The behaviour of doramectin in the gastrointestinal tract, its secretion in bile and pharmacokinetic disposition in the peripheral circulation after oral and intravenous administration to sheep

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Sheep were 'compartmentalized' by surgically implanting cannulae in the rumen, abomasum and terminal ileum with a re-entrant cannula inserted between the cystic duct and the duodenum to monitor bile secretion. Doramectin, containing a trace of [^{3}H]-doramectin, was administered both intravenously (i.v.) and intraruminally (i.r.) at a dosage of 150 μg/kg. The pharmacokinetic behaviour of [^{3}H]-labelled products was determined in these pools, and also in peripheral plasma, urine and faeces. Parent doramectin was also determined in plasma, abomasal digesta fluid and bile. Following i.r. administration, [^{3}H] compounds were almost entirely associated with particulate digesta. A 14.5 h half-life in the rumen prolonged the presence of [^{3}H] in the abomasum. Doramectin appeared to be degraded in abomasal digesta because only 24% of abomasal [^{3}H] was attributed to the parent drug. Absorption of doramectin resulted in a systemic availability of 35%, of which 1.6 and 23.6% of the dose was contained in urine and biliary secretions, respectively. Following i.v. administration, almost negligible quantities of [^{3}H] were secreted into the rumen or abomasum and only 2.7% of the dose was excreted in urine, whereas 132% was secreted in bile. This indicated that approximately one-third of biliary metabolites were enterohepatically recycled with biliary metabolites, elevating the proportion of [^{3}H] in fluid digesta in the small intestine. Passage of the i.r.-administered drug through the gastrointestinal tract (GIT) resulted in virtually complete faecal excretion of [^{3}H] within 5 days, whereas the continued secretion of i.v.-administered [^{3}H] in bile prolonged the presence of [^{3}H] in the GIT, with faecal clearance not being complete for at least 10 days. This multi-compartmental study has provided more information on the behaviour of doramectin than can be obtained from examining drug disposition in the peripheral circulation alone. With this knowledge, it is anticipated that opportunities for improving drug performance will be identified.

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INTRODUCTION

The pharmacokinetic behaviour of anthelmintic compounds has been extensively described in the peripheral plasma pool, i.e. the central compartment, and is generally regarded to be the major descriptor of drug availability and efficacy (Lanusse & Prichard, 1993; Baggot & McKellar, 1994; Oukessou *et al.*, 1999). This infers that data derived from peripheral plasma measurements reflect the quantitative or qualitative disposition of active drug and/or metabolites in other compartments, including those

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associated with parasitic infection such as the abomasum, the small and the large intestine. While all compartments are interconnected, the respective rates of exchange and volumes of distribution can differ widely and the concentration or duration of drug/metabolite in one compartment may, in fact, bear very little relationship to another. Furthermore, as the situation is dynamic, metabolites of differing intrinsic potency are being formed and excreted, and their availability at different sites is undoubtedly influenced by numerous physiological factors.

Studies with benzimidazole compounds demonstrate a complex interaction between compartments, which is described by such factors as the drug's association with digesta material (Ali & Hennessy, 1995), the physico-chemical characteristics at respective sites in the gastrointestinal tract (GIT) such as ion-trapping (Lanusse & Prichard, 1993), gastric transit time (Taylor *et al*., 1992; Ali & Hennessy, 1995), the rate and extent of absorption (Toutain *et al*., 1997), drug secretion in gastric compartments (Steel *et al*., 1986), secretion in bile (Hennessy *et al*., 1987, 1989) and re-absorption processes, including enterohepatic recycling (Hennessy *et al*., 1993) to name a few. Macrocyclic lactone compounds would be expected to display some of the same characteristics that are observed with benzimidazole compounds, albeit at differing degrees of importance. Indeed, ivermectin was shown to be almost completely associated (97%) with the particulate phase of digesta and the flow rate of this complex through the GIT significantly influenced the duration of drug absorption by host and parasites (Ali & Hennessy, 1995). Similarly, Bogan and McKellar (1988) detected no ivermectin in abomasal digesta fluid and only low concentrations of ivermectin in abomasal mucosa following its subcutaneous administration. The latter study recorded low concentrations of ivermectin in duodenal fluid, but found that concentrations of ivermectin had increased threefold in ileal digesta fluid. There was a greater concentration of ivermectin in gastric mucosa throughout the intestine, implicating drug passage across the gut wall.

Following the subcutaneous administration of ivermectin to sheep, and moxidectin to cattle, very high concentrations of administered drugs were detected in post-mortem bile samples for 19–58 days post-treatment (Bogan & McKellar, 1988; Lifschitz *et al*., 1999). However, no quantitative data for either drug secretion in bile or the contribution of biliary-derived compounds in the GIT were available. Re-absorption of biliary-secreted oxfendazole metabolites provides significant contact with parasites of the upper and lower intestine (Hennessy *et al*., 1993), and the presence of large amounts of macrocyclic lactone compounds in intestinal mucus (Bogan & McKellar, 1988; Lifschitz *et al*., 1999) could, in part, reflect the absorption of biliary metabolites. The negligible quantity of ivermectin in small intestinal digesta fluid also suggests that biliary-secreted compounds associate with digesta particulate material.

From these observations, it is evident that significant quantities of orally administered macrocyclic lactones associate with particulate digesta and that this complex functions as a reservoir for exchange of drug into digesta fluid and for absorption. Biliary secretion and recycling processes are also influential in the presentation of absorbed or parenterally administered compounds to parasites of the GIT. Apart from limited data collected at necropsy, there is no record of the quantitative and qualitative disposition of macrocyclic lactone compounds in biliary and GIT compartments throughout the residence time of the anthelmintic.

This lack of knowledge invites detailed multi-compartmental examination of macrocyclic lactone anti-parasitic compounds to provide a more complete understanding of their behaviour. This study, therefore, sought to describe the kinetic and dynamic characteristics of doramectin in parasitologically important compartments of the ruminant.

MATERIALS AND METHODS

Experimental animals

Eight Border-Leicester Merino cross-bred wether sheep aged approximately 12 months and weighing 35–40 kg were purchased from a commercial supplier. On receipt, they were assessed by a veterinarian to be in good health and were given a single oral dose of 200 μg/kg ivermectin (Ivomec, Merial, Sydney, Australia) to remove any resident worm population. The sheep were transferred to an air-conditioned room and were restrained in individual metabolism cages that were fitted with a device to allow the separate collection of urine and faeces. The sheep were offered a daily ration of 600 g equal parts lucerne:wheaten hay, which was presented evenly over 24 h by an automatic feeder. Water was provided *ad libitum*. Because doramectin was to be determined in a multi-compartmental system, the effect of surgical cannulation on doramectin disposition was of prime consideration. Therefore, once the sheep had adapted to their accommodation and diet, phase 1 studies were initiated.

