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Fate of the anthelmintic, phenothiazine, in man

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1. Radiolabelled [^{35}S]-phenothiazine has been administered orally to two healthy adult male volunteers (6 mg kg^{-1} body weight). Faeces were the major route of excretion of radioactivity (68%), the remainder being eliminated via the urine (32%) with an estimated urinary half-life (biphasic) of 6–16 h. Over the 5 days of the study a complete recovery of radioactivity was achieved.

2. From urinary data, it was shown that metabolism occurred via ring carbon oxidation to form phenothiazone and thionol and via ring sulphur oxidation to form phenothiazine sulphoxide. The majority of urinary material (92%) was present in the form of conjugates of phenothiazine and phenothiazone. Only unchanged phenothiazine was detected in the faeces. Phenothiazine sulphoxide was reduced to phenothiazine during incubation with faecal homogenates.

Introduction

The synthesis of phenothiazine (thiodiphenylamine, dibenzothiazine) was first published in 1883 during researches into the chemical structure of methylene blue and its derivatives (Bernthsen 1883, 1885). It remained a chemical curiosity for 50 years until it was shown to possess several biological properties (antifungal, bacteriocidal, insecticidal, vermifugal) and then quickly became regarded as the panacea for parasitic infestation. It was the first of the 'modern' anthelmintics and was extensively used during the Second World War enabling the provision of many tons of desperately needed infection-free food and sheep intestines (catgut sutures/ligatures). However, its potential adverse reactions including haemolytic anaemia, dermatitis, photosensitization and neuromuscular incoordination (Mitchell 1994), coupled with the development of more potent agents, have now limited its use. Nevertheless, low-dose phenothiazine has been shown to be active against strains of nematodes that have become resistant to more recently developed products and this property, together with its relative cheapness, still secures it a place on the world scene (Kelly *et al*. 1981, Lyons *et al*. 1993). Indeed, it is quoted as the 'drug of choice' for treatment of caecal nematode (*Heterakis gallinae*) infestations in poultry (Jacobs *et al*. 1997).

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Despite its wide-scale application amongst many animal species, investigations of the fate of phenothiazine have been limited (Mitchell 1982) and data for man are scant. This is surprising, since vast amounts of the compound have been used and exposure in man, through accidental ingestion, inhalation and potential dermal absorption, has been inevitable. The few studies undertaken (DeEds *et al.* 1938, DeEds and Thomas 1941, 1942) have shown the presence of red/purple quinoidal compounds in human urine after its relatively harsh chemical treatment and suggest extensive metabolism, but no advantage has been taken of the use of radiolabelled material. Any estimate of the persistence of phenothiazine within man necessarily requires the inclusion of all metabolic products, whether known or not, and would be aided greatly by the incorporation of a readily quantifiable marker within the parent molecule. The present paper describes the fate of [^{35}S]-phenothiazine in two adult men.

Materials and methods

Chemicals

Phenothiazine was obtained from Sigma-Aldrich Ltd (Dorset, UK) and purified by recrystallization from diethyl ether (Smith 1938) then aqueous ethanol to give light tan crystals, m.p. 181–183 °C, UV absorption peaks at 254 and 317 nm, mass spectrum M^+ m/z 199 (mol. wt 199) (Shine and Mach 1965, McLafferty and Stauffer 1989). Radiolabelled phenothiazine was synthesized by refluxing elementary [^{35}S]-sulphur (Nycomed-Amersham, Amersham, UK) with diphenylamine in the molar ratio of 2:1.3 respectively, for 1 h at 180 °C in 1,2-dichlorobenzene under an atmosphere of nitrogen, 1% (w/w) iodine being added as a catalyst (Lannung 1941, Massie 1954, Coffey 1978). The evolved radioactive hydrogen sulphide was trapped in an aqueous solution of $FeCl_3$ (1 M). The solvent was removed by distillation, the excess diphenylamine precipitated in anhydrous ethereal solution by dry HCl gas and the phenothiazine recrystallized twice from aqueous ethanol to give light yellow crystals of specific activity 189.4 mCi mol^{-1}, radiochemical purity >98% (TLC), mixed m.p. 181–183 °C, with spectral properties as above.

