THE PHARMACOKINETICS OF DRUGS IN THE RUMINANT ANIMAL

A THESIS

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of

DOCTOR OF PHILOSOPHY

by

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1. Marriner, S.E., and Bogan, J.A. The influence of the rumen on the absorption of drugs: studies using meclofenamic acid administered by various routes to sheep and cattle. J. vet. Pharmacol. and Ther. 2, 109-116, 1979.

2. Marriner, S.E., and Bogan, J.A. Rumen by-pass. Letter to Vet. Rec. 105, 261, 1979.

3. Bogan, J.A., and Marriner, S.E. The action of benzimidazole anthelmintics. Proc. Ass. vet. clin. Pharm. and Ther., 1979; in press.

4. Bogan, J.A., and Marriner, S.E. Analysis of benzimidazoles in body fluids by high-performance liquid chromatography. J. Pharm. Sci. 69, 422-423, 1980.

5. Marriner, S.E., and Bogan, J.A. Pharmacokinetics of albendazole in sheep. Am. J. vet. Res. 41, 1126-1129, 1980.

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7. Marriner, S.E., Bogan, J.A., and Vandaele, W. Comparison of the pharmacokinetics of albendazole and its major metabolites after oral administration of albendazole as a suspension and as a paste formulation to sheep. Zbl. f. Veterinärmed (Reihe B), 1980, in press.

8. Marriner, S.E., and Bogan, J.A. The pharmacokinetics of fenbendazole in sheep. Am. J. vet. Res., 1980, in press.

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SUMMARY

The aim of this study was to investigate the effects of the rumen on the kinetics of drugs by measuring plasma and gastro-intestinal fluid concentrations of model drugs (weak acids and bases), after various routes of administration. The experimental animals used were Finn-Dorset sheep fitted with permanent ruminal, abomasal and duodenal cannulae, and the drugs chosen were meclofenamate, an acidic drug, and levamisole, fenbendazole, oxfendazole and albendazole which are basic drugs.

All of the drugs studied were absorbed to some extent after oral administration. Levamisole only was absorbed from the rumen; absorption from this part of the gastro-intestinal tract was much slower than that from the more distal regions. For the other drugs studied, absorption from the rumen was negligible.

Passive diffusion of drug from the systemic compartment into the rumen, either across the rumen wall or via the saliva, was found to occur with levamisole and meclofenamate, but not with the benzimidazole drugs or their metabolites. Levamisole administered subcutaneously, and benzimidazole metabolites formed in the systemic compartment were found to diffuse into abomasal fluid, reaching higher concentrations than those found in plasma at the same time.

The kinetics of the benzimidazole drugs were found to be very slow, the drugs persisting in plasma and gastro-intestinal fluid for up to 9 days. This was attributed to the low water and lipid solubility of the drugs compared with that of levamisole and meclofenamate. Passage through the rumen, of the drugs studied, was found to slow the rate of absorption compared with that of drug administered directly into the

abomasum. Oral administration of each of the drugs studied resulted, on almost every occasion, in some of the drug being delivered directly to the abomasum by the action of the reticular groove. The extent of ruminal by-pass was variable and usually small. It did not appear to be consistent for any individual animal.

High concentrations of benzimidazole metabolites were found in plasma and in abomasal fluid after oral, intra-ruminal and intra-abomasal administration of the parent drug. The site of metabolism of the benzimidazoles was investigated in vitro. On incubation with hepatic microsomes both sulfide drugs, fenbendazole and albendazole, were oxidised to sulfoxides and sulfones. Reduction of fenbendazole sulfoxide (oxfendazole), and albendazole sulfoxide, also occurred during incubation in vitro with liver microsomes; both sulfoxides were found to undergo reduction to the sulfides on incubation with fresh ruminal fluid. No oxidation or reduction was found during incubation of sulfides or sulfoxides with abomasal fluid.

The anthelmintic activities of albendazole and its two major metabolites, albendazole sulfoxide and albendazole sulfone were compared by administration of the compounds in feed to rats infected with Nippostrongylus braziliensis. Albendazole sulfoxide was found to have similar anthelmintic activity to albendazole, and albendazole sulfone was found to be non-active. The relevance of these findings to the suggested mode of action of these drugs is discussed.

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CHAPTER 1

INTRODUCTION and LITERATURE REVIEW

There are an estimated 95 million cattle and 94 million sheep in Western Europe, the vast majority of which will receive some form of drug treatment, whether it be therapeutic or prophylactic, at some time during their lives. In 1977 the estimated value of anthelmintic drugs alone used in these animals was in the region of £58 millions, and the world market value was something over £250 millions. It is a little surprising in view of this large, relatively constant market that so little is apparently known about the processes of absorption, distribution and elimination of drugs with regard to the ruminant animal, and in particular the effect of the rumen on both orally and parenterally administered drugs.

Little rationale is apparently used in the choice of dosage regimes which are largely determined on the basis of efficacy trials or on data from monogastric animals. A more reasonable approach would seem to lie in the determination of minimum effective concentrations of a drug and how to achieve these concentrations at the site of action of the drug. The oral route for administration of drugs is more convenient in many cases than the parenteral route avoiding pain, the risk of carcase damage from injection site necrosis, and also the necessity for sterility. For these reasons it is very widely used in monogastric species, but the possible effects of the rumen have precluded such wide usage in cattle and sheep, the exception being the anthelmintic drugs which are frequently administered as drenches.

Absorption of drugs from the gastro-intestinal tract of

man and other monogastric animals has been studied in some depth and the processes involved are relatively well understood. The epithelial lining of the stomach and small intestine behaves as a classical cell membrane which is postulated to consist of a bimolecular layer of orientated phospholipid molecules forming a lipid matrix with polar heads aligned at both surfaces. Globular protein molecules are interspersed, with polar groups protruding into the aqueous phase at each side, and non-polar groups embedded in the matrix.

The cell membrane is permeable to the unionized form of lipid soluble molecules in solution which are absorbed by passive diffusion along a concentration gradient. The rate of passage across the membrane is determined by the lipid solubility of the drug. For example, increasing rate of absorption of different barbiturates from the rat stomach is in the same order as their increasing lipid-to-water partition co-efficients (Schanker, Shore, Brodie and Hogben, 1957), and correlation between rates of absorption of several weak organic acids and bases from the rat small intestine, and the lipid-to-water partition co-efficients of their unionized form, has also been demonstrated (Hogben, Tocco, Brodie and Schanker, 1958).

Since the unionized form of most drug molecules is the fraction available for absorption, the imposed concentration gradient along which passive diffusion occurs depends upon the degree of dissociation of the drug. This in turn is governed by the dissociation constant of the particular drug and the pH of the surrounding medium. In general terms this means that only drugs which are weak acids or

weak bases are well absorbed since strong acids and bases are largely ionized at any pH encountered within the gut.

Weak acids such as barbiturates and some sulfonamides are readily absorbed from the stomach (pH 1-2) but weak bases such as quinine and aminopyrine are very poorly absorbed. If the pH of gastric contents is raised artificially basic drugs have an increased rate of absorption and acidic drugs a decreased rate of absorption (Schanker et al, 1957). The pH of the intestinal lumen is generally around 6.6 and so weak bases are much better absorbed from this less acidic part of the tract. There is evidence for the existence of a more acidic region at the actual site of absorption by intestinal cells which is maintained, to some extent independently of the pH of intestinal contents, at a pH of 5.3 (Hogben et al, 1959). This effective pH was thought by these authors to explain why only weak acids with a pk_a value greater than 3 and weak bases with a pk_a of less than 7.8 are well absorbed from the intestine; outwith these limits the low proportion of unionized drug severely reduces absorption.

The degree of ionization of any drug at a given pH is given by the Henderson-Hasselbalch equation -

for a weak acid,

$$\log \frac{\text{unionized acid}}{\text{ionized acid}} = pk_a - pH$$

and for a weak base,

$$\log \frac{\text{ionized base}}{\text{unionized base}} = pk_a - pH$$

where pk_a = negative logarithm of the acid dissociation constant.

Theoretically absorption across the gastro-intestinal mucosa occurs until the concentration of unionized drug in plasma is equal

to that in the gut (although for most drugs the total concentrations will be very different because the difference in pH causes different degrees of ionization). In practice this steady state equilibrium is not achieved because of distribution, metabolism and excretion of the drug, and passage of gut contents further along the tract. Steady state concentrations have been measured experimentally in anaesthetized rats and the concentrations found were in agreement with the theory that the epithelial border has a slightly lower pH than intestinal contents (Hogben et al, 1958). The intestine, due to the greater surface area of its brush border, is the major site of absorption for most orally administered drugs; as mentioned earlier basic drugs undergo very little absorption in the stomach.

The theory of passive diffusion of lipid soluble, non-ionized molecules does not, however, account for the rapid movement of small lipid insoluble molecules such as urea across membranes. It is suggested that there are aqueous channels in the membrane through which lipid insoluble molecules and ions can pass. The rate of transfer in this case is dependent on size of the molecule, and in the case of ions, the charge carried. Small molecules undergo more rapid transfer than large ones. Most drug molecules are too large for this process to be quantitatively important in absorption.

Active transport mechanisms for certain molecules such as glucose, amino acids, pyrimidines and inorganic ions are known to exist in the small intestine. For drugs with chemical structures very similar to these substances, there is evidence that absorption using these active transport processes can occur. Pyrimidines such

as 5-fluorouracil and 5-bromouracil have been shown to be actively transported across the intestinal epithelium by the process which transports the natural pyrimidines uracil and thymine (Schanker and Jeffrey, 1961), and several foreign sugars with structures similar to that of glucose are actively absorbed by the monosaccharide transport process of the intestine (Wilson and Landau, 1960).

This applies to relatively few drugs, however, and for the great majority passive diffusion of the lipid soluble unionized form is the only means of transfer across the gut wall.

ANATOMY OF THE RUMINANT STOMACH

The ruminant stomach in the adult animal is a voluminous sac occupying a large part of the abdominal cavity. The sac has four parts, the reticulum, rumen and omasum collectively termed the forestomach, and the abomasum frequently termed the true stomach.

The reticulum, the most cranial part, is largely continuous with the rumen and the two are often alluded to as the reticulo-rumen. The rumen has by far the largest volume of the four parts; it is partially divided into dorsal and ventral sacs by thick muscular invaginations of the walls, the anterior and posterior pillars of the rumen. Digesta enters the reticulo-rumen from the oesophagus at the cardia. The outflow of digesta from the reticulo-rumen is to the omasum via the small reticulo-omasal orifice situated on the medial wall of the reticulum, ventral to the cardia. Running from the oesophageal opening across the medial wall of the reticulum to the reticulo-omasal orifice is a groove, the oesophageal or reticular groove. The groove has thickened edges which on contraction form an extension of the oesophagus delivering ingested material to the omasum. The omasum is smaller than the reticulum in sheep; the reverse is true in cattle. A large omasal-abomasal orifice opens into the abomasum which is similar to the monogastric stomach.

The mucous membrane of the parts of the forestomach has differing arrangements, although it is lined throughout by stratified squamous epithelium which is largely keratinized. In the reticulum the arrangement of the mucosa is honeycomb-like, thrown up into shallow folds forming the 'cells' of the honeycomb. These cells

contain epithelial projections or papillae which are also present over much of the interior of the rumen. The mucous membrane lining the omasum extends into a series of deep folds or laminae, several different depths of which are recognized. These laminae also bear horny papillae, the function of which is almost certainly to reduce digesta to very fine particles.

The abomasal mucosa in contrast is lined by a simple columnar, glandular epithelium. It has fundic and pyloric regions similar to those of the monogastric animal. In the fundic region the mucosa forms extensive spiral folds, which greatly increase the surface area of the abomasum relative to that of the monogastric stomach.

FUNCTION OF THE RUMEN

The reticulo-rumen acts as a continuous fermentation vat in which vast numbers of micro-organisms degrade the dietary intake of the animal. The fermentation process produces the volatile fatty acids acetic, proprionic and butyric, from which the ruminant animal gets most of its energy supply. Ammonia, carbon dioxide and methane are also important end products of the fermentation. Conditions within the rumen are fairly stable under a given dietary regime. The pH generally is maintained at 5.5-6.5. The addition of large volumes of highly buffered alkaline saliva as much as 98-190 litres in 24 hours in cattle (Bailey, 1961), and 6-16 litres over the same period in sheep (Kay, 1960), maintains the volume of the contents.

Various estimations of the capacity of the reticulo-rumen have been made. Sisson and Grossman (1953) quote a figure of

25-60 US gallons (95-227 litres) in cattle and 4 US gallons (15 litres) in sheep. Warner and Flatt (1965) estimated a 500 kg cow to have as much as 100 kg of reticulo-rumen contents and a 50 kg sheep between 5 and 10 kg. Neff-Davis, Davis and Powers (1975) estimated the total gastro-intestinal tract and contents of the goat to comprise 20% of total body weight; by comparison the same figure for the dog is given as 4.6%.

The contents of the reticulo-rumen are largely water. The exact dry matter of the digesta varies according to the type of diet, frequency of feeding and also varies within different parts of the reticulo-ruminal sac. In cows fed hay once daily Evans, Pearce, Burnett and Pillinger (1973) found the dry matter of the contents from different sites to vary between 3% and 15%.

Samples from the dorsal sac had higher dry matter than those from the ventral sac, illustrating the imperfect mixing of the contents.

Mixing occurs by a regular sequence of contractions of the various parts of the reticulo-rumen which serves to expel contents into the omasum, as well as give maximum exposure to the microbial digestive enzymes. Further contractions are associated with rumination and eructation. The papillae of the forestomach play a part in the fine division of ingesta, as does the process of rumination by which the contents are re-masticated by the animal at intervals.

POSSIBLE EFFECTS OF THE RETICULO-RUMEN ON ADMINISTERED DRUGS

"The presence of a large fermenting bulk within the foregut constitutes a major factor by which the ruminant differs from other species in the distribution and fate of ingested and injected materials"

(Dobson, 1967).

The reticulo-rumen indeed constitutes a large bulk, and has a significant effect on the distribution of body fluids. Dobson (1967) estimated that the gut water of the ruminant forms a compartment equivalent to or greater in size than the rest of the extracellular fluid of the animal.

This large volume of fluid might be expected to affect the pharmacokinetics of both orally and parenterally administered drugs in a number of ways. The accessibility or otherwise of rumen fluid to parenterally administered drugs would make a considerable difference to the concentrations achieved after a given dose in other body compartments. Knowledge of the distribution of such drugs is of importance in the prediction of therapeutic dosage, especially if this is based on data from monogastric animals.

If passive diffusion of drugs between the reticulo-rumen and the systemic compartment does occur freely, then it would also be expected that weakly basic drugs would diffuse across the rumen wall in the unionized state, become ionized at the lower pH of ruminal fluid and thus accumulate in the rumen by this process of 'ion trapping'. Since many of the anthelmintics and antibiotics currently used are weak bases, the extent to which this occurs is also of importance.

Drugs entering the rumen by oral administration or from the systemic compartment are exposed to large numbers of micro-organisms $>10^{10}$ per ml (Warner, 1962) which have considerable powers of degradation. Certain drugs are known to be susceptible to microbial

activity. Chloramphenicol is one (Theodorides, Dicuollo, Guarini and Pagáno, 1968) and trimethoprim another (Nielson, Romváry and Ramussen, 1978). Passage of material through the rumen is slow, and so the duration of exposure to micro-organisms of both solid drugs and those in solution can be considerable. Balch (1950) found that the concentration of stained hay in the rumen of cattle after its addition to the feed had fallen by 50% only after 40-48 hours had elapsed, and stained hay was detectable in the rumen for 5 days or more. Grovum and Williams (1973) found the average half-life of a fluid marker in the rumen of sheep to be 604 minutes and that of a particulate matter marker to be 811 minutes. The half-lives of the same markers in the abomasum were much shorter, 17 and 37 minutes for fluid and particulate matter respectively.

As well as the possible effects of microflora on orally administered drugs the large volume of reticulo-ruminal fluid has a dilution effect on dissolved drug so that the concentration would be much lower than if the equivalent dose was administered to the abomasum or to the monogastric stomach. Since the rate of absorption by passive diffusion is dependent partly on concentration gradient, even if absorption occurred freely from the rumen then it would be expected to be slower than that from the true stomach/small intestine. If the rumen has little or no absorptive capacity for drugs, the delivery of drug to the site of absorption (abomasum/small intestine) is slowed, so that the net effect is the same. For drugs with rapid elimination an excessively slow rate of absorption may mean that therapeutic concentrations are not obtained in the systemic

compartment in ruminant species.

Conversely the slow passage of orally administered drugs through the reticulo-rumen would increase the length of time available for dissolution of drugs administered in the form of solids or suspensions. For drugs of low solubility this effect, together with the large volume of the contents, may lead to an increased bio-availability of such drugs in the ruminant compared with the mono-gastric animal, in which low solubility and shorter gastro-intestinal retention time can lead to incomplete absorption.

THE RETICULAR GROOVE

The reticular (oesphageal) groove is an anatomical feature of the ruminant stomach which allows swallowed liquids to pass directly from the oesophagus to the reticulo-omasal orifice, thus allowing it to by-pass the reticulo-rumen. In the newborn animal the reticulo-rumen and omasum are small in relation to the abomasum, undeveloped and apparently functionless. It has long been known that in young suckling ruminants ingested milk passes directly to the abomasum.

The function of the groove in the older animal and the stimulus for its closure have been the subject of several investigations. The possibility of delivery of drugs, particularly anthelmintics, directly to the abomasum, avoiding dilution and possible degradation by the reticulo-rumen contents was realised by many early workers (Clunies Ross, 1934, 1936; Mönnig and Quin, 1935; Watson, 1941).

It was thought by Colin (1886) that closure of the groove during rumination was the means of delivery of digesta from the reticulo-rumen to the omasum and abomasum. Schalk and Amadon (1928), however, using animals with rumen cannulae reported that, in contrast to suckling animals, the groove did not regularly close in adult ruminants. Both these authors, and Trautmann and Schmitt (1933), observed that closure of the groove became less marked as the animal grew older, increasing amounts of milk passing to the rumen. Wester (1926) also noted that closure of the groove occurred more readily in younger animals. He postulated that constituents

of the milk, primarily sodium ions, were responsible for eliciting the response by contact with the mouth. From his experiments in cattle he concluded that solutions of sodium salts, particularly sodium bicarbonate, could effect closure of the groove in animals up to 2 years old.

The idea that metal salts could effect contraction of the reticular groove was perpetuated by Clunies Ross (1931, 1934) who found that sodium bicarbonate had little effect in sheep but that administration of solutions of copper salts, especially copper sulphate, regularly resulted in passage of liquids directly to the abomasum. He thought that groove closure was a response to contact of the copper ions with buccal and pharyngeal mucous membranes, and reported that the solution when delivered to the oesophagus via a stomach tube passed mainly to the reticulo-rumen. Clunies Ross (1931) also investigated the effects of starvation, and found that the withholding of food and water, particularly the latter, caused subsequently administered drenches to pass directly to the abomasum in most sheep. He further observed that where dye indicators were delivered to the abomasum, either by use of copper solutions or after starvation, they passed also, to a variable extent, into the small intestine.