Phase 1 study

The sheep were randomly divided into two groups of four and weighed. Doramectin (150 μg/kg) was administered orally to one group (oral Dectomax formulation Pfizer Animal Health) and intravenously (i.v.) into the right jugular vein of the second group. The i.v. dose was prepared by dissolving pure doramectin aqueous micelle powder in a minimal volume of organic stock solvent (Pfizer Animal Health, New York, USA), and then diluting with physiological saline to a concentration of 150 μg doramectin/0.1 mL. The formulation was sterilized by filtering through a 0.2 μm sterile pyrogen-free filter (FP 030/3; Schleicher & Schuell, Dassel, Germany). Blood samples were collected by jugular venipuncture into heparinized evacuated 'vacutainer' blood collection tubes (Bectin Dickinson, Sydney Australia) at 1, 2, 4, 6, 9, 12, 16, 24, 30, 36, 48, 56, 72, 80 and 96 h, and then 5, 6, 7, 8, 9, 10, 12, 14, 21 and 28 days after dose administration. For i.v. administration, additional

samples were taken at 5, 15 and 30 min with all samples during the first 6 h taken from the left jugular vein. Plasma was removed following centrifugation at $550 \times \boldsymbol{g}$ for 20 min and stored at -11 °C. On completion of phase 1, the animals were rested in a group pen for 2 weeks prior to surgical cannulation.

Phase 2 study

The sheep were each surgically fitted with sampling cannulae in the reticulo-rumen, the pyloric region of the abomasum and in the terminal ileum about 10 cm proximal to the ileo–caecal junction. The bile duct was ligated at the pancreas and a re-entrant cannulae of 1.575 mm inside diameter (ID) × 3.175 mm outside diameter (OD) medical grade silastic tubing (Dow Corning Corporation, Midland MI, USA) was positioned between the cystic duct and the duodenum. The flow rate of bile was continuously monitored through the exteriorized cannula section using a self-regulating positive displacement pneumatic pump described by Hennessy *et al*. (1987) and mounted on the animal's side. When the sheep had completely recovered from surgery and were consuming their daily food ration for at least 10 days, the doramectin treatments were repeated.

Dose preparation and administration

On the day prior to drug administration, the sheep were weighed and doramectin was administered by intraruminal (i.r.) injection for the group treated orally in phase 1 and by i.v. injection for the other group. Each dose was prepared for the specific animal's weight. For i.r. administration, the commercial formulation (oral Dectomax formulation) was fortified with a trace of $[^3H]$-doramectin (specific activity 1.68 mCi/mg doramectin, radiopurity $>96\%$; Pfizer Animal Health, New York, USA), with the $[^3H]$ being located at the '5' position of the doramectin molecule. The dose, which contained 6.0 μCi $[^3H]$-doramectin/kg, was administered via the rumen cannula at a rate of 150 μg doramectin/kg. The i.v.-administered dose, containing an equivalent amount of radioactivity, was prepared by dissolving pure doramectin aqueous micelle powder in a minimal volume of organic stock solvent containing $[^3H]$-doramectin and then diluting with physiological saline to give a final concentration of 150 μg doramectin/0.1 mL. The i.v. formulation was sterilized by filtering through a 0.2 μm sterile pyrogen-free filter. The appropriate dose (0.1 mL/kg) was prepared for the specific bodyweight of each animal and was injected into the left jugular vein.

Sample collection and preparation

Jugular blood, bile, rumen, abomasal and ileal digesta, as well as urine and faeces, were collected 1, 2, 4, 6, 9, 12, 16, 24, 30, 36, 48, 56, 72, 80, 96 and 120 h after dose administration. For i.v. administration, additional blood samples were taken 5, 15 and 30 min after dosing, the blood being collected from the right vein during the first 6 h. To eliminate non-representative sampling of rumen digesta prior to complete mixing of the i.r.-administered dose had occurred, rumen sampling began after 2 h. At each sampling time thereafter, multiple samples were collected from different sites within the rumen using a tubular probe and combined. Sampling from the rumen ceased after 120 h, whereas the remaining compartments continued to be sampled 6, 7, 8, 9, 10, 12, 14, 21 and 28 days after dose administration.

To minimize the possible exchange of $[^3H]$ between fluid and fibre digesta phases, all sample preparation was completed within 30 min of collection. Rumen digesta were filtered through a 0.5-mm nylon cloth to remove large, low density fibrous material. The filtrate was then weighed and centrifuged at $12\,000 \times \boldsymbol{g}$ for 20 min and the supernatant, designated as the fluid phase of digesta, was collected. The centrifuged pellet was re-suspended to the original filtrate weight with distilled water and mixed with the previously removed fibrous material. This was designated as the particulate or fibrous phase of rumen digesta. Abomasal and ileal digesta were collected directly from the sampling cannulae and were separated by centrifugation into fluid and particulate phases as described for the rumen filtrate. The flow rate of bile was continuously monitored between sampling times, the bile being collected from a three-way stopcock positioned between the output from the pump and the return to the duodenum.

The volume of urine and wet weight of faecal pellets collected between sampling time points was recorded and a representative subsample of each material was taken. Urine was stored intact, whereas faeces were mixed with about four volumes (w/v) of distilled water and macerated into a smooth paste. All collected samples were stored at -11 °C until analysis.

Determination of $[^3H]$ content in samples

Plasma, bile, urine and rumen, abomasal and ileal digesta fluid (0.5 mL) were mixed with an equal volume of distilled water and 10.0mL 'Ultima Gold' scintillant (Packard Instrument Company, Meriden, CT, USA). To solubilize rumen, abomasal, ileal particulate digesta and faeces macerate, approximately 0.4 g wet weight of the particulate material was digested for 2 h at 50 °C with 1.0 mL Soluene 350 (Packard, USA). Thereafter, 0.5 mL isopropyl alcohol was added and the samples were incubated for a further 2 h at 50°C. They were allowed to cool and were then bleached with 0.2 mL 30% hydrogen peroxide. Ten millilitres of 'Hionic Fluor' scintillant (Packard, USA) was added and the samples were stored in darkness for 72 h to dissipate chemiluminescence. The quantity of $[^3H]$ in fluid and particulate material was then determined using a Packard Tri-Carb model 2000CA scintillation spectrometer incorporating appropriate quench, chemiluminescence and recovery corrections. Recovery of $[^3H]$ from fluid and particulate material was $>$ 96%, while counting efficiency for $[^3H]$ in plasma, rumen and abomasal fluid was 45–60%. For bile, it was 15–35% and for ileal fluid it was 10–20%. The counting efficiency for digested particulate material was 45–55%. Application of the specific activity of $[^3H]$-doramectin dose allowed determination of the concentration of total $[^3H]$-labelled doramectin and metabolites per unit of the respective sample matrix.

For simplicity, combined quantities of [^{3}H]-doramectin and [^{3}H] metabolites in each sample are hereafter presented as the quantity of [^{3}H] per unit volume or weight of matrix.