Phenothiazine sulphoxide (phenothiazine-5-oxide) was prepared by oxidizing phenothiazine with 3-chloroperbenzoic acid in cold dry acetone (Beckett *et al.* 1975) to give light green crystals, m.p. 247–248 °C, UV absorption peaks 229, 271, 303 and 336 nm, IR sharp band at 1070 cm^{-1}, mass spectrum M^+ m/z 215 (mol. wt 215) (McLafferty and Stauffer 1989). Phenothiazone (phenothiaz-3-one) was synthesized by the oxidation of phenothiazine with ferric chloride in hot water (Pummerer and Gabner 1913, Houston *et al.* 1949a). The crude residue was extracted with hot ethanol and the crystalline product obtained by chilling (Granick *et al.* 1940). Further purification by elution from aluminium oxide columns with chloroform, followed by preparative thin-layer chromatography (TLC) (2.0 mm silica gel G) in carbon tetrachloride/acetone, 4/1 (v/v), gave red crystals, m.p. 161–163 °C, UV/vis. absorption peaks at 236, 258, 272, 368 and 508 nm, mass spectrum M^+ m/z 213 (mol. wt 213) (McLafferty and Stauffer 1989). Thionol (7-hydroxyphenothiaz-3-one) was prepared by refluxing phenothiazine with 80% (v/v) sulphuric acid at 160–165 °C for 6 h, the residue being poured into excess iced-water and filtered (Houston *et al.* 1949b, Granick and Michaelis 1947). The crude product was purified by elution with 10% (v/v) aqueous NH_4OH from aluminium oxide columns, followed by preparative TLC (2.0 mm silica gel G) in carbon tetrachloride/acetone, 4/1 (v/v), to give red/purple crystals, m.p. >300 °C, UV/vis. absorption peaks at 232, 276 and 592 nm, mass spectrum M^+ m/z 229 (mol. wt 229).

Human excretion study

Two healthy male volunteers (aged 27 and 28 years, weight 70.5 and 74 kg, height 1.85 and 1.80 m) were given a single oral dose of [^{35}S]-phenothiazine (423 mg (40.3 μCi) and 444 mg (42.3 μCi); dose-rate 6 mg kg^{-1} body weight) in a gelatin capsule with water (100 ml) during the early morning at least 2 h after a light breakfast. The subjects were non-smokers and no alcohol or medication was permitted for 1 week before or during the study. Urine was collected for known intervals for the first 0–24 h and then daily for the following 4 days, all samples being stored in darkened bottles at −70 °C until analysis. Faeces were collected daily for 0–5 days directly into lyophilization containers (plastic drums) and then stored at −70 °C until processing. Ethical approval for the study was obtained from the local ethics committee, following consultation with the local radiation protection panel.

Faecal metabolism

The possible contribution to metabolism by the lower gut contents was investigated by the separate incubation of phenothiazine, phenothiazine sulphoxide, phenothiaz-3-one and thionol (50 mg) with aqueous human faecal homogenate (2 ml) in sealed tubes under nitrogen at 37 °C for 48 h (Scheline 1966). The solutions were then lyophilized, extracted with methanol and analysed by TLC and HPLC as described below. Incubates with phosphate buffer (0.1 m, pH 7.4) instead of homogenates were included as controls.