Further work in sheep by Mönnig and Quin (1935) and Watson and Jarrett (1941), and in cattle by Riek (1954) added support to the theory that chemical and/or mechanical stimulation of the mouth by solutions of metal salts was responsible for eliciting closure of the groove. Stimulation of pharyngeal receptors by the swallowing of fluids was shown to cause reflex contraction of the groove in

de-cerebrate calves by Comline and Titchen (1951).

Watson (1941) performed detailed studies of reticular groove function in lambs and made the significant discovery that behavioural factors were of great importance in initiation of the mechanism. He disproved earlier suggestions that factors such as the temperature (Troutmann and Schmitt, 1933), composition of the liquid (Wester, 1926) and posture assumed by the animal during drinking (Wise, Anderson and Miller, 1942) influenced the site of delivery of the liquid. Watson found that provided the lambs were eager to suck or drink, the reticular groove closed and the liquid passed to the abomasum. As the desire to suck declined, more frequent passage to the forestomach was found to occur. The same author also observed that liquid taken merely in order to quench thirst, almost always passed entirely to the reticulo-rumen, even in lambs as young as 14-15 days old.

The introduction of intensive rearing methods stimulated more recent work on reticular groove function. In young fast-growing ruminants in which requirements for protein cannot be fully met by the supply of microbial protein, interest was aroused in the possibility of by-passing the rumen with high quality protein supplements which could be utilized more efficiently by the animal directly than via microbial degradation.

Ørskov and Benzie (1969) re-examined the earlier work with salt solutions, but failed to demonstrate regular closure of the groove in adult sheep with suspensions of protein with or without added salt solutions, or with milk. They found, however, that in lambs trained to drink milk from a bottle or from a trough protein suspension

could be later substituted for the milk and closure of the groove maintained even when the animals became adult. When the same suspensions were taken to quench thirst, however, they passed to the forestomach, thus confirming the earlier observations by Watson (1941). The fact that the same suspensions could be taken by mouth and regularly have different destinations suggested to these authors that the stimulus to groove closure was not chemical or mechanical stimulation of the mouth or pharynx. They considered that it might be a conditioned reflex, and this possibility was subsequently tested (Ørskov, Benzie and Kay, 1970). Catheters were inserted into lambs and suspensions of barium sulphate injected. When the animals were standing quietly at rest, the barium sulphate went to the forestomach. If, however, the animal was shown the bottle or trough from which it was regularly fed, and the stockman associated with feeding, the barium sulphate passed to the abomasum. Feeding the liquid ad libitum to the lambs caused the reflex to disappear.

Lawlor, Hopkins and Kealy (1971) performed similar experiments with lambs, and discovered that in lambs which were slow to learn to suck or were reluctant to suck at all, milk more frequently passed to the reticulo-rumen than the abomasum.

The degree to which the reticular groove operates during administration of drugs by drenching and the possible effects of reticulo-ruminal by-pass are not well documented. Differences in plasma concentrations of drug following oral and intra-ruminal administration to adult cows have been observed, however, and were attributed to part of the oral dose being delivered directly to the abomasum (Aitken and Sanford, 1975). The differences at times later than two hours after

administration were not marked, and it is probable that the extent of by-pass was not great.

Circumstantial evidence for the occurrence of ruminal by-pass has come from stucies with the flukicidal drug clioxanide which has been shown to be poorly effective in sheep when administered intra-abomasally (Boray and Roseby, 1969). These authors found that intra-ruminal administration of the drug was highly effective in all sheep, but that oral administration resulted in a wide variation in efficacy within the treated group, and they suggested that all or part of the dose had passed via the reticular groove to the abomasum. By contrast a similar, more recent study with the same drug by Campbell and Brotowidjoyo (1975) showed similar efficacy of the drug when given by the oral and intra-ruminal routes although they also found a much reduced efficacy of the intra-abomasally administered dose.

ABSORPTION FROM THE RUMEN

The stratified squamous epithelium which lines the ruminant forestomach is very different histologically from the generally accepted view of what constitutes an absorptive surface. Whether the keratinized epithelium has developed simply as protection against the irritant nature of the ruminant diet, or whether part of its function is in fact to prevent the rapid, indiscriminate absorption of nutrients which may be detrimental to the microflora, which have their own requirement for nutrients, is speculative.

The histology of the rumen has been described by Barcroft, McAnally and Phillipson (1944). These authors found the keratinized layer of the epithelium to be in many areas quite thin, and they also described the basal layer of the epithelium as being of columnar cells. Electron microscopy has shown the presence of intercellular pores (Hydén and Sperber, 1965) which it has been suggested may facilitate the absorption of some solutes. The papillae of the forestomach have an extensive underlying capillary network which may be of importance in the considerable absorption of volatile fatty acids which occurs from the reticulo-rumen (Barcroft et al, 1944; Danielli, Hitchcock, Marshall and Phillipson, 1945). It has been shown that the areas of the rumen wall with large numbers of papillae absorb more VFA than areas with only small numbers of papillae (Aafjes, 1972) and that the development of ruminal papillae which occurs in the growing animal is necessary before appreciable absorption can occur (Sutton, McGilliard and Jacobson, 1963).

The mechanism of absorption of volatile fatty acids appears

to be that of passive diffusion along a concentration gradient. Gray (1948) showed that the pH of the reticulo-ruminal contents has a marked effect on the rate of absorption, being much reduced at pH values of 7 and above. The theory of passive diffusion is also supported by the fact that if the contents of an isolated reticulo-rumen are replaced by solutions devoid of VFA, there is passage of VFA from the blood into the rumen to a final concentration approximating to that in plasma.

No evidence has been found for the existence of active transport mechanisms for the absorption of VFA despite numerous investigations. The same situation applies to ammonia which may also be absorbed from the rumen (McDonald, 1948). Ammonia itself has been shown to undergo more rapid absorption than the ammonium ion (Hogan, 1961; Bloomfield, Kearley, Creach and Muhrer, 1963).

Active transport mechanisms do exist for the absorption of selected ions. Sodium and, under certain conditions, chloride ions have been shown to be absorbed against both concentration and electrochemical gradients (Dobson, 1959). The same author found that the rumen wall is relatively impermeable to calcium, magnesium and phosphate ions.

The ability of the rumen to absorb drugs has been the subject of investigation by several workers. Trautmann (1933), one of the earliest investigators, by measuring rates of saliva production showed that pilocarpine and atropine were absorbed through the epithelium of an exteriorized cup of rumen mucosa. This was confirmed by Rankin (1940) who also demonstrated an increase in blood glucose concentration after administration of dextrose into a rumen pouch, and the presence

of iodide in saliva after administration of potassium iodide into the tied-off reticulo-rumen.

The same author showed that sufficient strychnine and sodium cyanide could be absorbed from the tied-off organ to kill a sheep within one hour of administration.

The effect of differences in pH on the rates of absorption of various sulfonamides, salicylic acid and p-toluidine was examined by Austin (1962). In his study, the reticulo-omasal orifice of atropinized and in some cases anaesthetized calves was obstructed with an inflatable balloon and the test drugs mixed with buffered solutions of various pH, which were placed in the washed out reticulo-rumen. Maintenance of the required pH was by the addition of 1M HCl or NaOH. The change in concentration of the test drug relative to that of a non-absorbed volume indicator was then recorded at various intervals. In the experiments with salicylic acid, measurable blood concentrations of the drug were also reached.

The results showed that, in general, the drugs examined disappeared from the ruminal solution most rapidly at the pH values which favoured the existence of the unionized non-dissociated form. Sulfanilamide was the exception in having slower absorption from solutions of increasing acidity despite the fact that it is a weak acid. No satisfactory explanation for this paradox was found. The integrity of the ruminal epithelium was found to be largely destroyed after contact with the more alkaline of the solutions used in these experiments, and the effect of other solutions with pH values outwith the normal range of rumen contents on the permeability of the rumen to drugs is unknown.

A similar experimental model was used by Jenkins, Davis and Boulos (1975) in their studies on drug transfer across the ruminal wall of goats. These animals were in most cases conscious. Oesophageal and reticulo-omasal orifices were temporarily blocked and the washed out rumen filled with buffered Krebs Ringer phosphate solution at the desired pH. This fluid was continuously circulated extracorporeally by pump. The rate of disappearance of various drugs at different concentrations and at different pH values was then measured. The pH values used were less extreme than those used by Austin (1962), and mostly within the normal range of ruminal fluid. Diffusion of intravenously administered drugs into the reticulo-rumen from plasma under conditions of constant plasma concentration was also studied using this model.

These authors demonstrated that the rate of transfer of pentobarbitone, antipyrine, salicylate and quinine across the rumen wall was proportional to the imposed concentration gradient. Rates of disappearance were also pH dependent. Increasing the degree of ionisation of the drug by altering the pH of the surrounding medium lowered the rate of absorption. At pH values within the physiological range normally encountered within the rumen, 75% absorption occurred after 2-4 hours or more. The authors suggested that the rate of mixing in the normal rumen is very much slower than that provided by this model and therefore absorption is likely to be slower also, and that it would be unwise to deduce absolute figures from such a model.

All four drugs studied in these experiments were found in

the rumen following intravenous infusion. Pentobarbitone and antipyrine reached steady state concentrations close to those predicted from their pk_a values and the pH of the two compartments, but salicylate and quinine did not. Ruminal concentrations of salicylate were lower than those predicted, and those of quinine higher.

Ion trapping was also observed by these authors. Salicylate failed to reach steady state concentration during infusion with an alkaline ruminal solution over a ten hour period, the drug simply accumulating in the rumen. The same was true of quinine infused under conditions of an acidic ruminal solution.

These results agreed with earlier conclusions that passive diffusion was the mechanism of transfer of drugs between plasma and ruminal fluid. The poor agreement between observed and theoretical steady state ratios of salicylate was attributed to another mechanism of absorption for this drug, probably diffusion through aqueous pores. The reason for the similar findings with quinine was not apparent.

Further work in animals with surgically isolated rumen was performed by McManus, Washko and Tocco (1966), again using goats. These authors found slow absorption of thiabendazole to occur from the rumen after removal of most of the solid contents, but considered the process to be limited by the relatively low aqueous solubility of the drug at ruminal pH.

Diffusion of intravenously administered drug across the isolated rumen wall has been demonstrated for various sulfonamides, antipyrine and ephedrine (Corker and Stowe, 1967). These drugs were detected in ruminal fluid within ten minutes of intravenous

injection. Tetracyclines were found not to distribute into the rumen.

These studies demonstrate that under certain conditions drug absorption through the rumen wall can occur, that the mechanism is almost certainly passive diffusion and that therefore the process is governed by the same factors - water/lipid solubility, degree of ionisation and concentration gradient that apply to passage of drugs across any biological membrane. They do not, however, assess the importance of this process in the normal intact animal where such factors as ruminal motility, the nature of the digesta or variations in ruminal blood flow which are known to occur may all influence absorption.

Studies in non-anaesthetized intact animals are relatively few, and the results are not always consistent with those in the experimental models referred to above. For example, Davis and Westfall (1972) found negligible concentrations of salicylate in the plasma of goats after administration of the sodium salt of the drug via a stomach tube and this they attributed to poor absorption of the drug. These authors used dose rates of up to 133 mg/kg which would have been expected to give rise to higher concentrations in the rumen than those used by Jenkins et al (1975) in their isolated rumen preparations, in which significant absorption was found to occur.

In cattle, however, the bio-availability of salicylate after oral administration of aspirin was found to be 70%, based on comparison of plasma concentrations after oral and intravenous administration (Gingerich, Baggot and Yeary, 1975) and it appears that there are species differences among ruminants as there are among monogastric

animals.

Reports of direct comparative studies of drug absorption between ruminant and non-ruminant species in the literature are few. Those that there are have mostly been performed with the sulfonamide group of antibacterial drugs. Stableforth and Hignett (1942) found that absorption of orally administered sulfanilamide was slower in cows and in a sheep than in horses or dogs, and Stewart and Paris (1962) and Neumann (1964) found similar results with sulphamethoxy-pyridazine in their respective studies with cattle, horses and dogs, and cattle, dogs and pigs.

Although in these studies only plasma concentrations of the drug, which are dependent on rates of distribution, metabolism and excretion as well as rate of absorption, were measured, the differences in the time taken to achieve maximum plasma concentrations in ruminant and non-ruminant species lead to the conclusion that absorption is generally slower in the ruminant.

The aims of the present study were

1) to assess the role of the rumen as a possible site for drug absorption
2) to explore the possibility that weakly basic drugs accumulate in ruminal fluid by diffusion from the systemic compartment
3) to assess the influence of the reticular groove on the kinetics of orally administered drugs.

These aims were to be achieved by studying the distribution of various weak acids and weak bases between plasma and the different parts of the gut after various routes of administration to sheep fitted with

permanent ruminal, abomasal and duodenal cannulae.

CHAPTER 2

MATERIALS AND METHODS

ANIMALS

The sheep used during this study were Finn-Dorset cross females or castrated males, aged between 8 and 14 months at the beginning of the experiments. The sheep were fitted with permanent ruminal, abomasal and, in some cases, duodenal cannulae. They were kept indoors, fed hay and water ad libitum and a commercial sheep concentrate ration was fed once daily.

CANNULAE

Rumen cannulae used initially were modified household sink waste fittings made of heavy inflexible plastic. These proved unsuccessful, and in two of the three sheep were rejected within 3 months of insertion. Attempts were made to replace the original cannulae with ones of a flexible rubber type; this was reasonably successful in one animal although there was persistent leakage around the barrel for several months.

It seemed probable that these early failures were due to the weight of the cannula and its flanges being too great for the body wall to support. Cannulae were therefore made to the same design but of a lighter weight Teflon material. None of these was rejected up to 2½ years after insertion, and none of them leaked.

Abomasal and duodenal cannulae were of the same material, and were similarly well tolerated. The design and dimensions of the cannulae can be seen in Fig. 1 (1). Fig. 1 (2) shows a ruminal cannula 1 year after insertion.

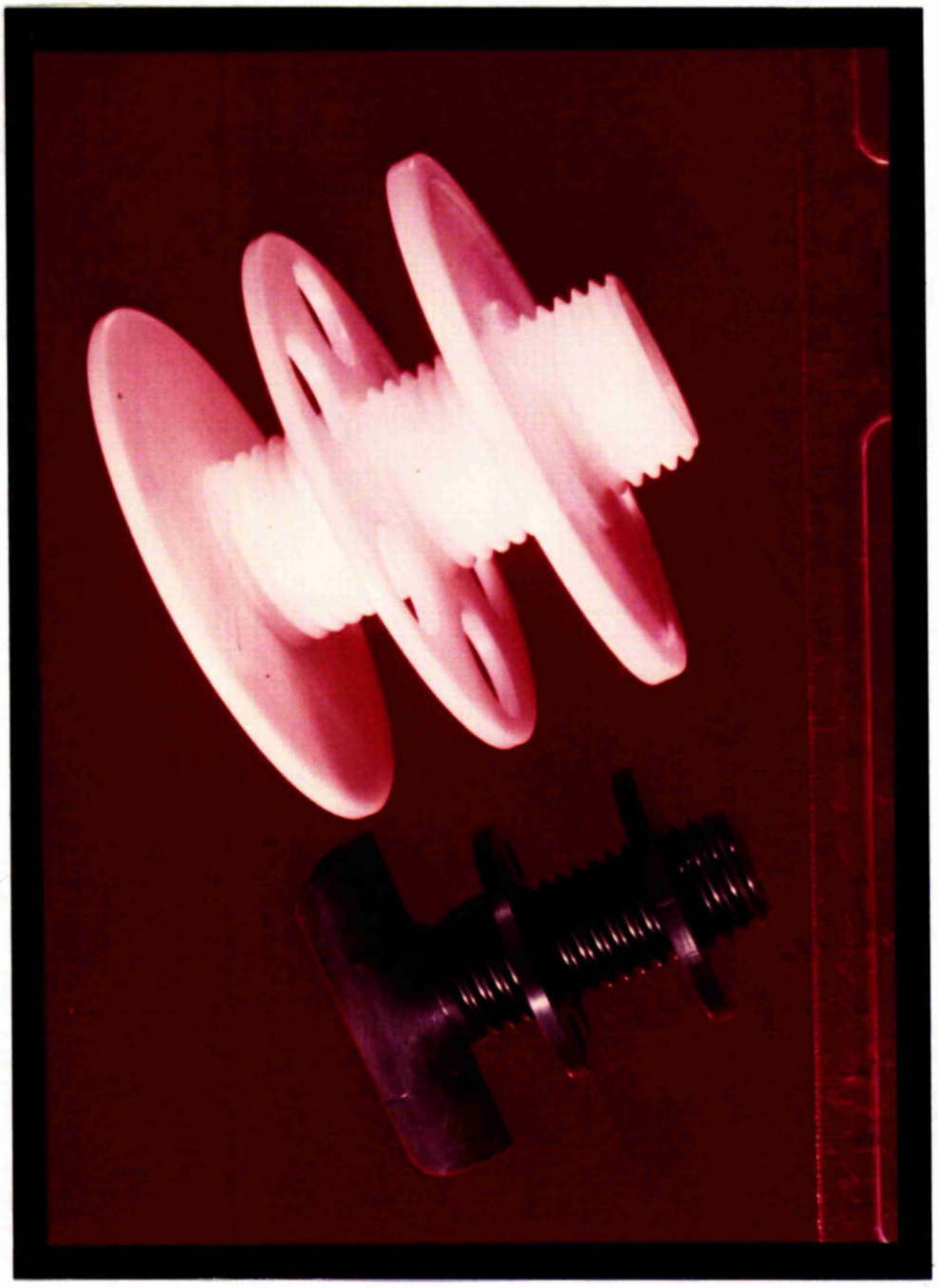


Fig. 2 (1) The design and dimensions of abomasal/duodenal and ruminal cannulae

Fig. 2 (2) A ruminal cannula one year after insertion

SURGERY

Animals were starved overnight before surgery. Anaesthesia was induced with intravenous sodium pentobarbitone (20 mg/kg) and maintained by incremental doses as required. Intubation was carried out if there appeared to be any respiratory difficulty or likelihood of regurgitation of rumen contents, but generally this was not required.

Cannulae were fitted using standard surgical techniques (Hecker, 1974) and positioned as follows: ruminal cannulae in the posterior part of the dorsal sac, abomasal cannulae in the fundus of the abomasum and duodenal cannulae approximately 8 cm from the pylorus in the proximal part of the duodenum.

At least four weeks elapsed between ruminal and abomasal/duodenal cannulation in any animal. The same period was allowed before the animals were used for any experiments.

SAMPLING TECHNIQUES

Blood samples

Blood samples were taken from the jugular vein using heparinised syringes (Monovettes, Sarstedt) and 20 gauge 1" needles.

Rumen fluid

In the early experiments the animal was restrained on its right side. Rumen contents were then withdrawn, via the cannula, using a 10 ml syringe with one side of the barrel cut away to form a ladle. Later, to facilitate single handed sampling, the animal was left standing during the procedure.

Abomasal and duodenal fluid

Initially a blunt, soft plastic catheter (St. Peter's catheter) attached to a wide necked syringe was used to withdraw abomasal and duodenal fluid. This was discarded when it was found to be impossible to obtain repeat samples without causing haemorrhage into the gut. Instead a small beaker was held under the barrel of the cannula and samples obtained by normal passage of gut contents.

In all cases the first part of the sample was not collected as this portion may have been stationary in the barrel of the cannula and been unrepresentative of the contents.