High performance liquid chromatography (HPLC) analysis of doramectin

Doramectin was determined in plasma, bile and abomasal fluid after pre-extraction and derivatization following a method modified from that described by Nowakowski *et al.* (1995). Reference doramectin as well as the 22,23 dihydro, 23 hydroxy doramectin internal standard (UK-71,647) were provided by Pfizer Animal Health (USA). The derivatized samples were analysed by reverse phase HPLC using a Waters (Waters Associates, Milford, MA, USA) 4 μm Nova-Pak 3.9 × 150 mm analytical steel column. The analytes were eluted isocratically with a mobile phase of 50/30/20 (volume/volume/volume) acetonitrile/tetrahydrofuran/water at a flow rate of 1.2 mL/min using a Waters Model 510 solvent pump. The fluorescent derivatives of doramectin and the internal standard were detected using excitation and emission wavelengths of 360 and 470 nm, respectively, using a Waters Model 470 scanning fluorescence detector. Waters 'Millennium-32' data handling software was used for instrument control, data logging, peak integration and sample quantitation. The recovery of doramectin was 91–95% from plasma, 89–92% from abomasal fluid and 92–98% from bile. The assay system provided a limit of detection and quantitation of 0.06 and 0.16 ng/mL, respectively, in plasma, abomasal fluid and bile with an accuracy of estimation of 97–99%. The respective precision (% coefficient of variation) of assay at the lower and upper limits of quantitation was 0.48–5.39% for plasma, 0.49–3.22% for abomasal fluid and 2.64–4.83% for bile.

Data analysis

The pharmacokinetic parameters for [^{3}H] in rumen fluid and particulate digesta, and for [^{3}H] and doramectin in plasma were determined by fitting observed data to the pharmacokinetic modelling program 'WinNonLin' (Statistical Consulting Inc., Pharsight Corportation, Cary, NC, USA). For rumen digesta, a single compartment model with bolus input and first order output was used. For plasma, a two compartment model with first order output and micro constants as primary parameters was used for both routes of administration. For i.v. administration, a single bolus input was used, whereas for i.r. administration the absorption into the central compartment followed first order input with no lag time provided. Pharmacokinetic parameters were accepted when the correlation coefficient (r^2) of the curve fit exceeded 0.95 and the percentage coefficient of variation was within 20%. In other compartments, C_{max} and t_{max} were observed values and the area under the concentration with time curve (*AUC*) was calculated using the trapezoidal method.

The secretion rate of [^{3}H] and doramectin in bile at each time point was the product of the concentration and the mean biliary flow rate during the preceding and following sampling periods. The integral of secretion rate with time provided the total quantity of [^{3}H] and doramectin, which was secreted in bile over the experimental period.

The Student's t-test was used to examine differences in pharmacokinetic parameters for doramectin in plasma for each dose route between phases 1 and 2. A value of $P < 0.05$ was considered significant.

RESULTS

Phase 1

The mean disposition of doramectin in peripheral plasma following oral and i.v. administration is shown in Table 1. Following i.v. administration, doramectin was rapidly distributed ($t_{1/2\alpha}$ of 0.99 h) through a volume of 5.1 L/kg, reaching a C_{max} of 139.8 ng/mL. The terminal elimination half-life ($t_{1/2\beta}$) was slower at 64.9 h, resulting in an *AUC* of 3215 ng·h/mL. Following oral administration, doramectin was absorbed at a half-life ($K_{01}t_{1/2}$) of 17.2 h, reaching a C_{max} of 6.81 ng/mL after 27 h. The extended absorption process from the GIT resulted in a distribution half-life ($t_{1/2\alpha}$) of 16.1 h with a longer elimination half-life ($t_{1/2\beta}$) of 128.9 h. The mean *AUC* for orally administered doramectin was 797 ng·h/mL, reflecting a systemic availability (F) of 25%.

Phase 2

Disposition in the rumen. Only total [^{3}H] metabolites were determined in rumen digesta; summary data are presented in Table 2, while Fig. 1a and b shows the mean concentration with time profiles for the two dose routes and digesta phases. From as early as 2 h after i.r. administration, over 90% of [^{3}H] was associated with particulate digesta, this association exceeding 97% within 72 h. Fluid and particulate-associated [^{3}H] flowed from the rumen with half-lives of 10.7 and 14.5 h, respectively, resulting in mean residence times (MRT) of 15.5 and 21.0 h, respectively. Very low concentrations of [^{3}H] detected in rumen fluid and particulate material following i.v. administration indicated that there was minimal secretion of absorbed [^{3}H] metabolites into the rumen (Fig. 1a and b). By 7 days after i.r. and i.v. administration, the concentration of [^{3}H] in rumen fluid and particulate digesta had decreased to negligible levels.

Disposition in the abomasum. Table 3 presents the concentration of doramectin in abomasal fluid and [^{3}H] in abomasal particulate digesta. The concentration with time profiles for each of these two parameters are shown in Fig. 2a and b, respectively. The large percentage of i.r. dose, which associated with particulate digesta and flowed from the rumen, resulted in a maximum [^{3}H] concentration of 475 ng/g wet weight of abomasal particulate digesta within 12 h of administration. Only a small proportion of [^{3}H] in the abomasum was present in the fluid phase, reaching a maximum concentration of 32 ng/mL 6 h after drug

Table 1. Mean ($\pm$SD) pharmacokinetic disposition ($\pm$SD) of doramectin in peripheral plasma following oral and i.v. administration of 150 μg doramectin/kg in phase 1 and of [^{3}H] and doramectin following i.r. and i.v. administration in phase 2. Parameters are generated from data fitted to a two-compartment model with first-order (i.r. and oral) or bolus (i.v.) input and first-order output using the WinNonLin pharmacpokinetic modelling program

Parameter	Phase 1 (oral)	Phase 2 (i.r.)		Phase 1 (i.v.)	Phase 2 (i.v.)	
	Doramectin	[^{3}H]	Doramectin	Doramectin	[^{3}H]	Doramectin
$K_{01}t_{1/2}$ (h)	17.17 ± 2.62	11.59 ± 4.39	17.75 ± 4.86	–	–	–
C_{max} (ng/mL)	6.81 ± 1.38	6.31 ± 1.59	4.01 ± 1.53	139.76 ± 70.17	214.12 ± 79.84	147.54 ± 36.78
t_{max} (h)	26.84 ± 4.77	32.21 ± 11.14	31.95 ± 10.93	0	0	0
AUC (ng · h/mL)	797.2 ± 281.7	1300.6 ± 162.2	710.8 ± 198.4	3215.0 ± 683.8	3630.8 ± 1225.4	2036.2 ± 548.6
$t_{1/2\alpha}$ (h)	16.09 ± 3.02	0.76	15.71 ± 2.02	0.99 ± 0.53	0.71 ± 0.59	1.18 ± 1.15
$t_{1/2\beta}$ (h)	128.86 ± 4.06	164.27 ± 34.13	183.01 ± 87.01	64.87 ± 16.80	$88.93 \pm 31\ 23$	78.56 ± 38.77
V_{ss} (L/kg)	–	–	–	5.07 ± 1.49	5.15 ± 0.82	5.72 ± 1.68
MRT (h)	–	–	–		123.50 ± 44.60	101.2 ± 46.4
$AUC_{dor}/AUC_{[3H]}$		0.54 ± 0.10			0.63 ± 0.09	
F (%)	25	35	34	–	–	–

$K_{01}t_{1/2}$, half-life of absorption; C_{max}, maximum concentration; t_{max}, time of C_{max}; AUC, area under concentration with time curve; $t_{1/2\alpha}$, half-life of α elimination phase; $t_{1/2\beta}$, half-life of β elimination phase; V_{ss}, volume of distribution; MRT, mean residence time; F, systemic availability. For each route of administration (i.v. or oral/i.r.), there were no significant differences for each parameter of doramectin between phases 1 and 2.

administration. Over the experimental period, 96% of [^{3}H] in abomasal digesta was associated with particulate matter. Of the amount of [^{3}H] present in abomasal fluid, about 24% was attributed to doramectin (i.e. about 1% of the total radioactivity).