Quantification of radioactivity

Aliquots of urine and HPLC eluant (100 µl–1.0 ml) and areas of silica from thin-layer plates were added directly to vials containing scintillation fluid ('Ecoscint', National Diagnostics, NJ, USA) and analysed by liquid scintillation spectrometry using a Packard model 385 scintillation counter (Ambac Industries, Inc., Downers Green, IL, USA), efficiency being determined by automatic external standardization and previously prepared quench-correction curves. Faeces were lyophilized, ground to a mixed powder, weighed and triplicate samples (about 100 mg) combusted in oxygen (Harvey Biological Material Oxidizer, ICN Tracer Labs, High Wycombe, Surrey, UK), the [$^{35}SO_2$] so produced being trapped in an alkaline scintillation mixture containing 2-phenylethylamine and then analysed as above.

Chromatography

Thin-layer chromatography (TLC) was carried out in nitrogen flushed tanks at 4 °C in the absence of light on plates coated with silica gel G (20 × 20 cm, 0.4 mm thick; Merck, Darmstadt, Germany) using solvents A (chloroform/acetone, 9/2 v/v), B (carbon tetrachloride/acetone, 4/1 v/v) and C (hexane/acetone, 3/2 v/v). Phenothiazine and its derivatives were detected under UV light and by spraying the dried plates with either (1) 50% v/v aqueous H_2SO_4, (2) 1% (w/v) I_2 in methanol, (3) 0.8% w/v HgI_2 in ethanol (with gentle warming), (4) 5% (w/v) $AgNO_3$ in 10% (v/v) aqueous NH_4OH or (5) 1% w/v $PdCl_2$ in 60 ml 0.1 M HCl plus 40 ml ethanol (Elliot 1959).

High-performance liquid chromatography (HPLC) was undertaken using a Perkin-Elmer LC sample processor ISS200 (PerkinElmer Analytical Instruments, Buckinghamshire, UK) connected to a Dionex quaternary gradient pump (Dionex Ltd, Camberley, Surrey, UK) in line with an LDC SpectroMonitor 5000 photodiode array detector (LDC/Milton Roy, Riviera Beach, FL, USA) and a fraction collector (LKB Bromma; Pharmacia LKB, Piscataway, NJ, USA). Data acquisition was by Chromquest chromatography software (ThermoFinnigan, Hemel Hempstead, Hertfordshire, UK). A reverse phase Zorbax Rx-C8 (5 µ, 250 × 4.6 mm) analytical column (Phenomenex UK Ltd, Macclesfield, Cheshire, UK) was used together with a gradient elution programme at an overall solvent flow rate of 1 ml min^{-1}. The mobile phase consisted of aqueous acetic acid (0.1% v/v) with its acetonitrile component increasing linearly from an initial 5% to 90% over 15 min, thereafter rapidly returning to 5% during the next 3 min and remaining at these proportions for another 7 min (a total run time of 25 min).

Investigation of metabolites

Urine (0–24 h) was examined both neat (concentrated by lyophilization where necessary) and following incubation with a β-glucuronidase/sulphatase mixture (type H-1 from *Helix pomatia*, Sigma-Aldrich) in acetate buffer (equal vol.; 0.05 M, pH 5) at 37 °C for 18 h (to hydrolyse any conjugates present). Incubations with standard phenolphthalein glucuronide or *p*-nitrophenol sulphate confirmed activity of this enzyme preparation. Lyophilized powdered faeces (0–48 h) were extracted with cold methanol (3 × 1 ml g^{-1}). The combined extracts were then filtered and decreased in volume by rotary evaporation at room temperature under reduced pressure. The extracted faecal residues were lyophilized and combusted as described above to detect any residual radioactivity. Known amounts of [^{35}S]-phenothiazine added to control faeces and then extracted gave recoveries of 88.6 ± 4.2% ($n = 6$) and the results obtained were corrected for this.