TREATMENT OF SAMPLES

All samples were centrifuged at 1,000 x g for 10 minutes. The blood plasma was taken off from the red cells. Supernatant fluid from the gut samples was filtered (Whatman No. 4 filter paper). All samples were then deep frozen and stored at -20^{o}C until assayed.

ASSAY METHODS

MECLOFENAMATE

The concentrations of free, unconjugated meclofenamate in plasma and gastro-intestinal fluid were measured by the spectrofluorimetric method of Glazko (1966) with some minor modifications to the original method.

Total meclofenamate, comprising free meclofenamate, ester conjugates of meclofenamate, and also conjugated metabolites of meclofenamate can be measured using the same method after first freeing the conjugates by incubating with alkali at room temperature. This was not done as the metabolites are not differentiated from parent drug by this procedure and since neither they nor conjugated meclofenamate are biologically active, they were of less interest than the free drug.

REAGENTS

1) Sodium chloride 0.25M (Analar)
2) Hydrochloric acid 1M (Analar)
3) Carbon tetrachloride (spectroscopic grade)
4) Trichloroacetic acid 50% w/v in carbon tetrachloride.

Procedure

To 1 ml of sample in a 15 ml ground glass stoppered test tube was added 6 ml of 0.25M sodium chloride solution. The pH was adjusted to 2 with 0.3 ml of 1M hydrochloric acid, and 5 ml of carbon tetrachloride added.

The tube was shaken for 10 minutes on a slow speed

rotary mixer, and subsequently centrifuged at 1,000 x g for 5 minutes.

3 ml of the organic layer was removed to a clean test tube and 4 drops of trichloroacetic acid solution (50% w/v in carbon tetrachloride) added. After 10 minutes the fluorescence was measured (360 nm excitation, 425 nm emission) on a Farrand Mark 1 fluorimeter.

Standards were prepared by adding known amounts of drug to blank plasma and gastro-intestinal fluid over a range of concentrations and taking these through the procedure. Unknown samples were read from the calibration curve obtained from the standards.

BENZIMIDAZOLES

Since fenbendazole was chosen as one of the drugs in this study, the method of Düwel, Hajdu and Damm (1975) for its estimation in plasma was investigated. However this method using fluorescence spectrophotometry was found to be unsuitable for a number of reasons.

Firstly, the fluorescence versus drug concentration plot became non linear at concentrations much lower than those which might be anticipated in the gut after normal doses. Thus samples would have had to be diluted, possibly several times, to be read.

Secondly, blank plasma values obtained were high relative to the concentrations measured after recommended oral dosage.

Thirdly, it was found to be impossible to achieve consistent results from repeat samples, especially in the case of gastro-intestinal fluid.

In retrospect, it was fortunate that an alternative method was found as the metabolites of fenbendazole have a similar fluorescence and would not have been differentiated from the parent drug by this method.

Since the only other published work on fenbendazole was performed using radio-labelled drug (Christ, Kellner and Klopffer, 1974) which also does not differentiate parent drug from metabolites,it was decided to develop a chemical assay if possible. Gas-liquid chromatography (GLC) was considered

briefly, but in view of the extremely low solubility of the drug in both water and organic solvents, and its low volatility, it was not surprising that no chromatographic peaks were obtained on a number of gas chromatographic columns (SE-30; OV-17; Carbowax) using maximum concentrations of fenbendazole in methanol.

High performance liquid chromatography (HPLC), a relatively new technique, has recently found a wide range of applications in the field of drug analysis and on theoretical grounds (low water and lipid solubility) was thought to be applicable to the benzimidazoles.

A number of HPLC solvents were tried using an ODS Hypersil column. The solvents included various mixtures of methanol/water, methanol/ammonium carbonate and methanol plus acid. It was found that satisfactory peaks were obtained with methanol/ ammonium carbonate mixtures.

For the extraction of fenbendazole from body fluids several organic solvents were tried, including ether, hexane, ethylacetate, chloroform and dichloromethane. Each solvent was used to extract the drug from aqueous solutions of various pH values ranging from 6 to 10. Ether was found to be the most suitable solvent as it gave a good extraction and was evaporated rapidly at relatively low temperatures. Fenbendazole was readily extracted at all the pH values used. Since fewer interfering peaks on the chromatograms arose from the use of a pH 7.4 phosphate buffer than with any other, this was the

buffer chosen.

Under these extraction conditions, plasma and abomasal fluid gave chromatograms which were free of interfering peaks. Some interfering substances were occasionally found in rumen fluid extracts, and therefore a method for the cleaning up of rumen fluid samples was sought. Since benzimidazoles are basic drugs, standard methods for cleaning up basic drugs using a back extraction into dilute hydrochloric acid were tried. However it was found that unacceptably high losses occurred using these methods, and on further investigation it was found that the partition co-efficient of fenbendazole between ether and aqueous solutions so favoured the organic layer that acid extraction removed less than 15% of the drug from the organic layer.

Other methods for the clean up of ether extracts involved washing of the extract with various buffers and with hexane, but none of these significantly reduced the amount of contamination of the samples and were abandoned.

The following method for benzimidazole analysis was found to be highly satisfactory for plasma and abomasal fluid. The injection of large numbers of rumen samples did at times result in marked deterioration of column performance with loss of sensitivity and specificity. This necessitated cleaning and repacking of the top of the chromatographic column at intervals.

In addition, the large numbers of contaminant peaks in rumen fluid samples on occasions gave rise to considerable

difficulties in interpretation of chromatograms, particularly where low drug concentrations were being measured.

REAGENTS

All reagents were of analar grade.

1) Diethyl ether
2) Phosphate buffer pH 7.4 - 250 ml 0.2M potassium dihydrogen phosphate + 197.5 ml 0.2M sodium hydroxide per litre
3) Methanol - redistilled before use
4) Ammonium carbonate (0.05M) prepared using permanganate distilled water.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Pump:	Altex model 110
Detector:	Cecil CE 2012 variable wavelength UV spectrophotometer
Column:	100 mm x 8 mm Shandon Southern (prepacked)
Packing:	ODS Hypersil 10
Wavelength:	292 nm
Absorbance:	0.1 A.U.F.S.
Solvent:	Methanol:Ammonium carbonate 80:20
Flow rate:	1.5 ml/min

Under these conditions fenbendazole had a retention time of 4.2 minutes.

Procedure

To 4 ml of plasma or gastro-intestinal fluid in a 50 ml ground glass stoppered tube was added 4 ml of pH 7.4

phosphate buffer, and 20 ml of diethyl ether. The tube was stoppered and shaken for 10 minutes on a slow rotary mixer. 16 ml of the ether (upper) layer was removed to a 50 ml glass test tube using a 5 ml adjustable pipette. (The pipette tip required to be pre-wetted with the ether before accurate transfer could be effected). A further 20 ml of ether was added to the first tube and shaken as before. 20 ml of the ether was removed and combined with the first 16 ml. The ether was slowly evaporated on a dri-bath at 55^{o}C under nitrogen to an approximate volume of 6 ml, and transferred to a conical 10 ml glass tube. The 50 ml tube was rinsed three times with 1 ml of ether and the washings added to the 10 ml tube each time. The ether was evaporated to dryness, the walls of the tube rinsed down with 0.5 ml of ether and this again evaporated to dryness.

The residue was redissolved in an appropriate volume (50 µl for plasma samples) of methanol by tilting and rotating the tube in an ultrasonic bath. 5 µl of this extract was used for HPLC analysis.

The concentration of fenbendazole in the samples was calculated with the aid of calibration curves prepared by adding known amounts of fenbendazole to blank plasma or gastro-intestinal fluid to give an appropriate concentration range. These standards were extracted along with the samples each time and the peak height of fenbendazole in the samples compared with the calibration curve obtained.

Non-extracted standard solutions of fenbendazole in

methanol were also injected as a check on the performance on the column, and to estimate recoveries of the drug.

The same method was found to be applicable to all the benzimidazoles studied and Fig. 2 (3) shows a chromatogram of eight of the benzimidazoles. The methanol:ammonium carbonate ratio of the solvent can be adjusted as shown in Table 2 (1) to produce more practical retention times for any particular drug.

The method is sufficiently sensitive to measure plasma and gastro-intestinal fluid concentrations down to 0.02 µg/ml.

The accuracy and precision of the method was determined for each drug by adding known amounts of the drug to blank plasma and abomasal fluid. Samples of each known concentration were then assayed in triplicate (Table 2 (2)).

It was determined that the recovery losses were due to the extraction procedure rather than to benzimidazole degradation.

Recoveries for fenbendazole, oxfendazole and albendazole from plasma, abomasal fluid and ruminal fluid after allowing for the losses due to discarded solvent are shown in Table 2 (3).

Typical high performance liquid chromatograms for oxfendazole extracted from the body fluids using the method are shown in Fig. 2 (4).

SEPARATION AND IDENTIFICATION OF METABOLITES

Under the solvent system described above, the respective major metabolites of both fenbendazole (i.e. oxfendazole and oxfendazole sulfone Fig. 5 (2), and albendazole (i.e. albendazole sulfoxide, albendazole sulfone and albendazole-2-aminosulfone

Fig. 2 (3) High performance liquid chromatogram of oxfendazole (1), thiabendazole (2), cambendazole (3), mebendazole (4), oxibendazole (5), albendazole (6), fenbendazole (7) and parbendazole (8) using methanol:ammonium carbonate (0.005 M) 65:35. Five microlitres of a solution containing 10 µg/ml of each benzimidazole in methanol was used for injection

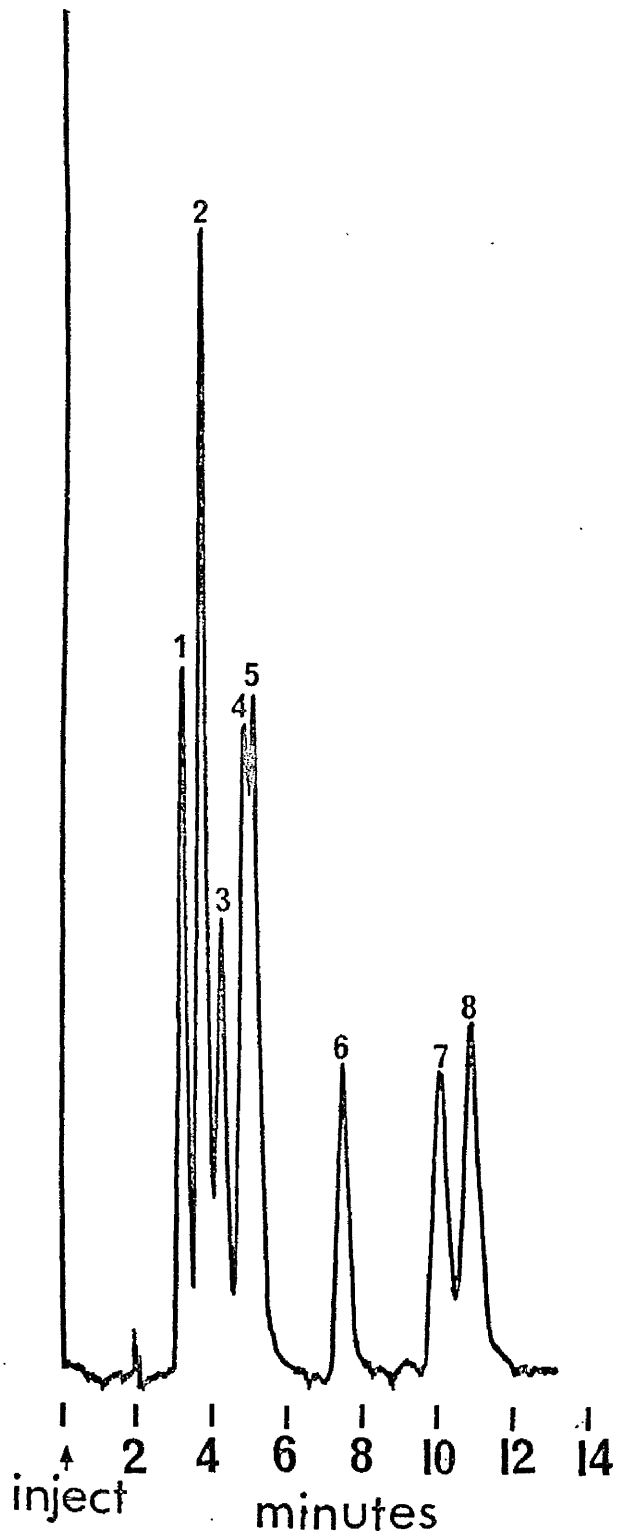

1
2
3
4
5
6
7
8
inject
2
4
6
8
10
12
14
minutes

TABLE 2 (1) RETENTION TIMES OF BENZIMIDAZOLES USING VARIOUS RATIOS OF METHANOL:AMMONIUM CARBONATE AS ELUTING SOLVENT FOR HPLC

Benzimidazole	Methanol:Ammonium Carbonate (0.05M)	Retention Times (mins)
Thiabendazole	65 : 35	4.1
Cambendazole	70 : 30	4.7
Parbendazole	85 : 15	3.6
Oxibendazole	75 : 25	3.8
Mebendazole	75 : 25	4.4
Fenbendazole	80 : 20	4.2
Oxfendazole	75 : 25	3.8
Albendazole	75 : 25	4.2

TABLE 2 (2) ESTIMATION OF BENZIMIDAZOLES IN PLASMA AND ABOMASAL FLUID

Fenbendazole added to Plasma (µg)	Fenbendazole Measured (µg)			Mean ± S.E.M.	S.E. as % of Mean	Ratio Assayed to Added
0.2	0.16	0.16	0.15	0.16 ± 0.003	2.1	0.80
0.4	0.30	0.32	0.28	0.30 ± 0.01	3.6	0.75
0.8	0.69	0.61	0.65	0.65 ± 0.02	3.6	0.81
1.5	1.21	1.19	1.30	1.23 ± 0.03	2.8	0.82
Albendazole added to Abomasal Fluid (µg)	**Albendazole Measured (µg)**			**Mean ± S.E.M.**	**S.E. as % of Mean**	**Ratio Assayed to Added**
1.0	0.86	0.90	0.89	0.88 ± 0.01	1.37	0.88
2.0	1.60	1.75	1.73	1.69 ± 0.05	2.8	0.85
4.0	3.20	3.36	3.45	3.34 ± 0.07	2.2	0.84
10.0	8.34	9.57	8.72	8.88 ± 0.36	4.1	0.89
Oxfendazole added to Abomasal Fluid (µg)	**Oxfendazole Measured (µg)**			**Mean ± S.E.M.**	**S.E. as % of Mean**	**Ratio Assayed to Added**
1.5	1.03	1.08	1.13	1.08 ± 0.03	2.7	0.72
7.5	5.90	6.20	6.10	6.07 ± 0.09	1.5	0.81
15	12.10	11.20	11.40	11.57 ± 0.27	2.4	0.77
30	24.50	24.20	26.10	24.93 ± 0.59	2.4	0.83

TABLE 2 (3) PERCENTAGE RECOVERY OF BENZIMIDAZOLES ESTIMATED BY ADDING KNOWN AMOUNTS OF DRUG TO BLANK PLASMA AND GASTRO-INTESTINAL FLUID AND TAKING SAMPLES THROUGH THE ANALYTICAL PROCEDURE

	% Recovery		
Benzimidazole	Plasma	Ruminal Fluid	Abomasal Fluid
Fenbendazole	83-100 (n=15)	82-105 (n=12)	83-112 (n=12)
Oxfendazole	70- 97 (n=10)	70- 98 (n=10)	72- 92 (n=12)
Albendazole	73-103 (n=10)	72- 97 (n=10)	77- 94 (n=10)

Fig. 2 (4) High performance liquid chromatograms of samples from a sheep dosed with oxfendazole 10 mg/kg

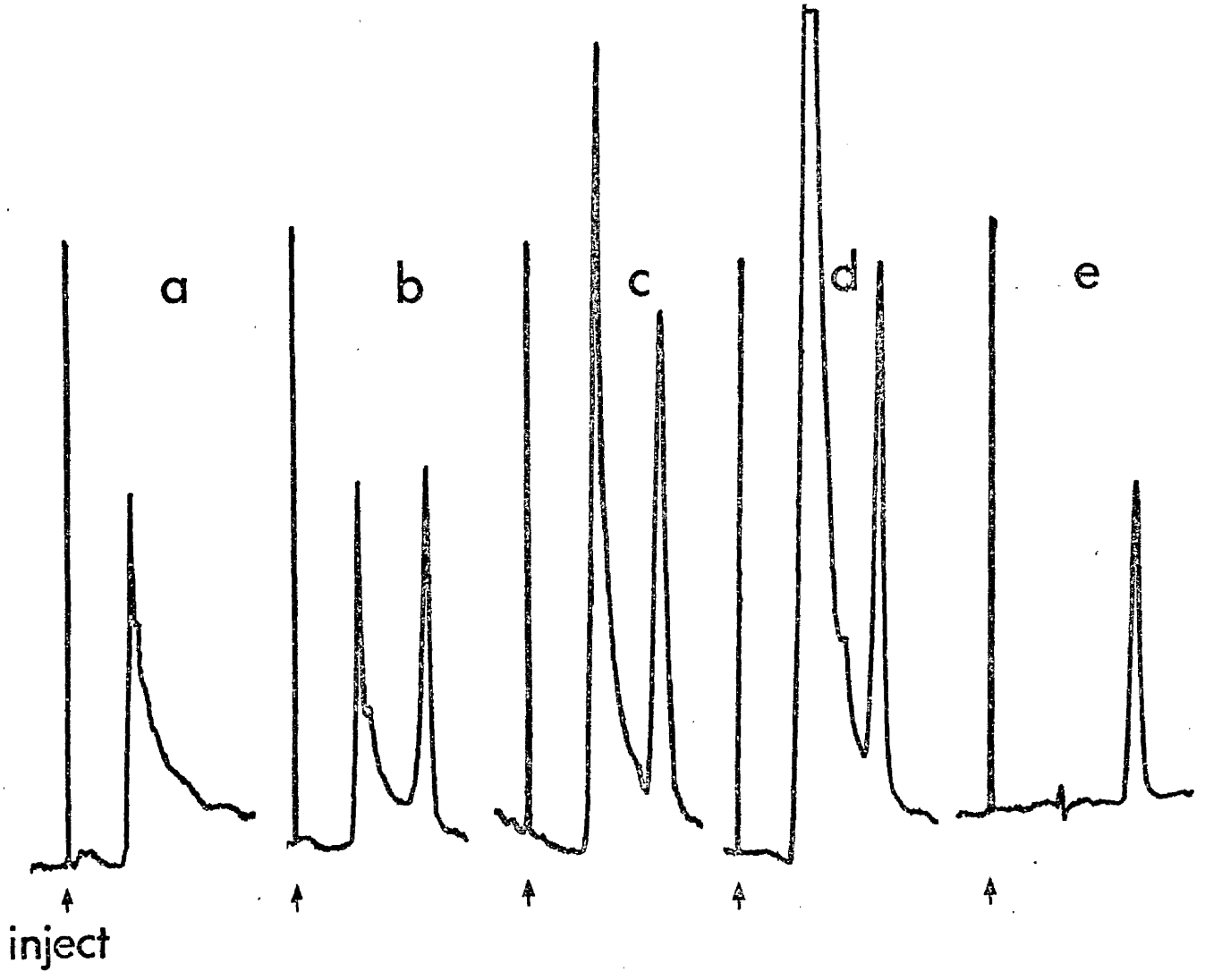
Key a) plasma before oxfendazole administration

b) plasma estimated to contain 0.16 µg/ml oxfendazole

c) abomasal fluid estimated to contain 0.25 µg/ml oxfendazole

d) ruminal fluid estimated to contain 0.25 µg/ml oxfendazole

e) oxfendazole standard in methanol (10 µg/ml)


a
b
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inject

Fig. 5 (8)) appeared as single chromatographic peaks. These peaks were well separated from that of the parent compound, having a much shorter retention time in both cases.