As is shown in Fig. 2a and b and in Table 3, negligible amounts of [^{3}H] were secreted into the abomasum following i.v. administration, which is indicated by the fact that only low concentrations of [^{3}H] were found in abomasal fluid (3.74 ng/mL) and particulate material (5.83 ng/g wet weight). Furthermore, only 6% of the AUC of [^{3}H] in abomasal fluid was attributable to the AUC of doramectin.

Disposition in plasma. Summary data of [^{3}H] and doramectin following i.r. and i.v. administration are presented in Table 1 and Fig. 3a and b. Following i.r. administration, total [^{3}H]-labelled metabolites appeared rapidly in plasma, displaying an absorption half-life ($K_{01}t_{1/2}$) of 11.6 h and reaching a C_{max} of 6.3 ng/mL within 32 h. The biphasic elimination profile indicated that [^{3}H] was distributed through two compartments; in addition, while the distribution half-life ($t_{1/2\alpha}$) of [^{3}H] was very rapid (0.8 h), the elimination half-lives ($t_{1/2\beta}$) of [^{3}H] and doramectin were much longer (164 and 183 h, respectively). The mean AUC for [^{3}H] was 1301 ng·h/mL, of which 54% was attributed to doramectin.

Following i.v. administration, the [^{3}H] profile in plasma, as is shown in Fig. 3b, had a $t_{1/2\alpha}$ of 0.71 h and a $t_{1/2\beta}$ of 88.9 h, describing a mean AUC of [^{3}H] of 3631 ng·h/mL distributed through a volume of 5.2 L/kg. The mean AUC of doramectin was 2036 ng·h/mL; within the first few hours of administration, the parent drug contributed to almost all of the [^{3}H] AUC. However, by 24 h, this contribution had decreased to about 63% of [^{3}H]-labelled metabolites in plasma. Without the continued absorption of drug from the passage of drug–digesta complex from the rumen, the terminal elimination half-lives of [^{3}H] and doramectin were of a shorter duration following i.v. administration compared with i.r. administration.

Comparison of the mean $AUC_{oral}/AUC_{i.v.}$ for sheep administered the same 150 μg/kg dosage indicated a 35% systemic availability of [^{3}H], which was similar to that observed (34%) for doramectin. There were no statistically significant differences between phase 1 and phase 2 studies for any parameter of doramectin, for either (i.v. or i.r./oral) dose routes.

Table 2. Mean ($\pm$SD) of [^{3}H] in rumen fluid and particulate digesta following i.r. administration of 150 μg doramectin/kg. Parameters are generated from data fitted to a single compartment model with bolus input and first-order output using the WinNonLin pharmacpokinetic modelling program

Parameter	Rumen fluid	Rumen particulate material
$K_{01}t_{1/2}$ (h)	10.71 ± 5.73	14.54 ± 5.21
C_{max}		
ng/mL fluid	76.88 ± 25.07	
ng/g wet weight particulates		2767.4 ± 877.9
MRT (h)	15.45 ± 8.27	20.98 ± 7.52
AUC		
ng · h/mL fluid	1070.7 ± 255.5	
ng/h/g wet weight particulates		$62\ 159 \pm 39050$
Total [^{3}H] (%)		
In fluid	2.08 ± 0.97	
In particulates		97.92 ± 0.87

$K_{01}t_{1/2}$, half-life of absorption; C_{max}, maximum concentration; t_{max}, time of C_{max}; AUC, area under concentration with time curve; MRT, mean residence time.

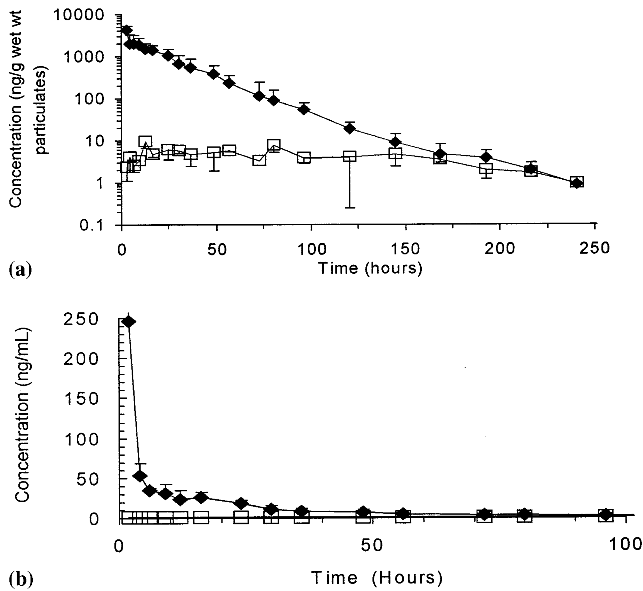

Fig. 1. (a) Mean concentration with time profiles of [^{3}H] in rumen particulate digesta following i.v. (□) and i.r. (◆) administration of 150 μg [^{3}H]-doramectin/kg to sheep. SD shown as error bars. (b) Mean concentration with time profiles of [^{3}H] in rumen fluid digesta following i.v. (□) and i.r. (◆) administration of 150 μg [^{3}H]-doramectin/kg to sheep. SD shown as error bars.

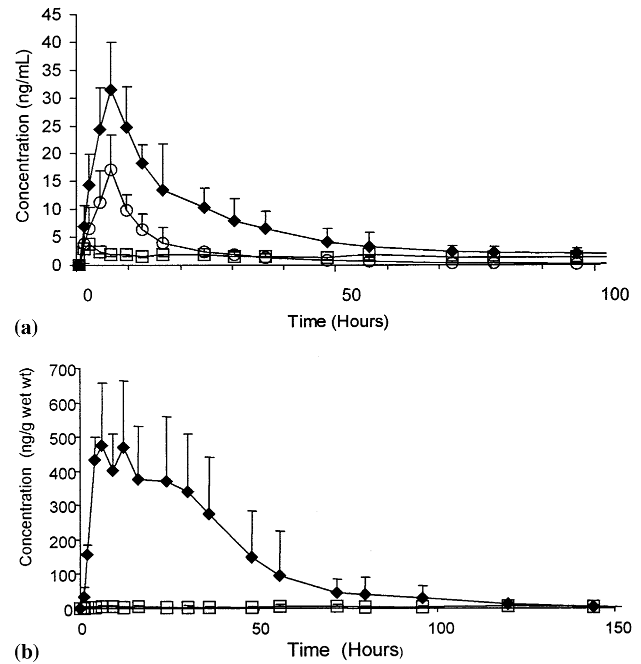

Fig. 2. (a) Mean concentration with time profiles of [^{3}H] (◆) and doramectin (○) following i.r. administration, and [^{3}H] (□) following i.v. administration in abomasal fluid digesta of 150 μg [^{3}H]-doramectin/kg to sheep. SD shown as error bars. (b) Mean concentration with time profiles of [^{3}H] in abomasal particulate digesta following i.r. (◆) and i.v. (□) administration of 150 μg [^{3}H]-doramectin/kg to sheep. SD shown as error bars.