These fluids (urine, incubates, extracts) were examined, as spots (10–50 µl) or streaks (100–500 µl), by TLC in the solvent systems A, B and C. On some plates where urine spots had been used (as opposed to streaks), once the non-conjugated material had been removed, cold HCl (9.7 M; about 100 µl) was applied to the origin, and the plates dried and rerun at an angle of 90 ° in the same solvent to give a two-dimensional separation. When developed and dried, the thin-layer plates were scanned directly for radioactivity using a Berthold Series 272 computerized radiochromatogram scanner (Berthold Instruments, Inc., Aliquippa, PA, USA) or otherwise cut into 0.3-cm strips and analysed for radioactivity as described above. Quantification was also undertaken by adding the relevant eluant fractions obtained from HPLC examination to scintillation fluid and counting as above. Authentic metabolites, dissolved in urine or methanolic extracts of faeces, were co-chromatographed by both TLC and HPLC to provide provisional identification. Radioactive areas removed from preparative plates (2 mm thick) were extracted with methanol, concentrated, redissolved in minimum quantities of methanol and, after filtration, examined by UV and mass spectral techniques. Similarly, the collection of relevant HPLC

eluant fractions, with their lyophilization and reconstitution in methanol, provided further samples for spectral analysis.

Spectrometry

UV and visible spectra were recorded in methanolic solution on a Pye-Unicam SP 1800 UV spectrophotometer (Pye-Unicam, Cambridge, UK). IR spectra were measured using a potassium bromide disc and a Pye-Unicam SP200 IR spectrophotometer (Pye-Unicam). Electron impact mass spectrometry was carried out on a Kratos MS80 instrument (Kratos Ltd, Manchester, UK) with Kratos D555 (data generator) computerized display and printout facilities. The sample was inserted directly into the ionization chamber at 70 eV with a source temperature of 200 °C.

Results

Excretion study

The studies showed similar results for both subjects. About one-third of the administered radioactivity was recovered from the urine, with the majority (about 26% dose, about 84% of that in the 0–5-day urine) being voided during the first day. The portion of the dose that was absorbed appeared to be so quite rapidly; with 9% of the radioactive dose (about 29% of that in the 0–5-day urine) being detected in the urine within 0–3 h. The remaining two-thirds of the radioactive dose was recovered from the faeces over 3–4 days with excretion tapering off towards the end of the study. Overall, excellent total recoveries (98.4 and 100.4%) were achieved (table 1).

Metabolism

Separate examination by TLC of the complete 0–24-h urine collected from each volunteer showed the presence of five areas of radioactivity at various distances along the plate from the origin to the solvent front. This pattern was identical for both subjects. For identification purposes the R_F obtained in all three TLC solvents systems are given in the order A, B and C.

Table 1. Excretion of radioactivity by two adult male subjects following the oral administration of [^{35}S]-phenothiazine (6 mg/kg body weight).

	Subject 1		Subject 2	
Period	Urine	Faeces	Urine	Faeces
Day 1				
0–3 h	8.4		10.2	
3–6 h	8.0		7.2	
6–9 h	3.3		4.8	
9–12 h	2.0		2.0	
12–18 h	2.7		1.8	
18–24 h	1.3		1.2	
Day 1	25.7	19.6	27.2	28.8
Day 2	3.6	32.0	4.2	29.8
Day 3	0.9	14.0	1.1	6.7
Day 4	0.2	4.2	0.1	0.3
Day 5	0.1	0.1	0.1	0.1
Subtotal	30.5	69.9	32.7	65.7
Total	100.4		98.4	