The two important metabolites (vide infra), the sulfoxide and sulfone, could not be separated using any variation of the methanol/ammonium carbonate solvent and a different solvent was necessary. It was found that methanol/water (in the same ratio as the methanol/ammonium carbonate mixture used for estimation of the parent drug) with the addition of a small amount of acid (1 µl of 1.1% w/v perchloric acid per ml of solvent) resulted in separation of the two compounds. Under these conditions the retention times of the sulfone metabolites were unchanged whilst those of the sulfoxides were increased.

Albendazole-2-aminosulfone, albendazole and fenbendazole were not detected under this system. (The equivalent 2-amino sulfone metabolite of fenbendazole was not available to us).

Albendazole-2-amino sulfone was separable from the other two metabolites of albendazole by using a 50:50 methanol: ammonium carbonate solvent, and under these conditions eluted in advance of the sulfoxide and sulfone.

Fig. 2 (5) shows high performance liquid chromatograms of albendazole and its metabolites in abomasal fluid using methanol/ammonium carbonate, and Fig. 2 (6) shows separation of the sulfoxide and sulfone using methanol/perchloric acid.

The shape of the sulfoxide peak using the methanol/ perchloric acid solvent was somewhat broader and flatter than

Fig. 2 (5) High performance liquid chromatogram of albendazole and its metabolites in abomasal fluid using methanol ammonium carbonate

a) abomasal fluid extract before drug administration
b) extract containing albendazole + metabolites
c) albendazole 10 µg/ml in methanol
d) albendazole sulfoxide 10 µg/ml in methanol

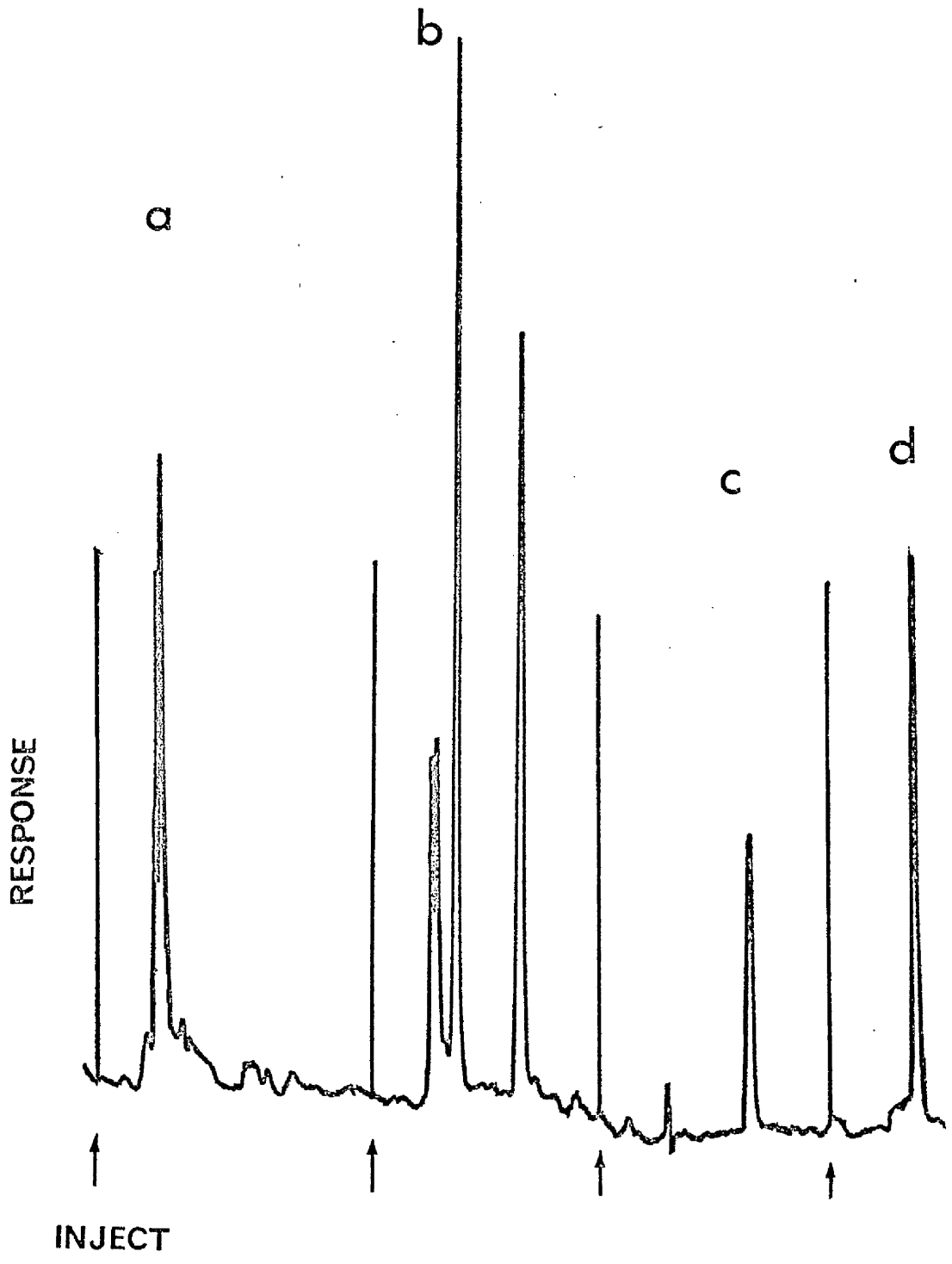
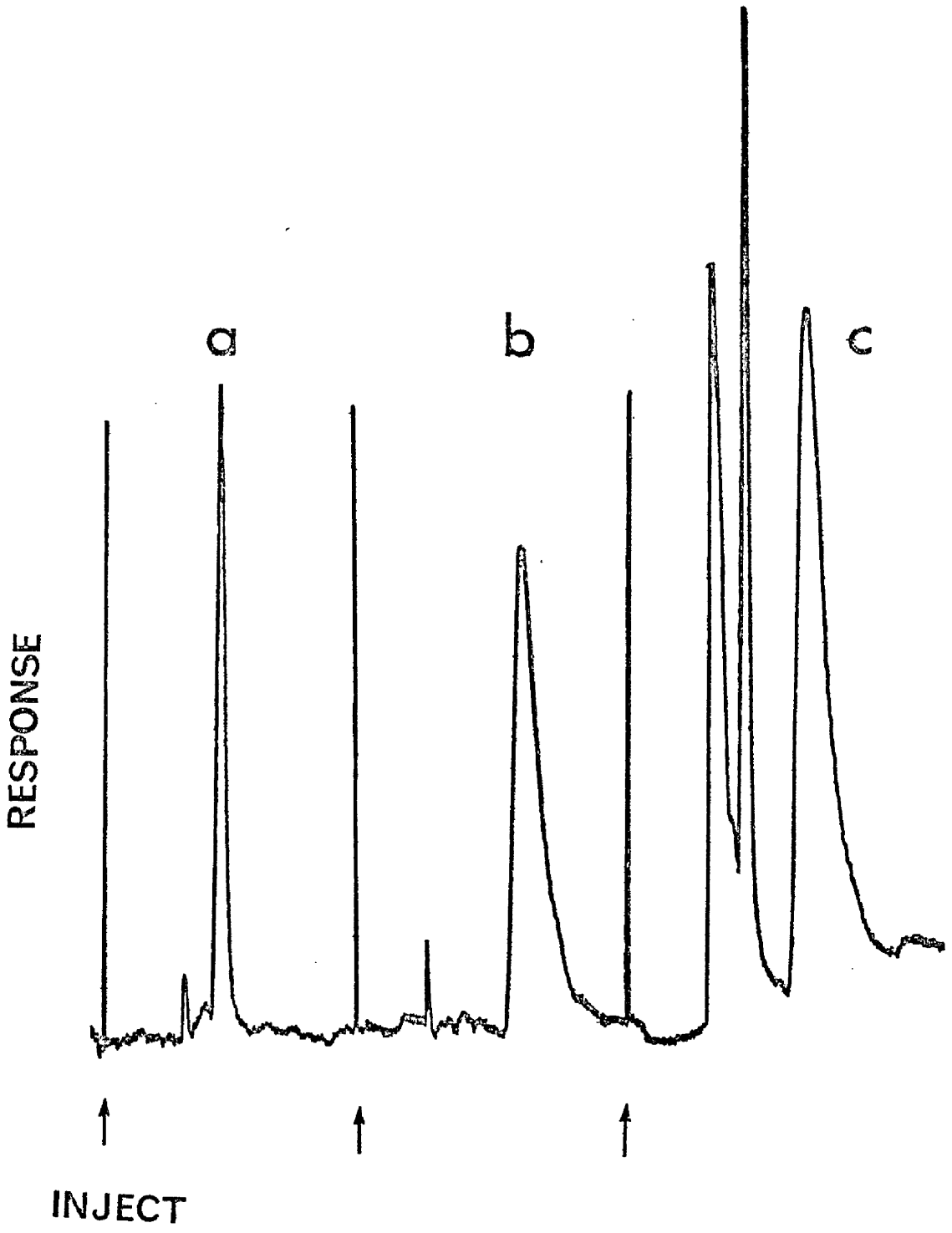

a
b
c
d
RESPONSE
INJECT

Fig. 2 (6) Separation of albendazole sulfoxide and albendazole sulfone using methanol/perchloric acid

a) sulfone 20 µg/ml in methanol
b) sulfoxide 50 µg/ml in methanol
c) abomasal fluid extract containing 0.95 µg/ml sulfoxide and 0.38 µg/ml sulfone


a
b
c
RESPONSE
INJECT

that of the same compound under methanol/ammonium carbonate. This resulted in reduced sensitivity of the method, and the limit of detection of sulfoxide was increased from 0.01 µg/ml under methanol/ammonium carbonate to 0.1 µg/ml. That of the sulfone under both solvents was 0.01 µg/ml.

LEVAMISOLE

Levamisole has been determined in bovine tissues and milk by cathode ray polarography (Holbrook and Scales, 1967) and in milk by gas-liquid chromatography (Smith, Pasarela and Wycroff, 1976). Cathode ray polarography, as well as being unavailable in this department, was known to be a relatively insensitive technique. The gas chromatographic method involved a lengthy clean up procedure, and it was thought that high performance liquid chromatography might well provide a more suitable technique for the analysis of levamisole. The HPLC solvents and ether extraction used for benzimidazole analysis were found to be equally suitable for levamisole and the following method was derived.

REAGENTS

All reagents were of analar grade.

1) Diethyl ether

2) Borate buffer pH 9

Solution A - Dissolve 0.746 g potassium chloride and 0.618 g boric acid in 180 ml of distilled water.

Solution B - 0.2N sodium hydroxide.

Mix solutions A and B in the approximate proportion of 9:1 to give pH 9 (pH meter).

3) Methanol - re-distilled before use

4) Ammonium carbonate (0.05M) prepared using permanganate distilled water

5) Britton Robinson buffer pH 10.7

Solution A - 11.3 ml glacial acetic acid
13.55 ml phosphoric acid (S.G. 1.7)
12.45 g boric acid
) per litre

Solution B - 1M sodium hydroxide.

Mix solutions A and B in approximate proportions 5:4 to give pH 10.7 (pH meter).

6) 0.1M hydrochloric acid

7) 1M sodium hydroxide.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Pump:	Altex model 110
Detector:	Cecil CE 2012 variable wavelength spectrophotometer
Column:	100 mm x 5 mm Shandon Southern
Packing:	ODS Hypersil 10 (Whatman)
Wavelength:	220 nm
Absorbance	0.1 A.U.F.S.
Solvent:	Methanol:ammonium carbonate 65:35
Flow rate:	1.0 ml/min.

Under these conditions levamisole had a retention time of 2.7 minutes.

Procedure for plasma

To 2 ml of plasma in a 50 ml ground glass stoppered test tube was added 2 ml of pH 9 borate buffer and 15 ml of ether. The tube was firmly stoppered and shaken for 10 minutes on a slow rotary mixer. 10.5 ml of the ether (upper) layer was removed to a 50 ml test tube using a 5 ml adjustable pipette. (The pipette tip required to be pre-wetted with the ether before accurate transfer could be effected).

A further 15 ml of ether was added to the first tube and shaken as before for 10 minutes. 15 ml of the ether (upper) layer was transferred to the second test tube, and the combined ether evaporated on a dri-bath at $55^{o}C$ under nitrogen to a volume of approximately 6 ml. This was transferred to a 10 ml conical glass tube. The 50 ml test tube was washed down three times with 1 ml of ether and the washings added each time to the 10 ml tube. This was evaporated to dryness, the walls washed with 0.5 ml of ether and again evaporated to dryness. The residue was taken up in 100 µl of methanol by rotating and tilting the tube in an ultrasonic bath. 5 µl of the extract was injected on to the HPLC column.

Procedure for abomasal and ruminal fluid

Abomasal fluid is strongly acidic (generally pH between 2.2 and 2.8 in these sheep) and levamisole, in contrast to fenbendazole, was well extracted into ether at alkaline pH only.

Since the addition of 2 ml of pH 9 borate buffer as used in the method for plasma raised the pH to only 7, it was found necessary to substitute a pH 10.7 Britton-Robinson buffer. This gave a satisfactory extraction, using the above procedure.

Rumen samples gave rise to many interfering peaks on the chromatograms and so a clean up step was found to be necessary as described below.

The sample was buffered and shaken twice with ether as for plasma. The ether layers were transferred to a 50 ml ground glass stoppered tube and shaken for 10 minutes with 3 ml of hydrochloric acid. The ether (upper) layer was discarded using suction, taking care not to disturb the interface. A further 15 ml of ether was added to the aqueous layer followed by 0.5 ml of sodium hydroxide. This was shaken for 10 minutes as above. 12 ml of the ether (upper) layer was removed to a 50 ml glass test tube. A further 15 ml of ether was added to the aqueous layer and shaken for 10 minutes as above. 15 ml of the ether (upper) layer was removed and combined with the 12 ml of ether. The combined ether was treated as described for plasma.

This back extraction into acid followed by a second forward extraction resulted in residues with negligible interfering substances.

The concentration of levamisole in the samples was calculated from calibration curves prepared by adding known amounts of levamisole to blank plasma or gastro-intestinal fluid to give a reasonable concentration range, viz 0.2-3.0 µg/ml for plasma.

These standards were extracted along with the samples and the peak height of the sample compared with the calibration curve prepared from the standards.

Non-extracted standards of levamisole in methanol were also injected as a check on the column performance, and to estimate recoveries of levamisole using this procedure.

Typical chromatograms of levamisole are shown in Fig. 2 (7). Recoveries were as follows:

Plasma	88% (81-98)	n = 30
Abomasal fluid	85% (78-96)	n = 28
Ruminal fluid	80% (74-89)	n = 25.

The precision and accuracy of the method were determined by adding known amounts of levamisole to plasma. Samples of each known concentration were then assayed in triplicate by both procedures. The results are shown in Table 2 (4).

It was determined that recovery losses were due to extraction losses rather than to degradation of the levamisole.

Fig. 2 (7) High performance liquid chromatograms of levamisole in methanol and in extracts of samples from a sheep dosed with levamisole 7.5 mg/kg

a) 5 microlitres of 5 µg/ml levamisole in methanol
b) 5 microlitres of 10 µg/ml levamisole in methanol
c) 5 microlitres of 15 µg/ml levamisole in methanol
d) 5 microlitres of 20 µg/ml levamisole in methanol
e) plasma before levamisole administration
f) plasma estimated to contain 0.42 µg/ml levamisole
g) ruminal fluid before levamisole administration
h) ruminal fluid estimated to contain 0.36 µg/ml levamisole

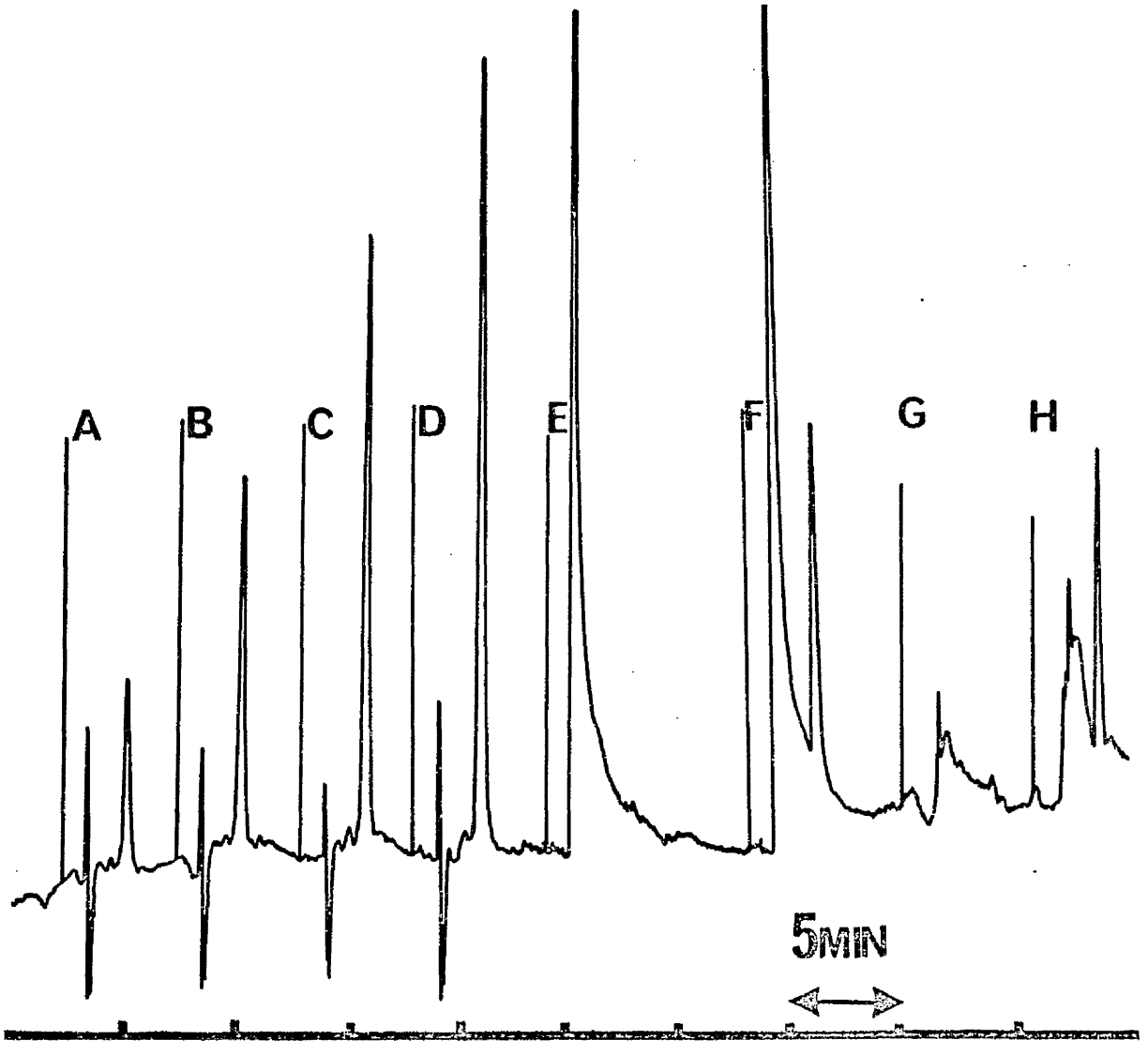

A
B
C
D
E
F
G
H
5MIN

TABLE 2 (4) ESTIMATION OF LEVAMISOLE IN PLASMA

Levamisole Added (µg)	Levamisole Measured (µg)			Mean ± S.E.M.	S.E. as % of Mean	Ratio of Assayed to Added
0.4	0.32	0.32	0.30*	0.31 ± 0.007	2.2	77.5
	0.30	0.27	0.29†	0.29 ± 0.009	3.0	72.5
1.0	0.82	0.78	0.84*	0.81 ± 0.02	2.2	0.81
	0.78	0.77	0.74†	0.76 ± 0.01	1.6	0.76
1.5	1.26	1.40	1.29*	1.32 ± 0.04	3.2	0.88
	1.26	1.15	1.19†	1.20 ± 0.03	2.7	0.80
2.0	1.60	1.56	1.68*	1.61 ± 0.04	2.2	0.81
	1.61	1.50	1.53†	1.55 ± 0.03	2.1	0.78

* Short procedure

† Using back extraction.