Secretion in bile. With the exception of an occasional high or low value, the flow rate of bile during the experimental period, and for the two routes of drug administration, was 0.62–1.52 mL/min. Table 4 provides details of the secretion of [^{3}H] and

Table 3. Mean (±SD) disposition kinetics of [^{3}H] and doramectin in abomasal fluid and [^{3}H] in abomasal particulate digesta following i.r. and i.v. administration of 150 μg doramectin/kg

Parameter	Fluid digesta		Particulate digesta
	[^{3}H]	Doramectin	[^{3}H]
i.r. administration			
C_{max}			
ng/mL fluid	31.55 ± 8.45	17.14 ± 6.29	
ng/g wet weight particulates			475.45 ± 183.67
t_{max} (h)	6.34 ± 1.91	6.34 ± 1.91	11.57 ± 8.88
AUC			
ng · h/mL fluid	31.55 ± 8.45	17.14 ± 6.29	
ng · h/g wet weight particulate			19 690.8 ± 9971.3
$AUC_{doramectin}/AUC_{[3H]}$	0.24		–
i.r. administration			
C_{max}			
ng/mL fluid	3.74 ± 0.31	<0.05	
ng/g wet weight particulates			5.83 ± 3.52
t_{max} (h)	3.25 ± 3.61	7	38.32 ± 30.88
AUC			
ng · h/mL fluid	343.5 ± 42.5	22	
ng · h/g wet weight particulate			998.3 500.1
$AUC_{fluid}/AUC_{particulates}$	0.06		–

C_{max}, maximum concentration; t_{max}, time of C_{max}; *AUC*, area under concentration with time curve.

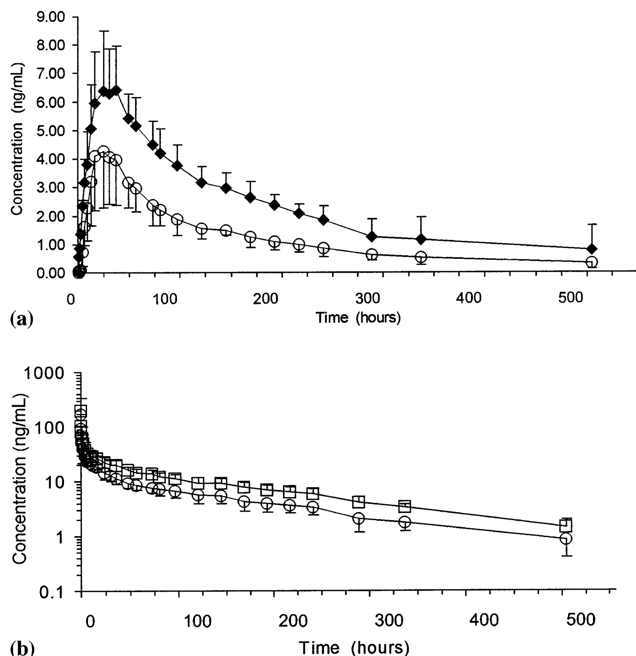

Fig. 3. (a) Mean concentration with time profile of [^{3}H] (◆) and doramectin (○) in plasma following i.r. administration of 150 µg [^{3}H]-doramectin/kg to sheep. SD shown as error bars. (b) Mean concentration with time profile of [^{3}H] (□) and doramectin (○) in plasma following i.v. administration of 150 µg [^{3}H]-doramectin/kg to sheep. SD shown as error bars.

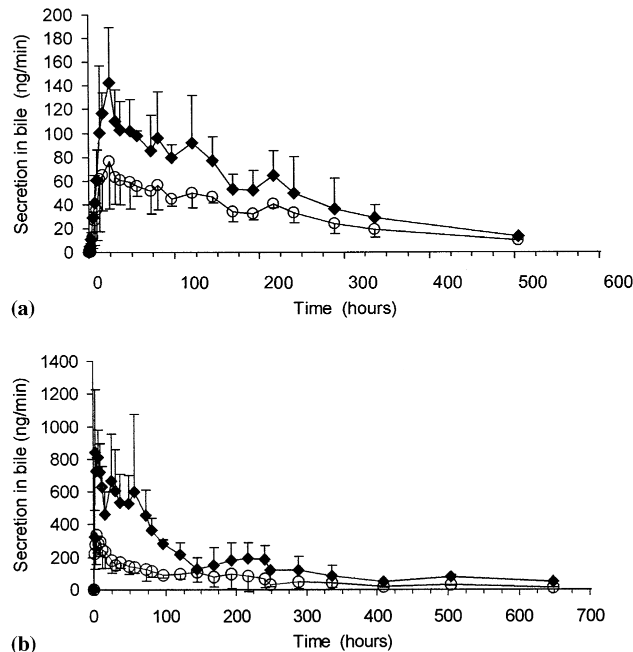

Fig. 4. (a) Mean secretion with time profile of [^{3}H] (◆) and doramectin (○) in bile following i.r. administration of 150 µg [^{3}H]-doramectin/kg to sheep. SD shown as error bars. (b) Mean secretion with time profile of [^{3}H] (◆) and doramectin (○) in bile following i.v. administration of 150 µg [^{3}H]-doramectin/kg to sheep. SD shown as error bars.

doramectin in bile. The mean secretion with time profiles following i.r. and i.v. administration are presented in Fig. 4a and b, respectively. Following i.r. administration, the [^{3}H] metabolites appeared rapidly in bile, reaching a maximum concentration of 195.4 ± 28.8 ng/mL by 30 h. Integrating bile flow rate and [^{3}H] concentration resulted in a maximum [^{3}H] secretion of 143.1 ± 46.1 ng/min, with a total of 23.6 ± 6.7% of the i.r.-administered [^{3}H] dose being secreted in bile over the experimental period. Doramectin followed a similar time profile as [^{3}H], with the maximum concentration of doramectin in bile (131.2 ± 32.7 ng/mL) similarly occurring 30 h after administration. Over the time course of the experiment, doramectin comprised up to 60 ± 7% of the quantity of [^{3}H] secreted in bile or 16% of the i.r.-administered doramectin dose.