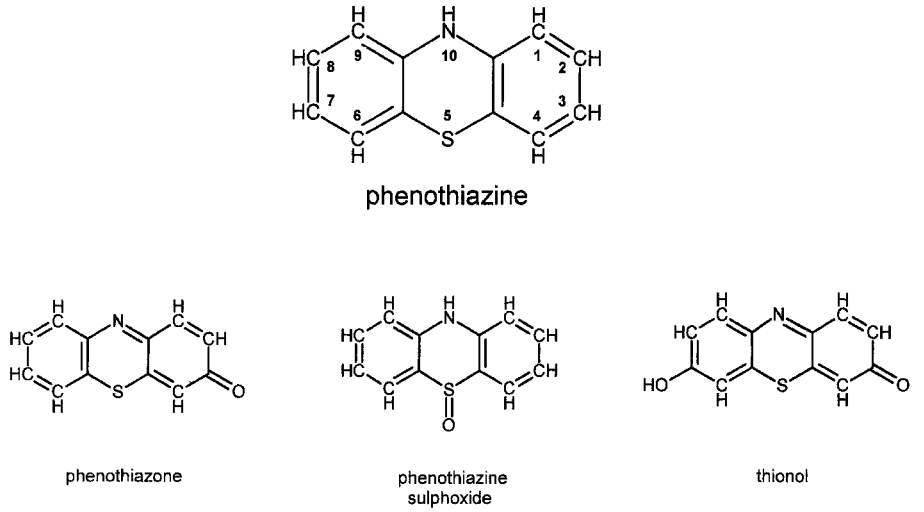

Figure 1. Structure of the anthelmintic, phenothiazine, and its oxidized metabolites, phenothiazone (phenothiaz-3-one), phenothiazine sulphoxide (phenothiazine-5-oxide) and thionol (7-hydroxyphenothiaz-3-one).

Area 1 (R_F *0.0, 0.0, 0.0) (conjugated material)*

The majority of radioactivity within the urine (91.0, 92.3%) remained on the TLC plate origin, where the urine sample had been applied, in all of the solvent systems examined. Two-dimensional chromatography following *in situ* acid hydrolysis of the material on the origin, and examination of urine following enzyme hydrolysis, both demonstrated no radioactivity at the TLC plate origin but displayed concomitant increases in phenothiazone (area 4) and phenothiazine (area 5). This general pattern was confirmed by HPLC examination.

Cold butanol extraction of powdered lyophilized urine provided a radioactive product that remained on the TLC origin and was unstable in concentrations of cold HCl > 0.5 M, decomposing to yield phenothiazine and glucuronic acid only, as detected by TLC (Elliot 1959). This conjugate was tentatively assigned the structure of phenothiazine *N*-glucuronide (Mitchell and Waring 1979). Rapid cooling of concentrated urine (by lyophilization) provided a crude white precipitate that turned purple on heating in air. This precipitate provided a radioactive product that remained on the TLC origin and decomposed in warm HCl (9.7 M) to yield phenothiazone and inorganic sulphate (Mitchell and Waring 1978). Infrared analysis (bands at 1100–1200 cm^{-1}, sulphur-oxygen bond vibrations, covalent sulphate) (Bellamy 1954, Avram and Mateescu 1972) and a mass spectrum (*inter alia*, mass peak at m/z 278, M^+-16) were consistent with the tentative assignment of this conjugate as leucophenothiazone sulphate (Collier *et al.* 1943, Clare 1962, Mitchell and Waring 1979).

Area 2 (R_F *0.26, 0.19, 0.42) (thionol)*

This radioactive metabolite co-chromatographed with authentic thionol (7-hydroxyphenothiaz-3-one) in all TLC solvent systems and by HPLC (R_T 5.8 min) following methanolic elution. The pink/purple tint was decolourized by covering

the TLC spot with a saturated solution of sodium dithionite. This reduces thionol to leucothionol (3,7-dihydroxyphenothiazine) thereby removing the quinoidal chromophore. Authentic thionol behaved in an identical manner. Absorption maxima were recorded at 232, 276 and 592 nm and mass spectral analysis gave a molecular ion at m/z 229 and a fragmentation pattern identical to the authentic compound (table 2).

Area 3 (R_F 0.35, 0.13, 0.37) (phenothiazine sulphoxide)

Co-chromatography of this radioactive excretion product was obtained with phenothiazine sulphoxide (phenothiazine-5-oxide) in all TLC solvent systems and by HPLC (R_T 4.0 min) following methanolic elution. Characteristic ultraviolet absorption maxima were observed at 229, 271, 303 and 336 nm. The molecular ion obtained for this metabolite was at m/z 215 with a fragmentation pattern identical to the authentic compound (McLafferty and Stauffer 1989, Sutherland *et al.* 2001) (table 2).