STATISTICS

Conventional statistical methods were used for calculating means ($\bar{x}$) and standard error of the mean (S.E.M.)

Intra-individual differences were tested for significance using Student's 't' test for paired samples (Snedecor, 1956)

Areas under the curve were calculated using Simpson's approximation (discrete).

CHAPTER 3

STUDIES WITH MECLOFENAMATE

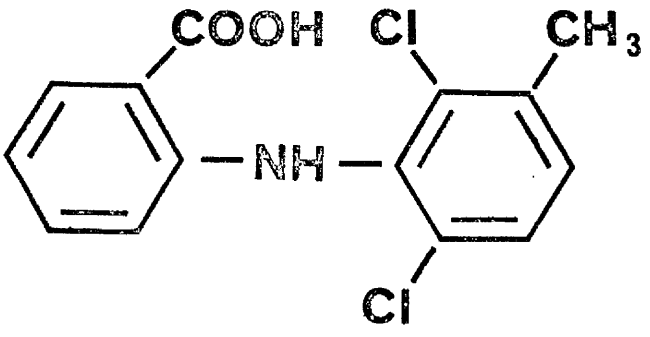
Meclofenamic acid is a non-steroidal anti-inflammatory drug which has been shown to possess anti-anaphylactic activity in cattle (Aitken and Sanford, 1969, 1972; Wells, Eyre and Lumsden, 1973) and in sheep (Alexander, Eyre, Head and Sanford, 1970). It is marketed for use in horses in the United Kingdom ("Arquel", Parke-Davis).

The structure of meclofenamic acid is shown in Fig. 2 (1).

Meclofenamic acid was used in this study as an acidic drug for which a simple, sensitive assay procedure was available.

Plasma concentrations of meclofenamate in cattle after intravenous, oral and intra-ruminal administration have been reported by Aitken and Sanford (1975). These authors found maximum concentrations in plasma occurring at 30 minutes and at 4-6 hours after oral dosing of both adult cattle and 4-6 month old calves. After intra-ruminal administration to two cows, a single plasma peak occurred at 8-12 hours.

Fig. 3 (1) The structure of meclofenamic acid


COOH
Cl
CH3
NH
Cl

EXPERIMENTS WITH MECLOFENAMIC ACID

Meclofenamic acid granules ("Arquel", Parke-Davis) were administered to three sheep as follows:

Experiment 1) Orally as a slurry in 150 ml of water using a drenching bottle. The dose rate was 20 mg/kg.

Experiment 2) Intra-ruminally via the ruminal cannulae at a dose rate of 20 mg/kg.

Experiment 3) Intra-abomasally via the abomasal cannulae at a dose rate of 10 mg/kg.

Blood and ruminal abomasal and duodenal fluid samples were taken prior to drug administration and at 10, 20 and 40 minutes and 1, 1½, 2, 3, 5, 7, 10, 14, 18 and 22 hours after administration.

Samples were taken and treated as described in Chapter 2. They were later analysed as described in Chapter 2 for the concentration of free meclofenamate.

RESULTS

The mean plasma and gastro-intestinal fluid concentrations of meclofenamate in the three sheep after oral, intra-ruminal and intra-abomasal administration are shown in Figs. 3 (2), 3 (3) and 3 (4) respectively and in Tables 3 (1) to 3 (12).

In all sheep after oral administration a bi-phasic pattern in plasma concentrations was observed; an initial maximum occurring within 40 minutes of drug administration and a second more sustained peak about 5 hours after administration.

After intra-ruminal administration the initial maximum

Fig. 3 (2) Mean plasma and gastro-intestinal fluid concentrations (µg/ml) of meclofenamate in three sheep after oral administration of meclofenamic acid (20 mg/kg)

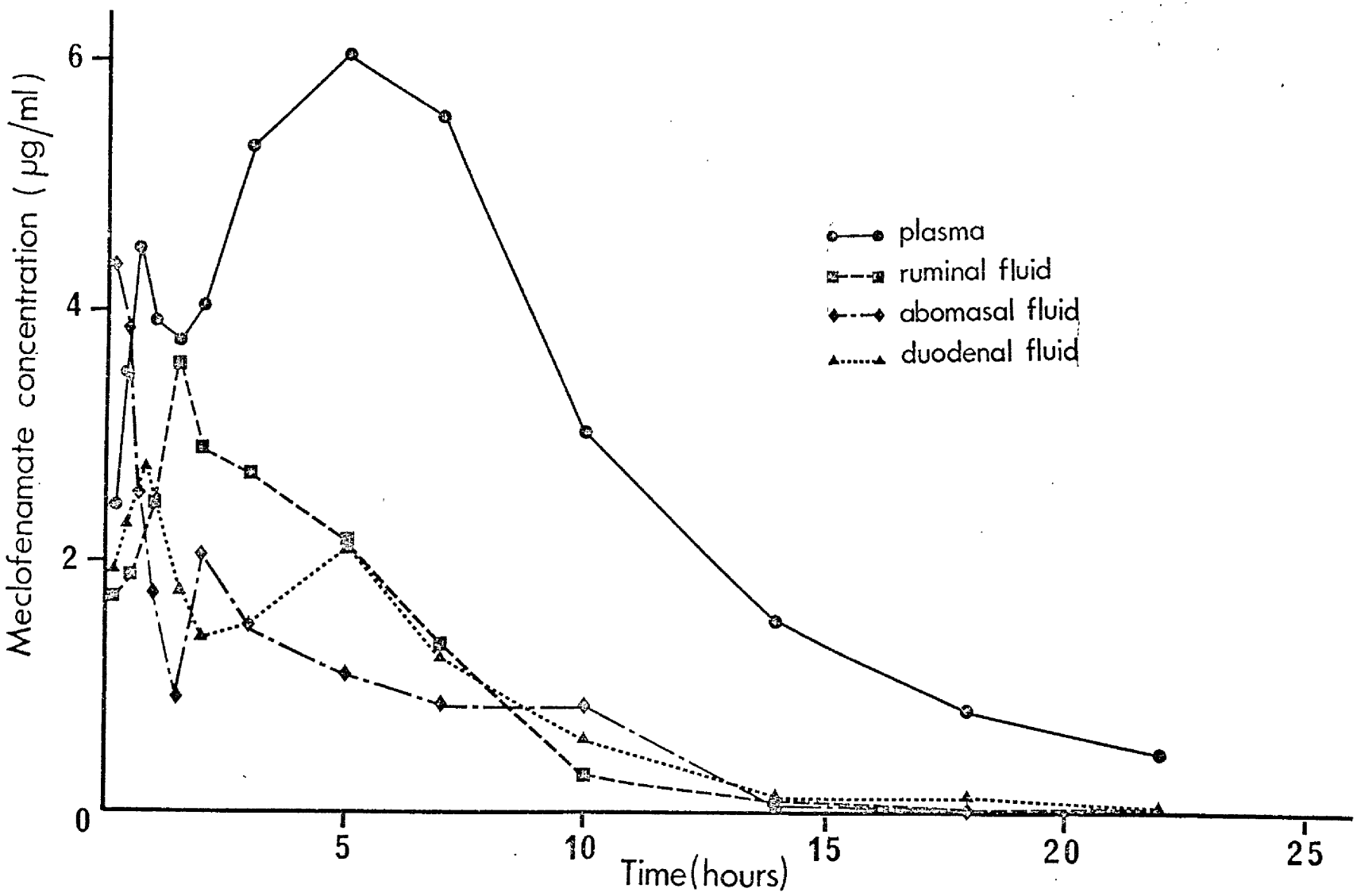

Meclofenamate concentration (µg/ml)
6
4
2
0
5
10
15
20
25
Time(hours)
plasma
ruminal fluid
abomasal fluid
duodenal fluid

Fig. 3 (3) Mean plasma and gastro-intestinal fluid concentrations (µg/ml) of meclofenamate in three sheep after intra-ruminal administration of meclofenamic acid (20 mg/kg)

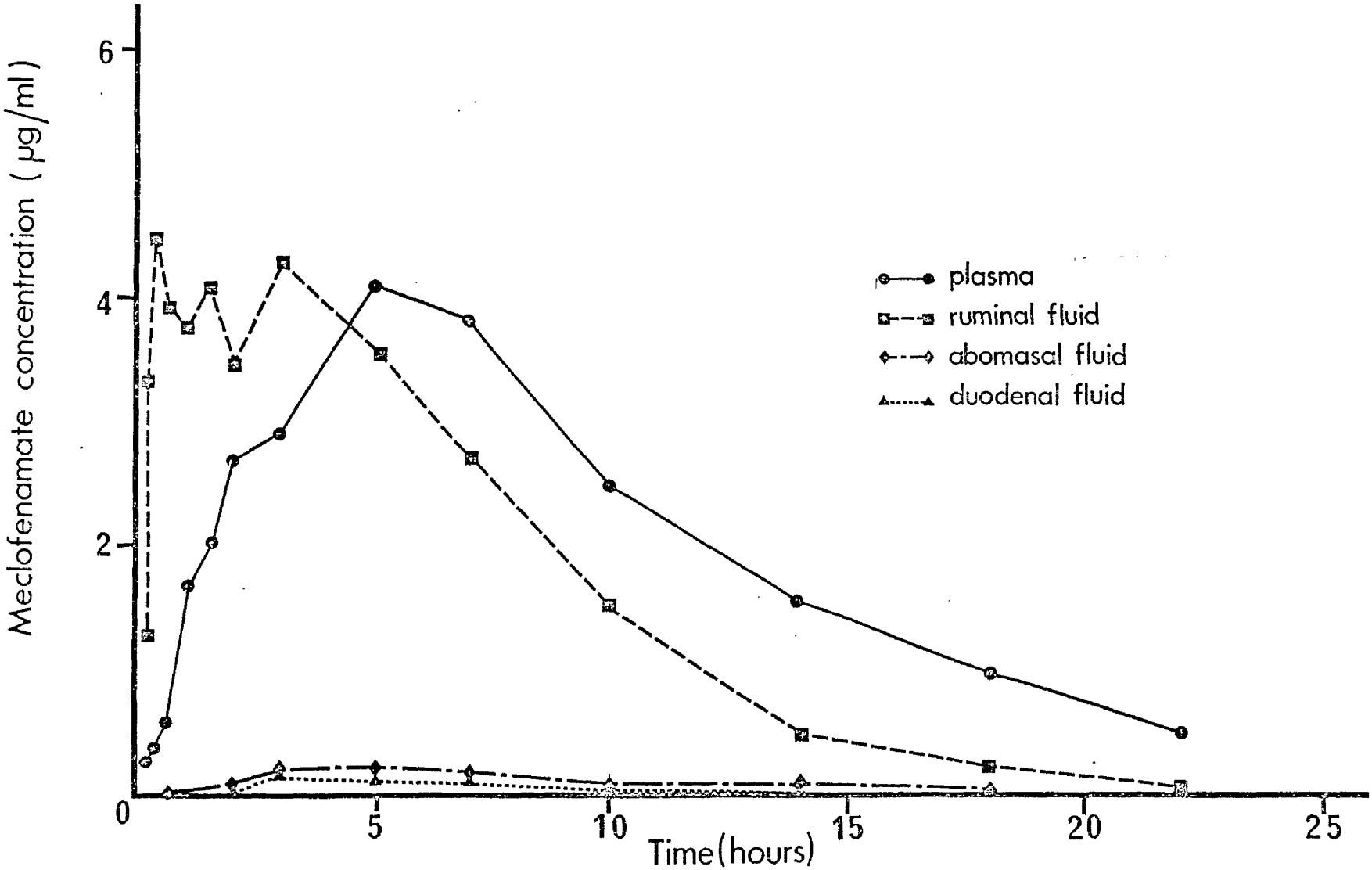

Meclofenamate concentration (µg/ml)
6
4
2
0
5
10
15
20
25
Time(hours)
plasma
ruminal fluid
abomasal fluid
duodenal fluid

Fig. 3 (4) Mean plasma and gastro-intestinal fluid concentrations (µg/ml of meclofenamate in three sheep after intra-abomasal administration of meclofenamic acid (10 mg/kg)

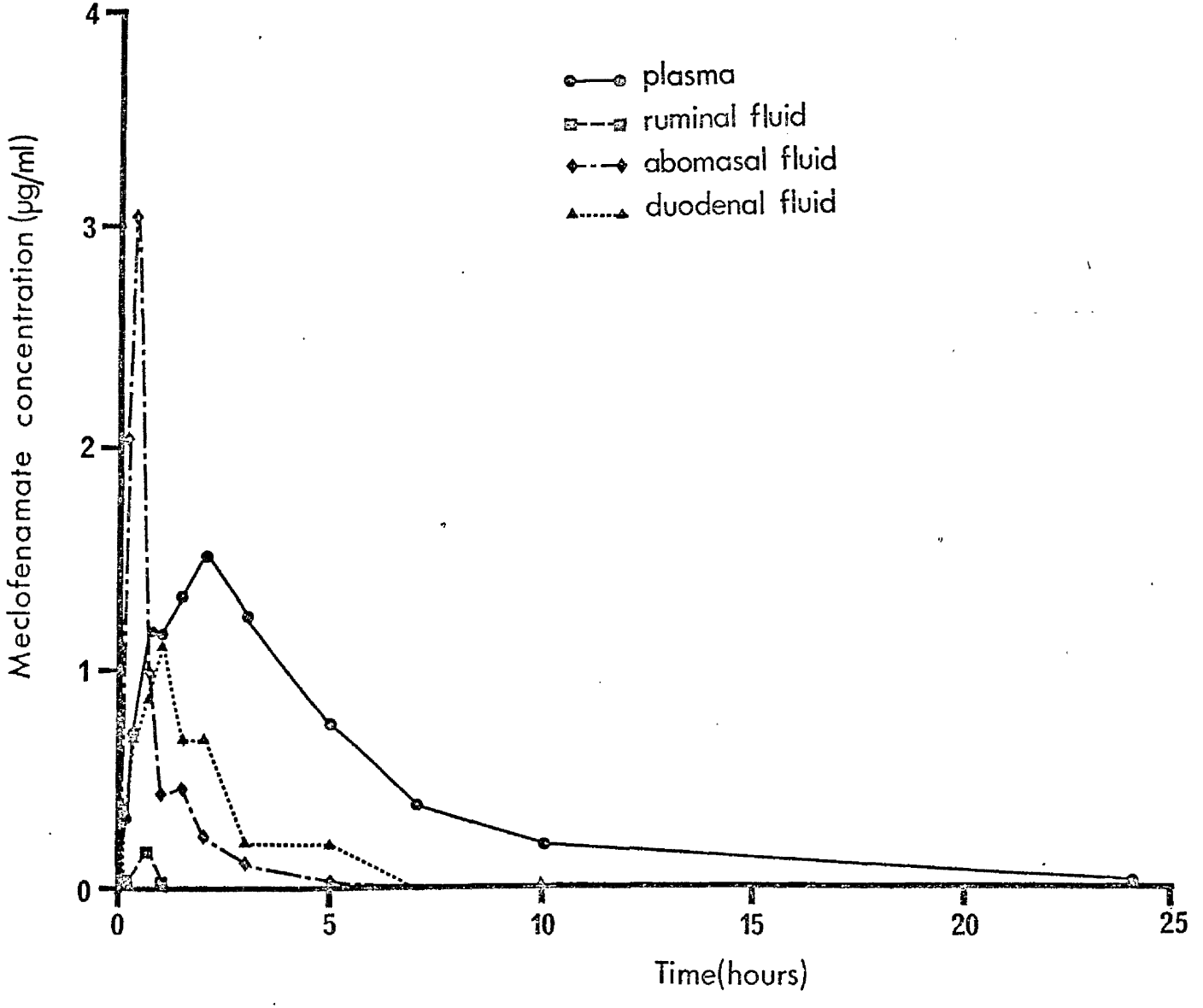

plasma
ruminal fluid
abomasal fluid
duodenal fluid
Meclofenamate concentration (µg/ml)
0
1
2
3
4
0
5
10
15
20
25
Time(hours)

TABLE 3 (1) PLASMA CONCENTRATIONS (µg/ml) OF MECLOFENAMATE AFTER ORAL ADMINISTRATION OF MECLOFENAMIC ACID TO 3 SHEEP (20 mg/kg)

Time	Sheep number 71	74	75	Mean ± S.E.M.
10 min	3.33	1.96	1.96	2.42 ± 0.46
20 min	4.89	3.01	2.64	3.51 ± 0.70
40 min	4.89	3.48	5.02	4.46 ± 0.49
1 hr	4.07	2.81	4.80	3.89 ± 0.58
1½ hr	3.31	2.65	5.25	3.74 ± 0.78
2 hr	3.82	2.54	5.71	4.02 ± 0.92
3 hr	6.15	2.98	6.68	5.27 ± 1.15
5 hr	9.32	3.25	5.45	6.01 ± 1.78
7 hr	8.06	3.60	4.96	5.54 ± 1.32
10 hr	5.53	1.43	2.07	3.01 ± 1.27
14 hr	2.24	1.11	1.07	1.47 ± 0.38
18 hr	1.35	0.41	0.66	0.81 ± 0.28
22 hr	0.64	0.41	0.29	0.45 ± 0.10