Following i.v. administration, there was an exceptionally rapid appearance of [^{3}H] and doramectin in bile, reaching a $C_{\max}$ of 839.2 and 431.7 ng/mL after 10.9 and 13.1 h, respectively (Table 4; Fig 4b). The rate of secretion of both entities decreased progressively to about 140 h, but then appeared to slow over the remainder of the experimental period. Over the experimental period, 132.0 ± 20.8% of the i.v.-administered [^{3}H] dose was secreted in bile, of which 58 ± 6%, amounting to 70% of the dose, was attributable to doramectin.

Disposition in ileal digesta. Only [^{3}H] was determined in ileal digesta; the mean disposition is shown in Table 5 and Fig. 5a and b. Comminution of digesta in the small intestine resulted in the ileal digesta being of fine particulate size, but of a more solid consistency than rumen or abomasal material. Very high con-

Table 4. Mean (± SD) disposition kinetics of [^{3}H] and doramectin in bile following i.r. and i.v. administration of 150 µg doramectin/kg

Parameter	i.v. administration		i.r. administration	
	[^{3}H]	Doramectin	[^{3}H]	Doramectin
$C_{\max}$ (ng/mL)	839.17 ± 142.44	431.70 ± 182.66	195.38 ± 28.77	131.19 ± 32.71
Maximum secretion rate (ng/min)	810.9 ± 169.7	335.1 ± 106.5	143.1 ± 46.1	76.56 ± 39.87
$t_{\max}$ (h)	10.9 ± 8.4	13.1 ± 2.3	30.0 ± 5.9	30.0 ± 5.9
Dose secreted (%)	132.02 ± 20.79	69.99 ± 12.69	23.55 ± 6.68	15.88 ± 3.01
$\text{Conc}_{\text{doramectin}}$/conc[^{3}H]	0.58 ± 0.06		0.60 ± 0.07	

$C_{\max}$, maximum concentration; $t_{\max}$, time of $C_{\max}$.

centrations of [^{3}H] in both fluid (292 ± 134 ng/mL) and particulate (824 ± 172 ng/g wet weight particulate digesta), respectively, were detected following i.r. administration, the C_{max} of [^{3}H] occurring earlier in fluid (18 h) than in particulate (25 h). In ileal digesta, 27% of [^{3}H] was in the fluid phase compared with only 4% in abomasal fluid. While almost negligible quantities of [^{3}H] were present in abomasal fluid or particulate digesta following i.v. administration, [^{3}H] appeared rapidly in ileal fluid with [^{3}H] C_{max} (129 ng/mL) occurring within 5.4 h of administration while particulate-associated [^{3}H] C_{max} (246 ng/g wet weight digesta) occurred 11.2 h after drug administration.

Table 5. Mean (± SD) disposition kinetics of [^{3}H] in ileal digesta following i.r. and i.v. administration of 150 μg doramectin/kg

Parameter	i.r. administration	i.v. administration
Fluid digesta		
C_{max} (ng/mL)	292.47 ± 134.12	129.94 ± 109.08
t_{max} (h)	17.91 ± 15.67	5.43 ± 3.21
AUC (ng · h/mL)	12 517.1 ± 5536.6	9389.1 ± 1906.4
Particulate digesta		
C_{max} (ng/g wet weight particulates)	824.13 ± 171.99	248.77 ± 94.77
t_{max} (h)	25.1 ± 10.0	11.2 ± 4.9
AUC (ng · h/g wet weight particulates)	46 898.8 ± 27 437.3	22 995.9 ± 7230.8
$AUC_{fluid}/AUC_{particulates}$	0.27	0.41

C_{max}, maximum concentration; t_{max}, time of C_{max}; AUC, area under concentration with time curve.

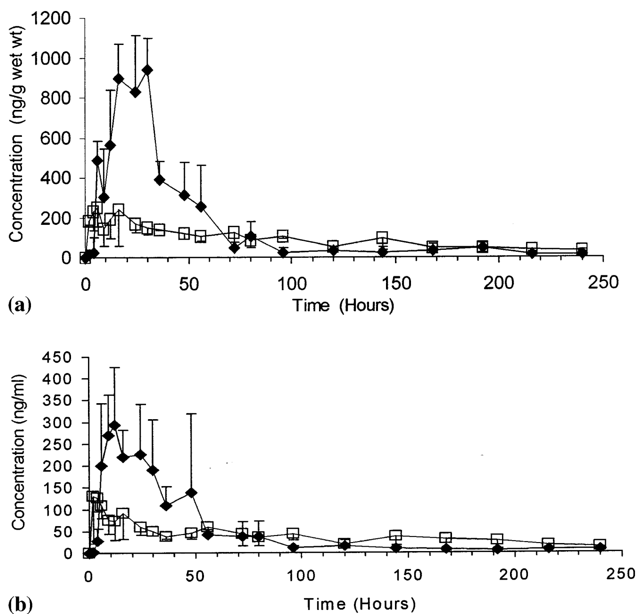

Fig. 5. (a) Mean concentration with time profiles of [^{3}H] in ileal particulate digesta following i.v. (□) and i.r. (◆) administration of 150 μg [^{3}H]-doramectin/kg to sheep. SD shown as error bars. (b) Mean concentration with time profiles of [^{3}H] in ileal fluid digesta following i.v. (□) and i.r. (◆) administration of 150 μg [^{3}H]-doramectin/kg to sheep. SD shown as error bars

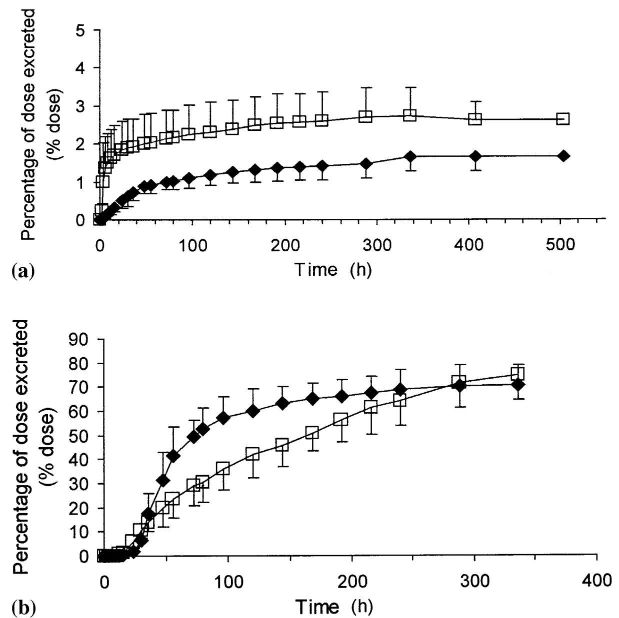

Fig. 6. (a) Mean cumulative excretion of [^{3}H] in urine following i.v. (□) and i.r. (◆) administration of 150 μg [^{3}H]-doramectin/kg to sheep. SD shown as error bars. (b). Mean cumulative excretion of [^{3}H] in faeces following i.v. (□) and i.r. (◆) administration of 150 μg [^{3}H]-doramectin/kg to sheep. SD shown as error bars.