Area 4 (R_F 0.87, 0.77, 0.75) (phenothiazone)

This radioactive compound was shown clearly to co-chromatograph with authentic phenothiazone (phenothiaz-3-one) in all three TLC solvent systems employed and also by HPLC (R_T 7.4 min) when eluted and examined. Absorption maxima were recorded at 236, 258, 272, 368 and 508 nm and mass spectral analysis of this material gave a molecular ion at m/z 213 and fragment ions identical to those obtained from the authentic compound (McLafferty and Stauffer 1989, Sutherland *et al.* 2001) (table 2).

Area 5 (R_F 0.96, 0.87, 0.91) (phenothiazine)

Co-chromatography with authentic phenothiazine was achieved for this radioactive metabolite in all three solvent systems used for TLC and also by HPLC (R_T 13.7 min) following elution and rechromatography. Distinctive colorations, produced by authentic phenothiazine, were also noticed with various TLC reagents; green with ammoniacal $AgNO_3$, blue with $PdCl_2$ and brown with I_2 and HgI_2. UV absorption maxima were recorded at 254 and 317 nm. Mass spectral examination gave a molecular ion at m/z 199 and an expected splitting pattern characteristic of this molecule (Shine and Mach 1965, McLafferty and Stauffer 1989) (table 2).

For both subjects, the majority of radioactivity present within the urine was in the form of conjugated material (91.0 and 92.3% of the total radioactivity in the 0–24 h urine corresponding to 23.4 and 25.1% of the administered radioactive dose). Non-conjugated metabolites together accounted for $< 10\%$ (9.0 and 7.7% in urine; 2.3 and 2.1% dose), with phenothiazine sulphoxide being the most prominent (table 3).

The only radioactive component found in methanolic extracts of faeces showed chromatographic and spectral properties identical to those of the parent compound, phenothiazine (table 2). Methanolic extraction, although efficient in removing [^{35}S]-phenothiazine added to control faeces, only removed $82.4 \pm 5.2\%$

Table 2. Chromatographic and spectral properties of phenothiazine and its derivatives.

	Chromatographic properties				Spectral properties		
	TLC R_f Solvent system					Mass spectral fragmentation (m/z)*	
Compound/metabolite	A	B	C	HPLC R_t (min)	Ultraviolet/visible absorption maxima (nm)	Molecular ion (base peak)	Other diagnostic ions
Phenothiazine	0.96	0.87	0.91	13.7	254, 317	199	198, 167, 154
Phenothiazine sulphoxide	0.35	0.13	0.37	4.0	229, 271, 303, 336	215	199, 198, 186, 167, 154
Phenothiazone	0.87	0.77	0.75	7.4	236, 258, 272, 368, 508	213	199, 197, 185/6, 135
Thionol	0.26	0.19	0.42	5.8	232, 276, 592	229	215, 213, 199, 186, 167

* Although interpretation of mass spectra may be complex, the majority of daughter ions observed might be explained by the loss of fragments to yield phenyl sulphide ($C_{12}H_{10}S$, mol. wt 186), carbazole ($C_{12}H_9N$, mol. wt 167) and diphenyl ($C_{12}H_{10}$, mol. wt 154); all degradation products readily observed during chemical reaction of phenothiazine in the presence of metal ions (e.g. Goske 1887, Orlow 1931, Shah *et al.* 1948). The ion at m/z 135 observed with phenothiazone may represent the loss of a benzene moiety (M^+, 213 – C_6H_6, 78).