TABLE 3 (2) RUMINAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER ORAL ADMINISTRATION OF MECLOFENAMIC ACID TO 3 SHEEP (20 mg/kg)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	0.81	4.17	0.16	1.71 ± 1.24
20 min	1.42	3.78	0.24	1.81 ± 1.04
40 min	2.61	5.49	0.38	2.83 ± 1.48
1 hr	2.95	4.07	0.41	2.48 ± 1.08
1½ hr	6.10	4.60	0.36	3.69 ± 1.72
2 hr	2.81	5.54	0.33	2.89 ± 1.50
3 hr	2.98	4.66	0.48	2.71 ± 1.21
5 hr	2.64	3.73	0.13	2.17 ± 1.07
7 hr	1.64	2.32	0.08	1.35 ± 0.66
10 hr	0.38	0.40	0.04	0.27 ± 0.12
14 hr	0.18	0.05	0	0.08 ± 0.05
18 hr	0.11	0.01	0	0.04 ± 0.04
22 hr	0	0	0	0

TABLE 3 (3) ABOMASAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER ORAL ADMINISTRATION OF MECLOFENAMIC ACID TO 3 SHEEP (20 mg/kg)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	3.87	0.84	8.41	4.37 ± 2.20
20 min	7.78	1.46	2.34	3.86 ± 1.98
40 min	4.69	0.94	1.90	2.51 ± 1.12
1 hr	2.91	1.03	1.22	1.72 ± 0.60
1½ hr	0.41	0.90	1.44	0.92 ± 0.30
2 hr	3.69	0.79	1.67	2.05 ± 0.86
3 hr	1.90	1.46	1.10	1.49 ± 0.23
5 hr	1.00	1.55	0.76	1.10 ± 0.22
7 hr	1.41	0.92	0.17	0.83 ± 0.34
10 hr	2.20	0.31	0.10	0.87 ± 0.66
14 hr	0	0.14	0.01	0.05 ± 0.05
18 hr	0	0	0	0
22 hr	0	0	0	0

TABLE 3 (4) DUODENAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER ORAL ADMINISTRATION OF MECLOFENAMIC ACID TO 3 SHEEP (20 mg/kg)

Time	Sheep number			Mean ± S.E.M.
	71	74	75	
10 min	3.47	0.76	1.65	1.96 ± 0.80
20 min	4.98	0.72	1.19	2.30 ± 1.35
40 min	3.22	1.92	2.75	2.63 ± 0.38
1 hr	2.55	3.14	1.59	2.43 ± 0.45
1½ hr	1.63	2.25	1.28	1.72 ± 0.28
2 hr	1.77	1.39	0.98	1.38 ± 0.23
3 hr	2.70	0.95	0.76	1.47 ± 0.62
5 hr	4.68	1.11	0.55	2.11 ± 1.29
7 hr	2.44	1.15	0.03	1.21 ± 0.70
10 hr	0.61	0.54	0.52	0.56 ± 0.03
14 hr	0.14	0.19	0.07	0.13 ± 0.03
18 hr	0	0.35	0.09	0.15 ± 0.10
22 hr	0.09	0.05	0	0.05 ± 0.02

TABLE 3 (5) PLASMA CONCENTRATIONS (µg/ml) OF MECLOFENAMATE AFTER INTRA-RUMINAL ADMINISTRATION OF MECLOFENAMIC ACID TO 3 SHEEP (20 mg/kg)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	0	0.20	0.45	0.22 ± 0.13
20 min	0.08	0.33	0.68	0.36 ± 0.17
40 min	0.54	0.53	0.68	0.58 ± 0.05
1 hr	1.98	2.35	0.65	1.66 ± 0.52
1½ hr	2.85	2.55	0.64	2.01 ± 0.69
2 hr	3.36	2.75	1.90	2.67 ± 0.42
3 hr	3.69	2.39	2.47	2.85 ± 0.42
5 hr	4.65	3.84	3.80	4.10 ± 0.28
7 hr	4.98	3.47	2.98	3.81 ± 0.60
10 hr	3.00	1.91	2.40	2.44 ± 0.32
14 hr	2.21	1.08	1.30	1.53 ± 0.35
18 hr	1.18	0.53	1.20	0.97 ± 0.22
22 hr	0.49	0.28	0.65	0.47 ± 0.11

TABLE 3 (6) RUMINAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER INTRA-RUMINAL ADMINISTRATION OF MECLOFENAMIC ACID TO 3 SHEEP (20 mg/kg)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	2.47	4.69	2.57	3.24 ± 0.72
20 min	4.66	3.96	4.85	4.49 ± 0.27
40 min	2.08	6.32	3.38	3.93 ± 1.25
1 hr	2.14	3.49	5.71	3.78 ± 1.04
1½ hr	2.28	4.44	5.58	4.10 ± 0.97
2 hr	3.04	4.14	3.29	3.49 ± 0.33
3 hr	4.58	5.30	2.89	4.26 ± 0.71
5 hr	5.58	2.54	2.65	3.59 ± 1.00
7 hr	3.65	2.80	1.71	2.72 ± 0.56
10 hr	2.25	1.16	1.05	1.49 ± 0.38
14 hr	0.64	0.30	0.44	0.46 ± 0.10
18 hr	0.28	0.10	0.25	0.21 ± 0.06
22 hr	0.15	0	0.08	0.08 ± 0.04

TABLE 3 (7) ABOMASAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER INTRA-RUMINAL ADMINISTRATION OF MECLOFENAMIC ACID TO 3 SHEEP (20 mg/kg)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	0.01	0	0	0
20 min	0	0	0	0
40 min	0	0.02	0	0.01 ± 0.01
1 hr	0	0	0	0
1½ hr	0	0.07	0	0.02 ± 0.02
2 hr	0.05	0.21	0.04	0.10 ± 0.06
3 hr	0.16	0.20	0.22	0.19 ± 0.02
5 hr	0.18	0.23	0.16	0.19 ± 0.02
7 hr	0.26	0.18	0.03	0.16 ± 0.07
10 hr	0.15	0.03	0.04	0.07 ± 0.04
14 hr	0.07	0.06	0	0.04 ± 0.02
18 hr	0	0	0.02	0.01 ± 0.01
22 hr	0	0	0	0

TABLE 3 (8) DUODENAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER INTRA-RUMINAL ADMINISTRATION OF MECLOFENAMIC ACID TO 3 SHEEP (20 mg/kg)

Time	Sheep number			Mean ± S.E.M.
	71	74	75	
10 min	0	0	0	0
20 min	0	0	0	0
40 min	0	0	0	0
1 hr	0	0	0	0
1½ hr	0.02	0.08	0	0.03 ± 0.02
2 hr	0.01	0.12	0	0.04 ± 0.04
3 hr	0.22	0.15	0.14	0.17 ± 0.03
5 hr	0.13	0.12	0.11	0.12 ± 0.01
7 hr	0.15	0.05	0.09	0.10 ± 0.03
10 hr	0.11	0	0.02	0.04 ± 0.03
14 hr	0	0	0.01	0
18 hr	0	0	0	0
22 hr	0	0	0	0

TABLE 3 (9) PLASMA CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER INTRA-ABOMASAL ADMINISTRATION OF MECLOFENAMIC ACID TO 3 SHEEP (10 mg/kg)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	0.55	0.07	0.34	0.32 ± 0.14
20 min	1.46	0.06	0.57	0.70 ± 0.41
40 min	2.32	0.42	0.74	1.16 ± 0.59
1 hr	1.80	0.98	0.68	1.15 ± 0.33
1½ hr	1.66	1.06	1.24	1.32 ± 0.18
2 hr	1.56	1.13	1.80	1.50 ± 0.20
3 hr	0.96	1.26	1.43	1.22 ± 0.14
5 hr	0.60	0.84	0.80	0.75 ± 0.07
7 hr	0.30	0.37	0.46	0.38 ± 0.05
10 hr	0.15	0.19	0.29	0.21 ± 0.04
24 hr	0.01	0.01	0.03	0.02 ± 0.01

TABLE 3 (10) RUMINAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER INTRA-ABOMASAL ADMINISTRATION OF MECLOFENAMIC ACID TO 3 SHEEP (10 mg/kg)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	0.06	0	0.05	0.04 ± 0.02
20 min	0.01	0	0.02	0.01 ± 0.01
40 min	0.24	0.19	0.08	0.17 ± 0.05
1 hr	0	0	0.07	0.02 ± 0.02
1½ hr	0	0	0.03	0.01 ± 0.01
2 hr	0	0	0.03	0.01 ± 0.01
3 hr	0	0	0.04	0.01 ± 0.01
5 hr	0	0.02	0	0.01 ± 0.01
7 hr	0	0	0	0
10 hr	0	0	0	0
24 hr	0	0	0	0

TABLE 3 (11) ABOMASAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER INTRA-ABOMASAL ADMINISTRATION OF MECLOFENAMIC ACID TO 3 SHEEP (10 mg/kg)

Time	Sheep number			Mean ± S.E.M.
	71	74	75	
10 min	2.26	3.37	0.46	2.03 ± 0.85
20 min	0.95	7.70	0.51	3.05 ± 2.33
40 min	0.82	1.58	0.55	0.98 ± 0.31
1 hr	0.23	0.47	0.55	0.42 ± 0.10
1½ hr	0.87	0.33	0.11	0.44 ± 0.23
2 hr	0.24	0.30	0.16	0.23 ± 0.04
3 hr	0.01	0.11	0.21	0.11 ± 0.06
5 hr	0	0.07	0.02	0.03 ± 0.02
7 hr	0	0	0	0
10 hr	0	0	0	0
24 hr	0	0	0	0

TABLE 3 (12) DUODENAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER INTRA-ABOMASAL ADMINISTRATION OF MECLOFENAMIC ACID TO 3 SHEEP (10 mg/kg)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	0.36	0.02	0.69	0.36 ± 0.19
20 min	1.03	0.55	0.44	0.67 ± 0.18
40 min	1.38	0.34	0.84	0.85 ± 0.30
1 hr	2.41	0.26	0.59	1.09 ± 0.67
1½ hr	1.23	0.35	0.41	0.66 ± 0.28
2 hr	1.12	0.20	No S	0.66 ± 0.46
3 hr	0.33	0.06	No S	0.20 ± 0.14
5 hr	0.08	0	0.50	0.19 ± 0.16
7 hr	0.02	0	0	0.01 ± 0.01
10 hr	0	0	0	0 0
24 hr	0.02	0	0	0.01 ± 0.01

plasma concentration was absent and the second more sustained peak was similar to that seen after oral administration.

Intra-abomasal administration resulted in a single plasma maximum occurring 2 hours after dosing. Maximum concentrations achieved by this route were lower and less sustained than by either of the other two routes.

The presence of meclofenamate in the abomasum within 10 minutes of oral administration was almost certainly due to functioning of the reticular groove reflex, since no drug was detectable in the abomasum after intra-ruminal administration for over 1 hour after administration.

The concentrations of meclofenamate in the rumen, abomasum, duodenum and plasma (Figs. 3 (1) to 3 (3)) were all consistent with this view that a proportion of orally administered meclofenamate was delivered directly to the abomasum.

The abomasal and duodenal concentrations of meclofenamate at times later than 2 hours after intra-ruminal administration were considerably lower than after oral administration. Although only mean values are shown, this was found in each individual sheep. These elevated concentrations after oral administration may have been due to slow passage and slow absorption of the proportion of the administered dose delivered to the abomasum, or, more probably, that some of the drug by-passing the rumen due to the reticular groove reflex was deposited in the omasum and that this amount was responsible for maintaining abomasal and duodenal concentrations. McEwan and Oakley (1978) have shown in calves given anthelmintic

drenches that, in those calves where the rumen was by-passed, some or all of the drench was deposited in the omasum.

This latter view is also supported by the results of experiment 3 where abomasal and duodenal concentrations of meclofenamate were much lower than after oral administration although in this case the entire dose was deposited in the abomasum.

Meclofenamate was detectable at very low concentrations in the earlier rumen samples after intra-abomasal administration. The maximum concentration in all sheep occurred at 40 minutes after administration. This was the only time at which drug was detectable in one animal.

DISCUSSION

There is evidence in these experiments that closure of the reticular groove occurred in all three animals to a varying degree, as estimated by abomasal fluid concentration 10 minutes after dosing.

The very low plasma concentrations found in all three sheep for over an hour after intra-ruminal administration would suggest that absorption from the rumen, if it occurs, must be very slow relative to the rate of transfer across other parts of the gastro-intestinal tract. At the pH of both plasma and rumen contents (in these sheep 5.6-7.0) meclofenamic acid (pk_a 3.76) is very extensively ionised but the theoretical equilibrium between plasma and rumen contents is about 10:1 in favour of plasma.

It is interesting that meclofenamic acid was present in the rumen after intra-abomasal administration. The ratio of plasma:rumen fluid concentrations was generally much higher than the predicted ratio of 10:1,

suggesting that diffusion across the ruminal epithelium was extremely slow. Concentration and excretion of acidic drugs in the alkaline saliva of ruminants has been demonstrated by Rasmussen (1963) and the large volumes of saliva produced by sheep (6-16 litres per day (Kay, 1980)) might account partially or totally for the concentrations found in the rumen.

From the results of Experiments 1 and 3 it is apparent that intra-abomasal administration of meclofenamic acid did not mimic the delivery of drug to the abomasum via the reticular groove. Even allowing for the fact that the dose given intra-abomasally was only half of that given orally the maximum concentrations in plasma after intra-abomasal administration were lower and occurred much later than the initial plasma maximum seen after oral administration and the reason for this is not clear.

Drug entering the abomasum via the groove does so at the omasal/abomasal orifice which is considerably anterior to the site of the abomasal cannula, and it may be that the higher plasma concentrations are a reflection of the greater surface area which is thus available for absorption. Alternatively, administration of the drug via the cannula may result in smooth muscle contraction of the abomasal wall with rapid expulsion of drug forward into the more alkaline intestine which is a less favourable environment for absorption of an acidic drug.

A third explanation is that dissolution of the drug in the abomasum is so slow that this limits the absorption. The solubility of meclofenamic acid is less than 0.03 mg/ml in water and in abomasal fluid (pH 2-3) is much lower. Orally administered drug may undergo

more rapid dissolution in the alkaline (pH 8.2) saliva and be immediately available for absorption.

In order to investigate this point it was decided to repeat Experiments 1 and 3 with the soluble sodium salt of meclofenamic acid.

EXPERIMENTS WITH SODIUM MECLOFENAMATE

The same three sheep were used as for the earlier experiments. The dose rate was equivalent to 20 mg/kg of meclofenamic acid both orally (Experiment 4) and intra-abomasally (Experiment 5). In both cases the appropriate volume of sodium meclofenamate (Parke-Davis experimental) was made up to 20 ml with water.

Drug administration and sampling were carried out as in Experiments 1 and 3.

RESULTS

Plasma and gastro-intestinal fluid concentrations of meclofenamate after oral and intra-abomasal administration of sodium meclofenamate are shown in Tables 3 (13) to 3 (20).

Fig. 3 (5) represents the mean concentrations in the different body compartments after oral administration.

The bi-phasic pattern of absorption seen after oral administration of meclofenamic acid was again evident. With the soluble form of the drug, however, the initial plasma maximum was much higher and occurred earlier, within 20 minutes of administration. The second maximum was much less marked and occurred later than after meclofenamic acid administration.

Intra-abomasally administered sodium meclofenamate gave rise to a single plasma maximum which occurred one hour after dosing. This was earlier than seen after meclofenamic acid but again later than the first plasma peak present after oral administration of the drug.

TABLE 3 (13) PLASMA CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER ORAL ADMINISTRATION OF SODIUM MECLOFENAMATE TO 3 SHEEP (20 mg/kg acid equivalent)

Time	Sheep number			Mean ± S.E.M.
	71	74	75	
10 min	10.47	4.45	9.17	8.03 ± 1.83
20 min	11.69	5.94	8.00	8.54 ± 1.68
40 min	8.38	4.98	6.76	6.71 ± 0.98
1 hr	5.00	3.55	5.66	4.74 ± 0.62
1½ hr	5.00	3.34	4.55	4.30 ± 0.50
2 hr	5.00	2.97	3.97	3.98 ± 0.59
3 hr	2.77	2.97	5.66	3.80 ± 0.93
5 hr	4.46	3.07	3.58	3.70 ± 0.41
7 hr	6.00	3.55	4.62	4.72 ± 0.71
10 hr	5.92	2.30	2.54	3.59 ± 1.17
14 hr	2.62	1.66	1.74	2.01 ± 0.31
18 hr	2.46	1.15	0.76	1.46 ± 0.51
22 hr	2.15	0.49	0.55	1.06 ± 0.54

TABLE 3 (14) RUMINAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER ORAL ADMINISTRATION OF SODIUM MECLOFENAMATE TO 3 SHEEP (20 mg/kg acid equivalent)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	0.04	1.63	6.66	2.78 ± 2.00
20 min	2.44	4.60	5.49	4.18 ± 0.91
40 min	9.29	4.72	6.50	6.84 ± 1.33
1 hr	13.89	5.33	11.13	10.12 ± 2.52
1½ hr	9.18	6.54	9.01	8.24 ± 0.85
2 hr	9.41	5.57	8.15	7.71 ± 1.13
3 hr	5.89	4.32	5.56	5.26 ± 0.48
5 hr	4.71	2.65	4.15	3.84 ± 0.61
7 hr	2.71	0.65	2.66	2.01 ± 0.68
10 hr	0.78	0.52	0.61	0.64 ± 0.08
14 hr	0.39	0.14	0.09	0.21 ± 0.09
18 hr	0.25	0.10	0.03	0.13 ± 0.06
22 hr	0.25	0.06	0.07	0.13 ± 0.06

TABLE 3 (15) ABOMASAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER ORAL ADMINISTRATION OF SODIUM MECLOFENAMATE TO 3 SHEEP (20 mg/kg acid equivalent)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	0	0.01	0.23	0.08 ± 0.08
20 min	0	0	0.37	0.12 ± 0.12
40 min	0.04	0.03	0.36	0.14 ± 0.11
1 hr	0.04	0.01	0.25	0.10 ± 0.08
1½ hr	0.04	0.03	0.22	0.10 ± 0.06
2 hr	0.09	0.04	0.12	0.08 ± 0.02
3 hr	0.10	0.01	0.24	0.12 ± 0.07
5 hr	0.11	0.05	0.24	0.13 ± 0.06
7 hr	0.08	0.01	0.06	0.05 ± 0.02
10 hr	0.02	0.03	0.03	0.03 ± 0.003
14 hr	0.16	0.01	0.05	0.07 ± 0.04
18 hr	0.06	0.01	0.03	0.03 ± 0.01
22 hr	0	0.02	0.03	0.02 ± 0.01

TABLE 3 (16) DUODENAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER ORAL ADMINISTRATION OF SODIUM MECLOFENAMATE TO 3 SHEEP (20 mg/kg acid equivalent)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	0	0	0.03	0.01 ± 0.01
20 min	0	0.03	0.31	0.11 ± 0.10
40 min	0.03	0.03	0.40	0.15 ± 0.12
1 hr	0.01	0.18	0.28	0.16 ± 0.08
1½ hr	0.04	0.01	0.20	0.08 ± 0.06
2 hr	0.05	0.03	0.23	0.10 ± 0.06
3 hr	0.09	0.02	0.15	0.09 ± 0.04
5 hr	0.14	0.04	0.04	0.07 ± 0.03
7 hr	0.10	0	0.05	0.05 ± 0.03
10 hr	0.10	0.01	0.02	0.04 ± 0.03
14 hr	0.09	0.01	0	0.03 ± 0.03
18 hr	0.03	0.01	0.02	0.02 ± 0.01
22 hr	0.03	0	0	0.01 ± 0.01

