dosing. Not more than 2%

### Table 1  Toluene-soluble and total radioactivity in plasma of two male volunteers following oral administration by capsule of \([^{14}\text{C}]\)-tiquinamide HCl (20 and 50 mg)

| Subject | Time after 
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ML</td>
<td>DM</td>
</tr>
<tr>
<td><strong>20 mg</strong></td>
<td><strong>50 mg</strong></td>
</tr>
<tr>
<td><strong>(\mu)g ([^{14}\text{C}])-tiquinamide HCl equivalents/ml plasma</strong></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.29 (0.19)</td>
</tr>
<tr>
<td>1</td>
<td>0.37 (0.18)</td>
</tr>
<tr>
<td>2</td>
<td>0.38 (0.15)</td>
</tr>
<tr>
<td>3</td>
<td>0.36 (0.10)</td>
</tr>
<tr>
<td>4</td>
<td>0.31 (0.07)</td>
</tr>
<tr>
<td>6</td>
<td>0.26 (0.04)</td>
</tr>
<tr>
<td>8</td>
<td>0.24 (0.02)</td>
</tr>
<tr>
<td>12</td>
<td>0.19 (0.01)</td>
</tr>
<tr>
<td>24</td>
<td>0.13 (&lt;0.008)</td>
</tr>
<tr>
<td>48</td>
<td>0.12 (&lt;0.008)</td>
</tr>
<tr>
<td>72</td>
<td>NS (NS)</td>
</tr>
<tr>
<td>96</td>
<td>NS (NS)</td>
</tr>
</tbody>
</table>

Values in brackets represent toluene-soluble radioactivity.
NS = No sample
Table 2: Cumulative urinary and faecal excretion of radioactivity in two male volunteers following oral administration by capsule of [14C]-tiquinamide HCl (20 mg and 50 mg)

<table>
<thead>
<tr>
<th>Time after dosing (h)</th>
<th>20 mg Subject</th>
<th>20 mg DM</th>
<th>50 mg Subject</th>
<th>50 mg DM</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>ML</td>
<td>DM</td>
<td>ML</td>
<td>DM</td>
</tr>
<tr>
<td></td>
<td>Urine % of radioactivity in administered dose</td>
<td>Faeces</td>
<td>Urine % of radioactivity in administered dose</td>
<td>Faeces</td>
</tr>
<tr>
<td>3</td>
<td>33.8</td>
<td>37.5</td>
<td>12.7</td>
<td>11.0</td>
</tr>
<tr>
<td>6</td>
<td>48.4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>59.6</td>
<td>60.2</td>
<td>NS</td>
<td>55.0</td>
</tr>
<tr>
<td>12</td>
<td>66.3</td>
<td>NS</td>
<td>56.9</td>
<td>78.0</td>
</tr>
<tr>
<td>24</td>
<td>73.0 &lt;1.6</td>
<td>66.0 &lt;2.1</td>
<td>60.8 NS</td>
<td>87.5 0.8</td>
</tr>
<tr>
<td>48</td>
<td>76.2 &lt;0.2</td>
<td>68.2 &lt;2.7</td>
<td>63.3 4.1</td>
<td>90.6 4.0</td>
</tr>
<tr>
<td>72</td>
<td>76.3 2.4</td>
<td>68.7 &lt;2.7</td>
<td>64.6 5.2</td>
<td>91.6 7.6</td>
</tr>
<tr>
<td>96</td>
<td>76.8 NS</td>
<td>69.1 1.7</td>
<td>65.7 5.6</td>
<td>92.0 NS</td>
</tr>
<tr>
<td>120</td>
<td>NS NS</td>
<td>5.9 NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>76.8 2.4</td>
<td>69.1 1.7</td>
<td>65.7 5.9</td>
<td>92.0 7.6</td>
</tr>
<tr>
<td>Grand total</td>
<td>79.2</td>
<td>70.8</td>
<td>71.6</td>
<td>99.6</td>
</tr>
</tbody>
</table>

NS = No sample

of radioactivity in urine was unchanged tiquinamide. Only 2-8% of the dose was excreted in faeces in 4 days.

The presence of at least two major metabolites was detected in urine after hydrolysis with β-glucuronidase/sulphatase. Neither of these metabolites had RF values corresponding to those of the nitrile, which would have resulted by loss of hydrogen sulphide from the thiooxoamide group. The chromatographic properties of one metabolite were similar to, though not identical with, those of 3-methyl-5,6,7,8-tetrahydroquinoline 8-carboxylic acid, the expected product of hydrolysis of the thioamide group of tiquinamide. Furthermore, it could be extracted into diethyl ether from acidified urine (pH 2), and is acidic in nature. A more detailed study of the nature of the major metabolites in progress and will be reported separately.

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Received March 1, 1976

SALIVARY ANTIPYRINE HALF-LIFE: A USEFUL MEASURE OF HEPATIC DRUG METABOLISM

The rate of disappearance of antipyrine from plasma after a single oral dose has been shown to be a useful indicator in man of the drug metabolising capacity of the liver (Vessell & Page, 1968). With the proliferation of new drugs and the increasing interest in the effect of these agents on bodily functions, newer methods of examining the drug metabolising capacity of man have been discovered, such as the urinary excretion of D-Glucaric acid and plasma glutamyl transpeptidase activity (Davidson, McIntosh & Ford, 1974). However, despite these new methods, measurement of plasma antipyrine half-lives remains a reliable method for assessing hepatic drug metabolism. The problem with measurement of the plasma antipyrine half-life is that frequent
blood samples are required over a 24 h period, which makes study of outpatients difficult. Recently, concentrations of a number of drugs (Huffman, 1975), including antipyrine (Vessel, Passananti, Glenwright & Dvorchik, 1975), have been measured in the saliva and we decided to see if salivary antipyrine half-lives could be correlated with the disappearance rate of antipyrine from plasma, thus allowing us to carry out drug metabolism studies on outpatients as well as inpatients.

Antipyrine in a dose of 10 mg/kg was given to six subjects (three normal volunteers and three patients with rheumatoid arthritis). Blood was collected 6 h, 12 h, 20 h and 26 h after ingestion. At the same time as blood was taken, saliva (3 ml) was collected in a container and centrifuged to remove debris. Blood was centrifuged at 2,000 rev/min, and the resultant plasma and saliva stored at 4° C until antipyrine estimation was carried out, using the method of Brodie, Axelrod, Soberman & Levy, 1949). After a period of at least 4 days, salivary antipyrine half-lives were repeated using the same dose of oral antipyrine.

From Table 1 it can be seen that there is no significant difference between the plasma and salivary antipyrine half-lives. The mean plasma/saliva ratio for antipyrine concentration in these patients was 0.839 ± 0.09 s.d.). Regression analysis showed that the correlation coefficient for antipyrine plasma v saliva half-lives was 0.76 and that for saliva (1) v saliva (2) half-lives was 0.98.

The advantage of measuring antipyrine in the saliva is that drug metabolism studies can be carried out in outpatients. Subjects can be given an oral dose of antipyrine and instructed as to when and how saliva samples are to be collected. Samples can be stored in the refrigerator and posted back in an envelope, thus negating another visit to the clinic.

The method shows a high degree of reproducibility and the collection of adequate amounts of saliva did not pose too great a problem in this study, despite the fact that three of the patients were elderly females with rheumatoid arthritis and would perhaps have been expected to have less saliva than normal.

The method is far more acceptable to patients because blood sampling is not required, though it was found to be less aesthetically pleasing to the medical and technical staff handling the saliva samples.

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Received February 11, 1976

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