Table 3. Excretion of metabolites in urine (0–24 h) following the ingestion of [^{35}S]-phenothiazine (6 mg/kg) by two male subjects

	Percentage radioactivity in urine (0-24 h)		Percentage of the administered radioactive dose	
Metabolite	Subject 1	Subject 2	Subject 1	Subject 2
Phenothiazine	2.4	1.9	0.6	0.5
Phenothiazine sulphoxide	4.0	3.3	1.0	0.9
Phenothiazone	2.1	1.5	0.6	0.4
Thionol	0.5	1.0	0.1	0.3
Phenothiazine conjugate*	53.2	62.2	13.7	16.9
Phenothiazone conjugate**	37.8	30.1	9.7	8.2
	100.0	100.0	25.7	27.2

The conjugates have been tentatively assigned the structures of *phenothiazine *N*-glucuronide and **leucophenothiazone sulphate.

of the radioactivity associated with the faeces. This meant that the chemical identity of around 10% (9.1%, 10.3%) of the radioactive material within the faeces (0–48 h) obtained from both subjects was unaccounted for; probably remaining bound to the cellulose and other polyphenolic components of the faeces. Incubation of metabolites with faecal homogenates produced no chemical alterations to phenothiazine or its derivatives except for the almost quantitative (90%+) reduction of phenothiazine sulphoxide to phenothiazine (data not shown).

Discussion

In agreement with the present results for man, other workers have reported low urinary recoveries following oral administration; 5 and 10% for sheep (Lipson 1940, Swales and Collier 1940), 14% for the horse (Swales *et al.* 1942), 23% for the rabbit (Benham 1945) and 25% for the cow (Guyton *et al.* 1976). In addition, traces of large doses of phenothiazine have been shown to remain within calves for between 5 and 15 days (Sundukov 1963) and in sheep for 14–16 days (Dobryina 1963). However, the proportion of the dose that does enter the body appears to be absorbed quickly from the upper gastrointestinal tract and then rapidly voided in the urine. The greatest rate of urinary excretion of a single dose of phenothiazine has previously been shown to occur within 0–9 h in the cow, sheep and horse (Swales and Collier 1940, Swales *et al.* 1942, Ellison and Todd 1957) and this is consistent with the findings presented here for man. Simple graphical plotting of the present 0–24-h urinary data was suggestive of a biphasic excretion pattern with an initial half-life of around 6 h (0–12 h period) and a latter half-life of 16 h (12–24-h period). However, it must be appreciated that there are only a few data points during this first day, and there are only two subjects. Recalculated urinary data from the literature gives half-life values ranging from 11 to 18 h for both the sheep and the horse (Swales and Collier 1940, Swales *et al.* 1942).

The delay in excretion via the gastrointestinal tract is indicative of a decreased transit time through the gut, perhaps assisted by enterohepatic cycling (DeEds and Thomas 1941, Davey and Innes 1942, Mitchell 1982). This cycling may be a mechanism that prolongs its residence and assists its mode of action as an anthelmintic. Local effects of phenothiazine on the gut may also produce a

decrease in gastrointestinal motility. Phenothiazine has been shown to inhibit horse serum cholinesterase (Collier 1942, Collier and Allen 1942) and interfere with neuromuscular transmission in the crab (Collier 1940b). This has led to the suggestion that phenothiazine acts, in part, by paralysing the infesting helminths, thereby causing their detachment from anchorage and promoting their passage through and expulsion from the gut (Collier 1942, Mackie and Raeburn 1952, Mackie 1953). Colonic impaction has been reported in horses that have received large doses of phenothiazine but, conversely, so has diarrhoea (Habermann *et al.* 1941, Wolfe and Dennis 1941). The two volunteers in the present study reported no gastrointestinal disturbances although gut irritation in man has been cited elsewhere (Nour El-Din 1946). Persistence of phenothiazine, observed in some animals as measured by trace amount in their urine, may be related to the relatively large doses that these animals have received (up to 1 kg in the horse; Habermann *et al.* 1941). The effective formation of a slow moving bolus within the gut and the slight but constant absorption from this reservoir mimics a multiple or continuous dosing situation which would persist for many days following the original oral administration.