TABLE 3 (17) PLASMA CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER INTRA-ABOMASAL ADMINISTRATION OF SODIUM MECLOFENAMATE TO 3 SHEEP (20 mg/kg acid equivalent)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	1.57	3.58	0.81	2.65 ± 1.48
20 min	5.80	4.69	0.54	3.68 ± 1.60
40 min	10.47	3.26	0.44	4.72 ± 2.99
1 hr	14.86	3.26	0.54	6.22 ± 4.39
1½ hr	9.74	4.33	0.25	4.77 ± 2.75
2 hr	4.73	5.14	1.26	3.71 ± 1.23
3 hr	2.16	3.50	2.31	2.66 ± 0.42
5 hr	2.36	2.67	1.98	2.34 ± 0.20
7 hr	1.57	1.38	1.16	1.37 ± 0.12
10 hr	1.39	1.09	0.47	0.98 ± 0.27
24 hr	0.39	0.27	0.13	0.26 ± 0.08

TABLE 3 (18) RUMINAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER INTRA-ABOMASAL ADMINISTRATION OF SODIUM MECLOFENAMATE TO 3 SHEEP (20 mg/kg acid equivalent)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	0.08	0	0	0.03 ± 0.03
20 min	0.06	0.51	0.01	0.19 ± 0.16
40 min	0.19	0.08	0	0.09 ± 0.06
1 hr	0.11	0	0.18	0.10 ± 0.05
1½ hr	0.02	0.04	0.03	0.03 ± 0.01
2 hr	0	0.01	0	0
3 hr	0.01	0	0	0
5 hr	0.03	0.05	0	0.03 ± 0.01
7 hr	0	0.04	0	0.01 ± 0.01
10 hr	0	0.02	0	0.01 ± 0.01
24 hr	0	0.01	0	0

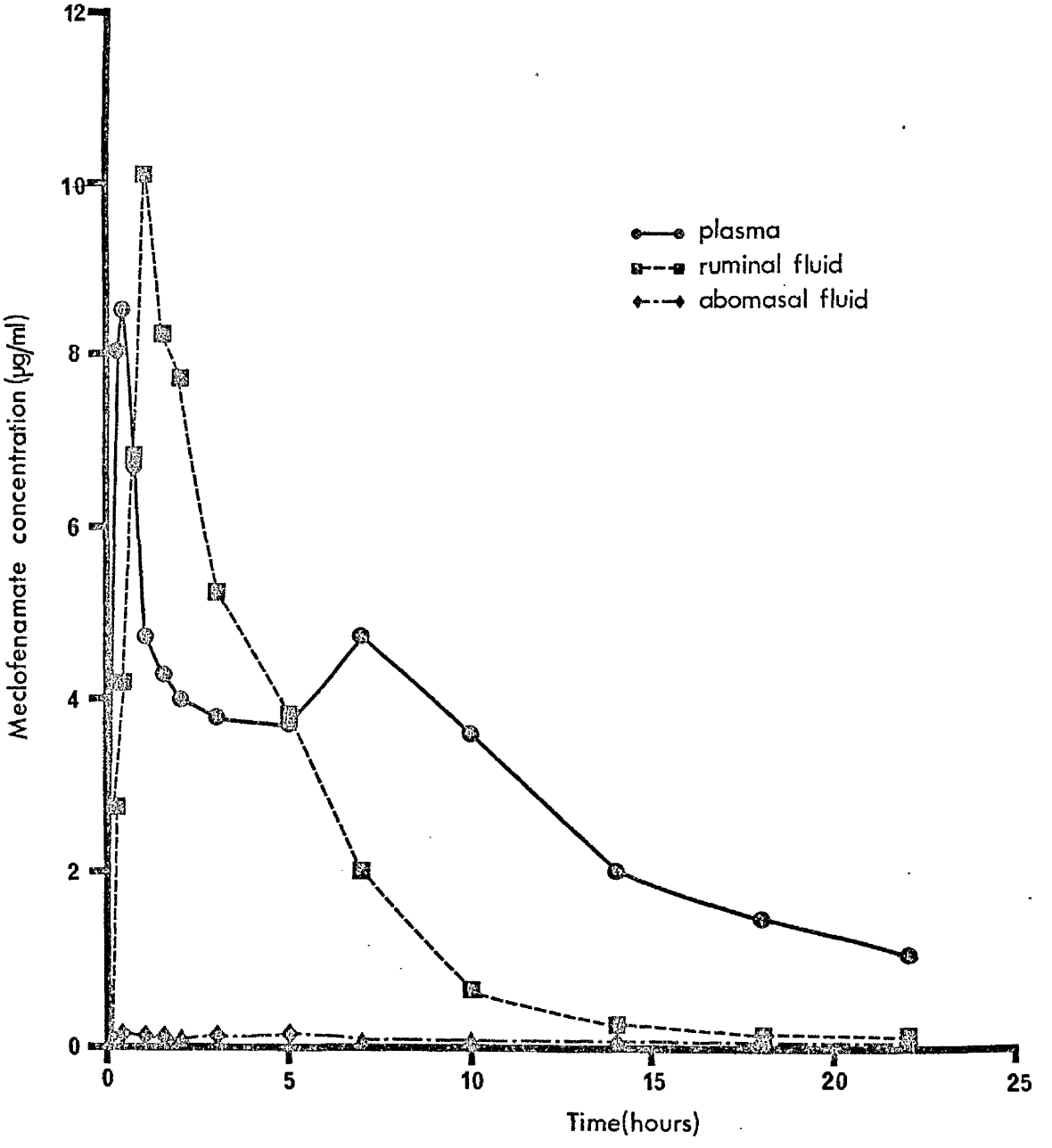
TABLE 3 (19) ABOMASAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER INTRA-ABOMASAL ADMINISTRATION OF SODIUM MECLOFENAMATE TO 3 SHEEP (20 mg/kg acid equivalent)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	7.85	33.29	2.05	14.40 ± 9.59
20 min	4.32	82.13	1.02	29.16 ± 26.50
40 min	1.64	18.80	1.20	7.21 ± 5.79
1 hr	0.57	4.11	0.41	1.70 ± 1.21
1½ hr	0.37	3.67	0.44	1.49 ± 1.09
2 hr	0.17	0.78	0.30	0.42 ± 0.19
3 hr	0.04	0.82	0.31	0.39 ± 0.23
5 hr	0.09	0.28	0.30	0.22 ± 0.07
7 hr	0.04	0.08	0.07	0.06 ± 0.01
10 hr	0.02	0.03	0.03	0.03 ± 0.003
24 hr	0	0.06	0	0.02 ± 0.02

TABLE 3 (20) DUODENAL FLUID CONCENTRATION (µg/ml) OF MECLOFENAMATE AFTER INTRA-ABOMASAL ADMINISTRATION OF SODIUM MECLOFENAMATE TO 3 SHEEP (20 mg/kg acid equivalent)

	Sheep number			
Time	71	74	75	Mean ± S.E.M.
10 min	2.42	18.41	1.51	7.45 ± 5.49
20 min	1.73	No S	2.14	1.94 ± 0.20
40 min	1.17	No S	1.58	1.38 ± 0.20
1 hr	0.60	4.62	1.21	2.14 ± 1.25
1½ hr	0.28	3.79	0.73	1.60 ± 1.10
2 hr	0.31	2.55	0.45	1.10 ± 0.72
3 hr	0.50	1.55	0.40	0.82 ± 0.37
5 hr	0.05	0.72	0.35	0.37 ± 0.19
7 hr	0.04	0.07	0.23	0.11 ± 0.06
10 hr	0	0.06	0.02	0.03 ± 0.02
24 hr	0.18	0.23	0.05	0.15 ± 0.05

Fig. 3 (5) Mean plasma and gastro-intestinal fluid concentrations (µg/ml) of meclofenamate in three sheep after oral administration of sodium meclofenamate (20 mg/kg)


12
10
8
6
4
2
0
0
5
10
15
20
25
Meclofenamate concentration (µg/ml)
Time(hours)
plasma
ruminal fluid
abomasal fluid

The maximum concentration achieved was also lower than that after oral administration.

DISCUSSION

The soluble sodium salt of meclofenamate administered either orally or intra-abomasally gives rise to more rapid absorption than the relatively insoluble acid form of the drug. Plasma concentrations with the sodium salt are well maintained. All three sheep had higher plasma concentrations at 10 hours and later times when dosed with the sodium salt than with the acid, suggesting that the low aqueous solubility of meclofenamic acid at the normal pH of the gut limits its absorption.

The difference between delivery of drug to the abomasum via the reticular groove and experimentally administering it via the abomasal cannula is again obvious. The lower total absorption and delayed occurrence of the maximum concentration compared with oral administration are similar to that seen in Experiments 1 and 3.

ABSORPTION OF MECLOFENAMIC ACID FROM THE ISOLATED RUMEN IN VIVO

In order to determine the rate and extent of absorption of meclofenamic acid through the rumen epithelium, an experiment was undertaken in which the reticulo-rumen was isolated from the abomasum by ligation of the omasal/abomasal junction. The drug was administered via the rumen fistula and plasma concentrations of meclofenamate determined.

MATERIALS AND METHODS

The animal used was one in which the rumen cannula was of an unsatisfactory type and had been rejected. The animal was therefore to be killed.

Anaesthesia was induced with halothane via a mask and maintained with a halothane/oxygen mixture via an endotrachael tube throughout the duration of the experiment.

The omasal/abomasal orifice was located via a right paracostal incision and clamped with bowel clamps. Double transfixion ligatures of thick surgical silk were used to tie off the junction. The clamps were removed and the abdominal incision closed.

A blood sample was taken before drug administration. 20 mg/kg of meclofenamic acid was given via the fistula. The rumen fistula was closed with bowel clamps throughout the duration of the experiment. A large bore needle was retained in the fistula to release the gas formed by ruminal fermentation. Blood samples were taken 10 minutes, 20 minutes and 40 minutes, and 1, 1½, 2, 3, 4, 5, and 6 hours after drug administration.

At 1 hour and 6 hours abomasal fluid samples were also taken in order to verify that there was no leakage of drug past the ligature.

After the 6 hour sample was taken, meclofenamic acid was administered at a dose rate of 10 mg/kg as a slurry in 50 ml of water via the abomasal cannula.

Further blood sampling was then carried out at 10, 20, 30, 40 and 60 minutes after intra-abomasal administration in order to ascertain whether or not the anaesthesia would significantly affect the absorption of the drug.

The animal was then killed by intravenous administration of sodium pentobarbitone (Euthatal, May and Baker) and the success of the surgical procedure verified at post-mortem. Plasma and abomasal fluid samples were assayed as described in Chapter 2 for meclofenamate.

RESULTS

The meclofenamate concentration in plasma was below the detectable limit in all samples up to 6 hours after intra-ruminal administration of the drug, as was that in both abomasal fluid samples.

Plasma concentrations of meclofenamate after intra-abomasal administration are shown in Table 3 (21).

DISCUSSION

Although no meclofenamate was detected in plasma samples after intra-ruminal administration, absorption of small amounts could have occurred at a slow rate such that because of redistribution, metabolism and excretion, the concentrations in plasma were always below the limit of detection of the analytical technique. Nevertheless

TABLE 3 (21) PLASMA CONCENTRATIONS (μg/ml) OF MECLOFENAMATE AFTER INTRA-ABOMASAL ADMINISTRATION OF MECLOFENAMIC ACID TO A SHEEP WITH A LIGATED OMASAL/ABOMASAL ORIFICE (DOSE RATE 20 mg/kg)

Time (mins.)	Plasma Concentration (μg/ml)
10	0.07
20	0.21
30	1.01
40	0.46
60	0.46

this experiment does demonstrate that absorption from the rumen, if it occurs at all, is negligible compared with absorption from the abomasum.

The maximum concentration of meclofenamate achieved after intra-abomasal administration of meclofenamic acid to this sheep was of the same order as those achieved in normal non-anaesthetised animals.

The effects of the anaesthetic and surgical procedure on absorption are difficult to determine; reduced/abolished motility of the gut would be the major effect, and reduced blood flow to the rumen is a frequent result of the tying off procedure (Annison, 1965).

CHAPTER 4

STUDIES WITH LEVAMISOLE

Levamisole (L-2,3,5,6-tetrahydro-6-phenylimidazo {2,1-6} thiazole) is widely used in several animal species as an anthelmintic. It has been shown to have good activity against adult and immature gastro-intestinal roundworms and against lungworms (Forsyth, 1966, 1968; Lyons, Drudge and Tolliver, 1968; Reid, Armour, Jennings and Urquhart, 1968; Ciordia and Baird, 1969).

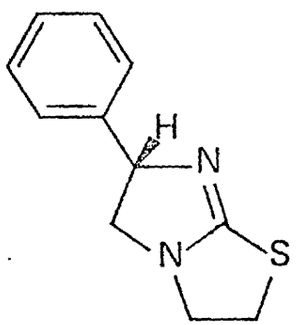
Levamisole is not effective against cestodes or trematodes (Walley, 1966). Reid, Duncan and Bairden (1976) have reported it to be effective against inhibited ostertagia spp in sheep but levamisole is ineffective against inhibited ostertagia ostertagi in cattle (Reid et al, 1968; Anderson, 1977). Levamisole is not ovicidal (Walley, 1966).

In the United Kingdom, levamisole is marketed as a drench, as a subcutaneous injection and as in-feed granules ("Nilverm", "Nemicide", I.C.I). It is available for use in cattle, sheep, goats, pigs and poultry.

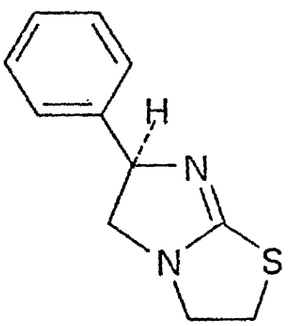
Fig. 4 (1) shows the structure of levamisole.

Although levamisole has been shown to inhibit the fumarate reductase system of nematodes (van den Bossche and Janssen, 1969; Prichard, 1973), it is generally thought to act primarily on the nervous system of the helminth. Wang and Saz (1974) found it to be effective against certain filarial worms which lack fumarate reductase. Aceves, Erlij and Martinez Maranon (1970), van Neuten (1972) showed a rapid contraction followed by paralysis of the worms which van Neuten (1972) suggested was due to stimulation of the ganglia of the

Fig. 4 (1) The structure of levamisole



S (-)



R (+)

levamisole

dexamisole

tetramisole

parasites. This author found that levamisole-induced contractions were inhibited by the ganglion-blocking agent hexamethonium and similar inhibition has been found with other ganglion-blocking drugs, namely mecamylamine and pempidine (Coles, East and Jenkins, 1975).

The paralysis caused by the drug allows expulsion of the parasite by the host gut or lung.

Eyre (1970) has shown tetramisole to have some anticholinesterase activity and this has been stated as a possible mode of action of levamisole (Meyer Jones, Booth and McDonald, 1977). Recent work, however, has shown that the cholinesterase-inhibitory action of levamisole is negligible (Henderson, 1980).

Levamisole was of interest in this study as a basic drug which is freely soluble at any pH encountered within the ruminant digestive tract as well as at the pH of blood plasma. It is the major anthelmintic used in ruminants in the United Kingdom, along with the benzimidazole drugs, and provides an interesting contrast to the latter group of compounds, both in its kinetics and its mode of action.

EXPERIMENTS WITH LEVAMISOLE

Plasma, abomasal fluid and ruminal fluid concentrations of levamisole were measured in four sheep after oral, intra-ruminal and subcutaneous administration of levamisole hydrochloride.

MATERIALS AND METHODS

In all experiments the dose was 7.5 mg/kg of the hydrochloride salt. Commercial "Nilverm" worm drench was used for oral and intra-ruminal administration and commercial "Nilverm" injection for subcutaneous administration. The oral dose was given using a syringe placed at the back of the tongue of the animal, intra-ruminal administration was via the cannula and subcutaneous injection was given over the ribs, behind the point of the elbow.

The three administrations were made at 14 day intervals, the same four sheep were used throughout and the routes used in any sheep on any experimental day were randomised.

Blood and gastro-intestinal fluid samples were taken as described in Chapter 2 before drug administration and at 10 minutes, 30 minutes, 1, 2, 4, 6, 8 and 24 hours after administration. Samples were later analysed for levamisole free base concentration as described in Chapter 2.

RESULTS

The plasma, abomasal fluid and ruminal fluid concentrations of levamisole after subcutaneous, oral and intra-ruminal administration are shown in Tables 4 (1), 4 (2) and 4 (3).