Following i.v. administration, the proportion of [^{3}H] in the fluid phase increased to 41%.

Excretion in urine and faeces. Renal clearance of [^{3}H] constituted a minor elimination pathway as only 1.6 ± 0.4% of the i.r.-administered and 2.7 ± 0.8% of the i.v.-administered [^{3}H] dose was found in urine. Most of the dose was cleared within 5 days of treatment (Fig. 6a). Faecal excretion was the predominant clearance route. A mean of 73.1 ± 7.5% of the i.r.-administered [^{3}H] was excreted in faeces, mostly 48–56 h after drug administration, with faecal excretion of [^{3}H] being about 90% complete by day 5 (Fig. 6b). Over the experimental period, a similar percentage (75.0 ± 10.3%) of the i.v.-administered [^{3}H] dose was excreted; however, the rate of clearance was more protracted as 90% excretion of the i.v.-administered [^{3}H] dose was not achieved until about 10 days had elapsed.

DISCUSSION

The pharmacokinetic disposition of doramectin in the peripheral plasma of cattle following intramuscular, subcutaneous and pour-on administration has been well documented (Wicks *et al.*, 1993; Nowakowski *et al.*, 1995; Lanusse *et al.*, 1997; Toutain *et al.*, 1997). However, those studies provide no information on the disposition of doramectin or its metabolic products in relation to other areas of physiological and parasitological importance. The present study used cannulated sheep as a model to provide a multi-compartmental description of doramectin over a 28-day residence time. This investigation is unique because

disposition in such a multi-cannulated animal has not been previously described for any anthelmintic compound, in any species. Of primary concern was the potential for the surgical intervention to adversely affect the general physiological condition of the sheep and, in turn, the disposition of doramectin. This concern was addressed in phase 1 of the study where the same animals (prior to cannulation) functioned as 'controls' to permit identification of any effect of cannulation on doramectin kinetics. Orally administered doramectin residence was considerably longer than that following i.v. administration; this is probably due to the extended absorption of doramectin from the digesta–drug complex as it flowed from the rumen. The phase 1 i.v.-administered results are in agreement with Gottschall's (1997) calculation of 5546 ng·h/mL for doramectin *AUC* following an i.v. dose of 300 μg/kg. While it cannot be assumed that there is a linear relationship between dosage and *AUC*, the *AUC* of 3215 ng·h/mL from the 150 μg/kg dose in phase 1 approximates half of the *AUC* calculated by Gottschall (1997).

In phase 2, i.r.-administered doramectin was almost completely associated with particulate digesta in the rumen and the 14.5-h half-life of this drug–digesta complex is consistent with the 12-h half-life of rumen particulate digesta determined by Ali and Hennessy (1995). After about seven half-lives (80–90 h), the concentration of fluid and particulate-associated [^{3}H] had decreased to negligible concentrations and it is apparent that the duration of presentation of orally administered doramectin to distal sites in the GIT is largely influenced by the passage of the particulate–drug complex from the rumen. It is not clear whether doramectin is absorbed from the rumen, but this is probably unlikely. Rumen function is largely the reduction of ingested cellulose and only small molecular weight substances, such as carbon dioxide, volatile fatty acids, sodium and ammonia, are known to be absorbed (Dobson, 1967). Not only does the large molecular size of doramectin make its absorption from the rumen unlikely, but the C_{max} of doramectin in plasma was not observed until 30–36 h after administration, a time when about 85% of the particulate-associated drug would have flowed from the rumen. The minimal amount of [^{3}H] associated with rumen particulate matter within 6-h of i.v. administration and the observation that these concentrations followed a similar time course as [^{3}H] in plasma suggest that parenterally administered doramectin does enter the rumen. However, the rumen is not regarded as a secretory organ and the presence of parenterally administered [^{3}H] may be due to entry in saliva.

Passage of doramectin associated with particulate digesta into the abomasum resulted in very high concentrations of [^{3}H] in abomasal particulate digesta within 12-h of drug administration. Again, this is consistent with the half-life of rumen particulate digesta, during which time about half of the i.r.-administered drug will have entered the abomasum, almost entirely associated with particulate material. Compared with the rumen, the lower pH in the abomasum increases the solubility of the virtually insoluble benzimidazole compounds (Lanusse & Prichard, 1993); however, abomasal pH did not facilitate exchange of [^{3}H] from particulate into fluid digesta. Most (96%) [^{3}H] in the present study remained in the particulate phase of the abomasal digesta. This complex is not available for direct uptake by the host or parasites, but most likely functions as a reservoir for exchange of drug into fluid. It is of interest to note that, in abomasal fluid, doramectin accounted for $<50\%$ of [^{3}H] within 36 h of drug administration, with this proportion decreasing thereafter. Hepatically metabolized [^{3}H] is unlikely to account for this observation because only minimal quantities of absorbed [^{3}H] are secreted into the abomasum. This suggests that doramectin may undergo some degradation in the rumen and/or abomasum, as reported by Prichard *et al.* (1985) for ivermectin.

It is significant that following i.v. administration there was very little [^{3}H] detected in abomasal digesta, with the total quantity (as [^{3}H] *AUC*) amounting to $<4\%$ of the [^{3}H] *AUC* determined in abomasal digesta following i.r. administration. Notwithstanding this, about 40% of [^{3}H] in abomasal digesta was in the fluid phase, a much greater proportion than following i.r. administration. This implicates the entry of [^{3}H] in gastric secretions rather than through passage of the small quantity, almost exclusively particulate-bound, from the rumen. The minimal amount of i.v.-administered [^{3}H] in gastric secretions is consistent with Bogan and McKellar's (1988) inability to detect ivermectin in the abomasal fluid of sheep, despite the subcutaneous administration of ten times the recommended dose. Those authors did report high concentrations of ivermectin in abomasal mucosa, indicating that absorbed or parenterally administered macrocyclic lactone compounds largely come into contact with parasites during passage across the abomasal mucosa. It does not preclude the possibility of biotransformation or degradation in the abomasal lumen.

The extended flow of drug–particulate complex from the rumen to the intestine prolonged the duration for absorption and, as might be expected, subsequently prolonged the elimination from plasma. Following i.r. administration, half-lives of 6.8 and 7.6 days were observed for [^{3}H] and doramectin, respectively. By way of comparison, elimination half-lives of 3.7 and 3.3 days for [^{3}H] and doramectin, respectively, were recorded following i.v. administration in this study, while a half-life of 4.5 days was recorded for doramectin following intramuscular administration in sheep (Gottschall, 1997). This half-life difference reflects the continued absorption of doramectin from the GIT following oral administration. Comparison of [^{3}H] and doramectin *AUC* following the two dose routes resulted in a systemic availability of 35 and 34%, respectively. Although a slightly elevated *AUC* of doramectin following i.v. administration in phase 1 studies was observed, this difference was not significant. No differences in the pharmacokinetic parameters for doramectin for either dose route were found in the phase 1 (pre-surgery) and 2 (post-surgery) studies. This provides sound evidence that surgical fitting of cannulae did not significantly affect doramectin behaviour. It is notable that doramectin *AUC* accounted for 54–63% of the [^{3}H] *AUC* in plasma by either route of administration, which is consistent with the 48 and 54% of [^{3}H] in liver and muscle, respectively, that Gottschall (1997) attributed to doramectin. This suggests considerable metabolism, but it is not clear whether the proportion of

doramectin and metabolic products in plasma are derived from direct absorption from the GIT or by systemic metabolism following absorption of the parent drug.