The pattern of metabolism found in man is in general agreement with that found for other species examined and there is nothing unusual or unexpected in the human profile. All of the phenothiazine metabolites identified in the present study have been previously reported (Mitchell 1982). The carbon oxidation products, phenothiazone and thionol have been demonstrated in the urine of the cow, dog, gerbil, horse, mouse, pig, rabbit, rat and sheep and the sulphur oxidation product, phenothiazine sulphoxide in the cow, guinea pig and sheep (Lipson 1940, Swales and Collier 1940, Taylor and Sanderson 1940, DeEds and Thomas 1941, Swales *et al.* 1942, Collier *et al.* 1943, Clare 1947, 1962, Richardson and Todd 1958, Mitchell and Waring 1979, Mitchell 1980). Conjugated forms of phenothiazine (phenothiazine-*N*-glucuronide) and phenothiazone (leucophenothiazone sulphate) have been also previously reported in the urine from the cow, gerbil, guinea pig, hamster, pig, rat and sheep (Lipson 1940, Swales and Collier 1940, Taylor and Sanderson 1940, DeEds and Thomas 1941, Swales *et al.* 1942, Collier *et al.* 1943, Clare 1947, 1962, Richardson and Todd 1958, Mitchell and Waring 1979, Mitchell 1980). The glucuronic acid conjugate of thionol appears to be absent from human urine, unlike that of the gerbil, mouse, rabbit and rat (Benham 1945, Mitchell 1980).

Phenothiazine is the only compound that has been positively identified as being present in the faeces of cows, guinea pigs, horses, rabbits, rats and sheep, presumably unabsorbed, although trace amounts of phenothiazone and thionol have been found in rat faeces and phenothiazone and the sulphoxide in guinea pig faeces (Collier 1940a, Lipson and Gordon 1940, Swales *et al.* 1942, Richardson and Todd 1958, Waddell 1959, Mitchell and Waring 1979, Mitchell 1982). Phenothiazone and phenothiazine sulphoxide have been detected in the gut of sheep distal to the bile duct and *in vitro* incubation experiments with rumen contents have shown that phenothiazine was not converted into any detectable derivatives, except for minute traces of phenothiazone ascribed to air oxidation (Waddell 1959), suggesting that the metabolites probably entered the enteric tract via the bile (Clare 1947, Harpur *et al.* 1950a, b). Phenothiazine and thionol have been observed in bile from one human subject but the harsh chemical detection methods employed did not permit differentiation between phenothiazine and its

N-glucuronide or even between the C-oxidation products (DeEds and Thomas 1941). No such metabolites made their way into the faeces to be detected in the present study.

By analogy with its water-soluble *N*-substituted derivatives, the carbon and sulphur oxidations of phenothiazine are presumably undertaken by cytochromes P450 as these positions present favourable electromolecular environments for these enzyme systems. Delocalization of electrons from the sulphur (and nitrogen) moiety into the adjacent unsaturated tricyclic ring system decreases the nucleophilicity of this heteroatom and renders it less susceptible to oxidation by the flavin monoxygenase family of enzymes. The lipophilic nature of phenothiazine (log $P_{(\text{heptane})}$ 3.88, log $P_{(\text{octanol})}$ 4.15; Cymerman-Craig and Warburton 1956, Leo *et al.* 1971) and its negligible solubility in water (both 51 and 125 μg $100\,\text{ml}^{-1}$ quoted; Davey and Innes 1942, Cymerman-Craig and Warburton 1956), although presumably aiding its uptake and retention within the cuticle of the parasite (Lazarus and Rogers 1951), prevents *in vitro* investigations and precludes any attempts at intravenous studies. Investigations are therefore limited and any useful data obtained concerning the interaction of phenothiazine with living systems are thus welcomed.

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