Large molecular weight compounds (> 300) are generally cleared in bile rather than in urine (Baggot 1988). Bogan and McKellar (1988) reported high concentrations of ivermectin in the bile of necropsied sheep and cattle, and recently Lifschitz *et al.* (1999) described the presence of moxidectin in the bile of necropsied cattle. Prior to the present study, this biliary presence was only confirmed in post-mortem samples and there has been no description of the absolute quantity, nor the secretion profile, of macrocyclic lactone compounds in bile throughout the residence time of the drug. This is surprising because the biliary route has been demonstrated to be a major route of elimination of oxfendazole (Hennessy *et al.*, 1985), triclabendazole (Hennessy *et al.*, 1987) and albendazole (Hennessy *et al.*, 1989). Lanusse *et al.* (1997) and Toutain *et al.* (1997) also identified biliary secretion as an important clearance pathway for macrocyclic lactone compounds. Estimation of biliary-secreted drugs is conventionally determined by withdrawing bile from a 'T' cannula implanted in the cystic duct; however, this provides no indication of bile flow rate or quantitative secretion of contained compounds. Acute preparations in which all bile is drained from an anaethesized animal eliminates any contribution of enterohepatic recycling. Secretion of bile is also influenced by the level of feed intake; however, by regulating feed intake in the present experiments, bile flow rate was relatively constant at about 1.0 mL/min, a rate that was consistent with previous observations using the same cannulation/pump technique (Hennessy *et al.*, 1993). Following i.r. administration, the secretion of [^{3}H] in bile reached a maximum by 24–30 h and followed a similar time course, but at a much greater concentration than that in plasma. For example, by 240 h after i.r. administration, when plasma concentrations of [^{3}H] had reduced to < 2 ng/mL, [^{3}H] was still being secreted in bile at a rate of 50 ng/min. The extended high concentration in bile is probably influenced by exchange of [^{3}H] from lipid reserves and the contribution of enterohepatic recycling into a relatively small volume of distribution.

Biliary secretion of 24% of the i.r.-administered dose was found in this study and considering that the systemic availability of [^{3}H] was 35%, this route is the major pathway for the clearance of doramectin. The similar doramectin:[^{3}H] concentration ratio in plasma and bile suggests that there was no further significant metabolism of doramectin prior to secretion in bile and lends further support to the suggestion that most doramectin metabolism occurs in the GIT lumen. In comparison, a similar proportion of a fenbendazole dose is secreted in bile; however, fenbendazole undergoes extensive hepatic metabolism, with its metabolite profile in bile being vastly different to that in plasma (Hennessy *et al.*, 1993).

Extremely high concentrations of [^{3}H] were present in bile within 4 h of i.v. administration. Similar to i.r. administration, [^{3}H] concentration in plasma had reduced to below 10 ng/mL after 240 h, whereas [^{3}H] was still being secreted in bile at a rate in excess of 100 ng/min. Excluding the amount of [^{3}H] contained in urine, abomasal (gastric) secretions, saliva and other non-specific secretions, as much as one-third of the 132% of the i.v. dose secreted in bile is enterohepatically recycled. Relating this observation to i.r. administration, some 8% of the 24% of dose secreted in bile may have been enterohepatically recycled. Describing the disposition of fenbendazole, Hennessy *et al.* (1993) demonstrated the minimal exchange of enterohepatically recycled fenbendazole metabolites from the bile:GIT:portal circulation 'pool' with peripheral plasma. This behaviour may similarly occur with doramectin and may explain, at least in part, the differences between biliary and circulating plasma concentrations.

Following i.r. administration, approximately 28% of [^{3}H] was present in the fluid phase of ileal digesta. This is in contrast with only 4% of [^{3}H] found in fluid flowing from the abomasum; the difference can be largely attributed to the secretion of [^{3}H] in bile. While some biliary-secreted [^{3}H] would presumably associate with particulate matter in the small intestine, this association did not seem to be as rapid or as complete as in more proximal regions of the GIT. This was more obvious following i.v. administration, where about half of the [^{3}H] remained in fluid at the terminal ileum, and is consistent with the threefold increase in ivermectin content found in ileal compared with abomasal fluid described by Bogan and McKellar (1988). Comminution of digesta in the small intestine resulted in very fine particulate material in ileal fluid and the high surface area of this material may be expected to associate large amounts of [^{3}H] and contribute to the high proportion of [^{3}H] in the 'fluid' phase. Nevertheless, the large proportion of [^{3}H] in intestinal fluid over an extended period, attributed to biliary secretion and enterohepatic cycling, is a source of drug for absorption by host and parasites and may assist in explaining the high and sustained activity of doramectin against intestinal worms (Vercruysse *et al.*, 1998).

Biliary elimination was the predominant excretion pathway, as urinary clearance was found to be unimportant. Even though the kinetic and dynamic relationship of [^{3}H] with digesta material differed throughout the GIT, the quantitative excretory balance was similar following either administration route. Following i.r. administration, the delay of about 24 h before faecal [^{3}H] was detected was consistent with the time for digesta to traverse the GIT. Because rumen digesta empties with a half-life of 12–14 h (Ali & Hennessy, 1995), the virtual complete passage of digesta-associated drug from the rumen occurred after seven half-lives, with the bulk of the drug–particulate complex being excreted within 4–5 days. The slower excretion over the following 5–14 days may be due to protracted clearance of lipid-associated drug via the bile, recalling that as long as 14 days after i.r. administration [^{3}H] was still being secreted in bile at a rate of some 30 ng/min. Following i.v. administration, the passage of digesta from the upper small intestine site of biliary secretion resulted in the presence of [^{3}H] in faeces within 6 h. Thereafter, the prolonged secretion of [^{3}H] in bile was reflected in a slower, but sustained, rate of excretion of [^{3}H] in faeces. It was not until some 14 days after i.v. administration, when the biliary secretion of [^{3}H] was almost

complete, that the cumulative faecal clearance profile plateaued at the same quantity as that following i.r. administration.

This comprehensive study has provided extensive information on the quantitative and qualitative behaviour of doramectin in the ruminant animal, information that cannot be obtained from examination of the disposition in peripheral blood alone. The broad assumption that drug disposition in the peripheral plasma pool reflects drug and/or metabolite concentration and activity at sites of parasitological importance, particularly at different sites in the GIT, has been shown not to be necessarily accurate. It is envisaged that studies of this type will be conducted more widely to assist in understanding the kinetics and dynamics of drug availability, to identify limitations in drug availability and to discover opportunities where the performance of anti-parasitic compounds may be increased.

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