Mean plasma and abomasal fluid concentrations by all routes

TABLE 4 (1) PLASMA CONCENTRATIONS OF LEVAMISOLE (μg/ml FREE BASE) AFTER SUBCUTANEOUS, ORAL AND INTRA-RUMINAL ADMINISTRATION TO 4 SHEEP (7.5 mg/kg HYDROCHLORIDE)

	Sheep name				
Time	Spot	Ger	Brian	Ben	Mean ± S.E.M.
SUBCUTANEOUS ADMINISTRATION					
10 min	1.35	1.25	0.71	1.13	1.11 ± 0.14
30 min	3.42	3.14	1.76	3.28	2.90 ± 0.38
1 hr	3.75	2.97	1.89	3.69	3.08 ± 0.43
2 hr	2.85	2.49	0.77	2.88	2.25 ± 0.50
4 hr	1.75	1.57	0.21	1.69	1.31 ± 0.37
6 hr	0.89	1.04	0.12	1.22	0.82 ± 0.24
8 hr	0.64	0.81	0.09	0.88	0.61 ± 0.18
24 hr	0.10	0.11	0.08	0.11	0.10 ± 0.01
ORAL ADMINISTRATION					
10 min	0.36	0.77	0.40	0.59	0.53 ± 0.09
30 min	0.68	0.48	0.38	0.46	0.50 ± 0.06
1 hr	0.78	0.59	0.38	0.46	0.55 ± 0.09
2 hr	0.93	0.84	0.42	0.51	0.68 ± 0.12
4 hr	1.04	0.97	0.13	0.62	0.69 ± 0.21
6 hr	0.95	0.95	0.04	0.90	0.71 ± 0.22
8 hr	0.82	0.85	0.05	0.75	0.62 ± 0.19
24 hr	0.16	0.17	0.00	0.00	0.08 ± 0.05
INTRA-RUMINAL ADMINISTRATION					
10 min	0.00	0.10	0.30	0.38	0.20 ± 0.09
30 min	0.23	0.30	0.64	0.65	0.46 ± 0.11
1 hr	0.59	0.48	0.52	0.72	0.58 ± 0.05
2 hr	0.88	0.71	0.35	0.67	0.65 ± 0.11
4 hr	1.10	0.98	0.28	0.85	0.80 ± 0.18
6 hr	0.91	1.15	0.17	0.89	0.78 ± 0.21
8 hr	0.84	0.99	0.15	0.74	0.68 ± 0.18
24 hr	0.15	0.42	0.06	0.15	0.20 ± 0.08

TABLE 4 (2) ABOMASAL FLUID CONCENTRATIONS OF LEVAMISOLE (µg/ml FREE BASE) AFTER SUBCUTANEOUS, ORAL AND INTRA-RUMINAL ADMINISTRATION TO 4 SHEEP (7.5 mg/kg HYDROCHLORIDE)

	Sheep name				
Time	Spot	Ger	Brian	Ben	Mean ± S.E.M.
SUBCUTANEOUS ADMINISTRATION					
10 min	1.72	1.51	1.23	0.85	1.33 ± 0.19
30 min	10.67	11.03	8.02	6.17	8.97 ± 1.15
1 hr	15.43	19.89	18.56	12.33	16.55 ± 1.69
2 hr	24.54	18.88	13.88	22.00	19.83 ± 2.29
4 hr	48.60	33.25	12.25	39.12	33.31 ± 7.70
6 hr	26.24	40.06	4.29	30.83	25.36 ± 7.59
8 hr	14.58	27.09	1.73	17.34	15.19 ± 5.23
24 hr	0.82	2.89	0.00	2.06	1.44 ± 0.64
ORAL ADMINISTRATION					
10 min	196.24	4.49	70.14	379.25	162.53 ± 82.47
30 min	201.72	13.50	100.72	340.52	164.12 ± 70.26
1 hr	119.18	65.75	67.44	265.66	129.51 ± 47.05
2 hr	106.34	18.22	43.16	202.15	92.47 ± 40.99
4 hr	45.44	24.22	9.89	94.75	43.58 ± 18.56
6 hr	22.70	27.32	7.82	35.90	23.44 ± 5.88
8 hr	14.63	30.78	4.92	17.83	17.04 ± 5.34
24 hr	1.60	4.24	0.18	1.25	1.82 ± 0.86
INTRA-RUMINAL ADMINISTRATION					
10 min	0.00	0.00	0.00	0.00	0.00
30 min	0.22	3.46	0.79	0.00	1.12 ± 0.79
1 hr	2.34	11.13	3.72	5.07	5.57 ± 1.94
2 hr	9.10	15.06	4.85	7.43	9.11 ± 2.16
4 hr	19.78	23.83	6.06	14.07	15.94 ± 3.85
6 hr	31.61	27.72	6.49	17.09	20.73 ± 5.65
8 hr	26.25	27.09	5.47	14.47	18.32 ± 5.16
24 hr	4.79	9.34	0.72	2.49	4.34 ± 1.86

TABLE 4 (3) RUMINAL FLUID CONCENTRATIONS OF LEVAMISOLE (µg/ml FREE BASE) AFTER SUBCUTANEOUS, ORAL AND INTRA-RUMINAL ADMINISTRATION TO 4 SHEEP (7.5 mg/kg HYDROCHLORIDE)

	Sheep name				
Time	Spot	Ger	Brian	Ben	Mean ± S.E.M.
SUBCUTANEOUS ADMINISTRATION					
10 min	0.00	0.00	0.00	0.00	0
30 min	0.00	0.00	0.00	0.00	0
1 hr	0.05	0.38	0.00	0.10	0.13 ± 0.08
2 hr	0.19	0.56	0.00	0.57	0.33 ± 0.14
4 hr	0.37	0.91	0.01	0.40	0.42 ± 0.19
6 hr	0.40	0.91	0.01	0.40	0.43 ± 0.18
8 hr	0.16	0.88	0.00	0.20	0.31 ± 0.19
24 hr	0.11	0.29	0.00	0.11	0.13 ± 0.06
ORAL ADMINISTRATION					
10 min	14.09	22.15	0.15	0.63	9.26 ± 5.38
30 min	16.68	11.81	2.83	9.96	10.32 ± 2.87
1 hr	8.31	9.91	3.73	6.51	7.12 ± 2.65
2 hr	1.16	7.17	4.50	12.49	6.33 ± 2.39
4 hr	0.87	4.05	2.58	2.33	2.46 ± 0.65
6 hr	0.58	2.87	1.67	0.71	1.46 ± 0.53
8 hr	0.00	1.77	0.91	0.47	0.79 ± 0.38
24 hr	0.00	0.36	0.06	0.13	0.14 ± 0.08
INTRA-RUMINAL ADMINISTRATION					
10 min	33.25	82.03	0.06	1074.11	297.36 ± 259.46
30 min	48.41	14.88	0.84	134.70	49.71 ± 30.04
1 hr	33.34	9.30	0.26	97.26	35.04 ± 21.88
2 hr	15.38	7.76	2.04	153.21	44.60 ± 36.31
4 hr	7.94	5.71	4.65	16.74	8.76 ± 2.75
6 hr	3.59	3.92	3.97	2.95	3.61 ± 0.23
8 hr	2.43	2.80	1.40	1.27	1.98 ± 0.38
24 hr	0.22	0.78	0.07	0.19	0.32 ± 0.16

are shown in Figs. 4 (2) and 4 (3) respectively.

Plasma concentrations were higher and the maximum concentrations achieved occurred much earlier after subcutaneous administration than by either of the other two routes. After oral administration and intra-ruminal administration plasma concentrations were similar except at ten minutes after dosing when all four sheep had higher concentrations after oral administration. This was probably due to the influence of the reticular groove reflex; high concentrations of levamisole were found in the abomasum of all the sheep by 10 minutes after the oral dose. No levamisole was detected in the abomasum after intra-ruminal administration until the 30 minutes sample in three sheep and until one hour in the fourth animal.

Abomasal fluid concentrations were highest when the drug was administered orally. Higher concentrations in abomasal fluid were found after subcutaneous administration than after intra-ruminal.

Ruminal fluid concentrations were significantly higher in all sheep after intra-ruminal administration than after oral dosing, supporting the suggestion that some of the orally administered drug by-passed the rumen.

Levamisole was detected in the ruminal fluid of all sheep after subcutaneous administration. The concentrations found varied widely in different animals.

The bio-availability of levamisole after subcutaneous administration was much greater than that after oral or intra-ruminal administration. The area under plasma concentration time curve was in the ratio 2.4:1.07:1.0 for subcutaneous, intra-ruminal and oral

Fig. 4 (2) Mean plasma concentrations (µg/ml) of levamisole in four sheep after subcutaneous, intra-ruminal and oral administration of levamisole (7.5 mg/kg)

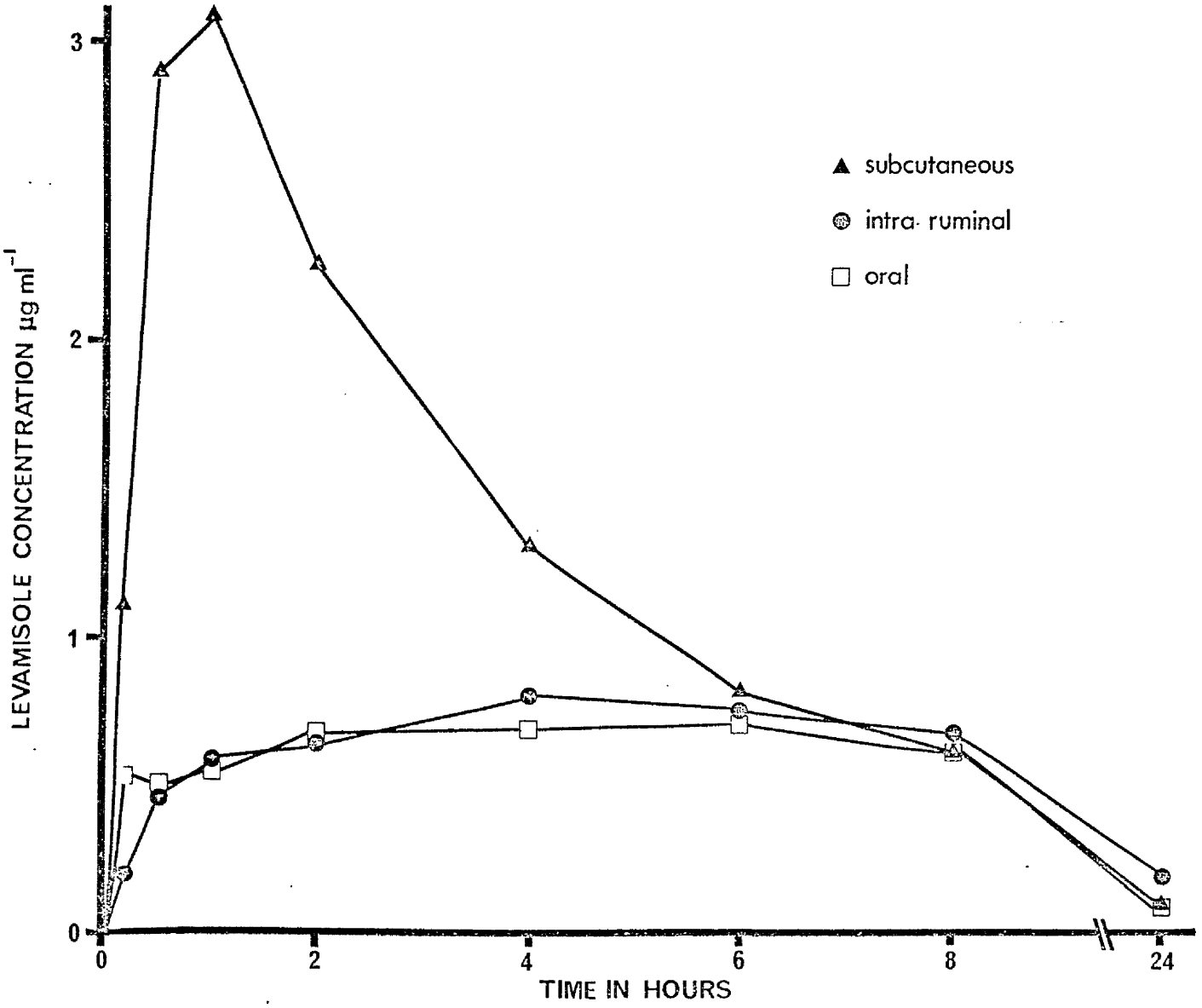
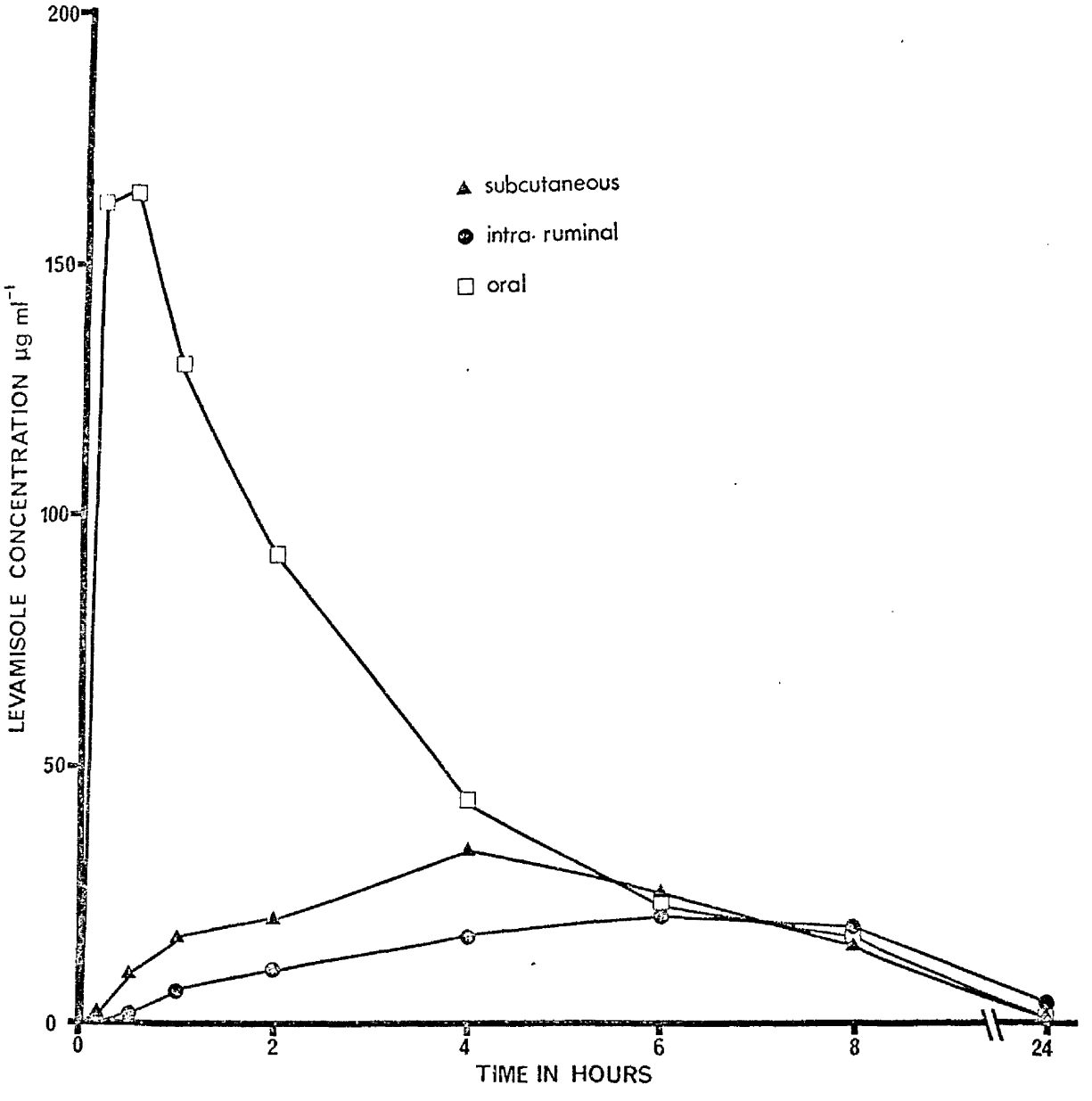

LEVAMISOLE CONCENTRATION µg ml⁻¹
3
2
1
0
0
2
4
6
8
24
TIME IN HOURS
subcutaneous
intra- ruminal
oral

Fig. 4 (3) Mean abomasal fluid concentrations (µg/ml) of levamisole in four sheep after subcutaneous, intra-ruminal and oral administration of levamisole (7.5 mg/kg)


200
150
100
50
0
LEVAMISOLE CONCENTRATION µg ml⁻¹
▲ subcutaneous
● intra· ruminal
□ oral
0
2
4
6
8
24
TIME IN HOURS

routes respectively.

DISCUSSION

Plasma concentrations of levamisole found in these sheep after oral administration were lower than those found by Allewijn and Demoen (1965) cited by Graziani and De Martin (1977) in a calf. These authors reported plasma concentrations of up to 6 µg/ml in a calf dosed orally with 10 mg/kg of tetramisole. The age of the calf was not given and since the peak plasma concentration occurred at 30 minutes, it seems probable that it was a pre-ruminant animal.

Dorn and Federmann (1976) in their comparative study of plasma concentrations after oral subcutaneous and pour on administration of levamisole to calves, found similar blood levels after oral administration to those reported here. Plasma concentrations after subcutaneous administration in these sheep, however, were much higher than those found by Dorn and Federmann (1976) after a similar dose.

Because of the mode of action of levamisole, it might be expected that it is the peak concentration of the drug at the site of action that is important in the determination of anthelmintic activity. If this is so, then these studies suggest that the subcutaneous route which achieves higher plasma concentrations (and therefore higher lung concentrations) would be the route of choice for treatment of Dictyocaulus infection; and the oral route which achieves higher abomasal concentrations would be the better route for treatment of gastro-intestinal helminthiasis.

The low abomasal fluid concentrations seen after intra-ruminal administration of levamisole suggest that the functioning of the reticular groove after oral administration, which was apparent in all four sheep, may be important in determining anthelmintic efficacy.

Ruminal fluid concentrations of levamisole in many of the samples after intra-ruminal administration of the drug were lower than would be expected from simple dilution of the drug by rumen contents. Dobson (1967) cites a 50 kg sheep as having a 5-10 kg of reticulo-ruminal contents. If a dose of 7.5 mg/kg of levamisole was evenly distributed throughout the ruminal contents, the expected concentrations would be about 37.5-75 µg/ml; in fact the concentrations found in these sheep were considerably lower, with the exception of some of the very early samples where mixing was obviously incomplete. This was particularly striking in the case of one animal (Brian) in which the maximum ruminal fluid concentration of levamisole measured was less than 5 µg/ml.

One possible explanation for these findings is that some adsorption of the drug to the fibrous material of the rumen occurred.

Since the assay procedure involved centrifugation of rumen samples followed by filtration of the supernatant fluid, any drug adherent to the solid material would not have been measured.

Alternatively levamisole may have been degraded to some extent by ruminal micro-organisms. The drug was not incubated with ruminal fluid <u>in vitro</u>, so this possibility cannot be discounted. No metabolites were evident on HPLC analysis of ruminal fluid extracts for the parent drug.

Either of these mechanisms, adsorption or degradation, may

have been responsible for the much lower bio-availability of levamisole when given orally and intra-ruminally than when administered by subcutaneous injection.

The third explanation for the low ruminal fluid concentrations found after intra-ruminal administration is that absorption of levamisole occurs through the ruminal epithelium. The evidence in favour of this explanation is that levamisole was detected in the plasma of three of the four sheep by 10 minutes after administration, but not in the abomasal fluid at this time.

Passive diffusion of levamisole into the gut after subcutaneous administration was observed. This accumulation of basic drug in the acidic regions of the gut by the process of 'ion trapping' was not unexpected. The theoretical equilibrium ratio of levamisole which has a pk_a of 8 between plasma (pH 7.4) and ruminal fluid (pH 6.6) and plasma and abomasal fluid (pH 2.5), would be approximately 1:5 and 1:63,000 in favour of the rumen and abomasum respectively for free unbound drug. The ratios measured in this study were at greatest 1:2.64 and 1:58 respectively for any animal.

Parenterally administered levamisole may have reached the rumen via the saliva rather than across the ruminal wall. At pH of saliva (8.2) levamisole would be expected to attain concentrations which at equilibrium (which is never achieved because of constant salivary excretion) would approach one-third of those in plasma.

CHAPTER 5

STUDIES WITH BENZIMIDAZOLES

The benzimidazoles are, along with levamisole, the most important broad spectrum anthelmintics currently marketed. Nine members of the group are available in the United Kingdom; all have a similar basic structure with differing side chain constituents at positions 2 and 5.

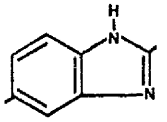
Fig. 5 (1) shows the structure of the different benzimidazoles and the species for which they are marketed in the United Kingdom.

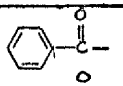
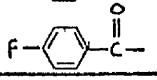
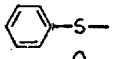
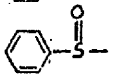
Thiabendazole, the first of the group, was introduced in 1961 and was followed by parbendazole, cambendazole, mebendazole and oxibendazole. These early benzimidazoles have good activity against mature gastro-intestinal nematodes in cattle, sheep and many other species (Baker and Douglas, 1962; Actor, Anderson, Dicuollo, Ferlauto, Hoover, Pagano, Ravin, Scheidy, Stedman and Theodorides, 1967; Baker and Walters, 1971; Brugmans, Thienpont, Van Wijngaarden, Vanparijs, Schuermans and Lauwers, 1971; Theodorides, Chang, Dicuollo, Grass, Parish and Scott, 1973). They are less effective against immature helminths and only mebendazole has activity against lungworms and tapeworms at normal dose levels.

The more recently marketed benzimidazoles - fenbendazole, oxfendazole and albendazole - are active at lower dosage rates and also have an extended spectrum of activity. Fenbendazole, introduced in 1974, was the first anthelmintic shown to be effective against inhibited larval stages of the important abomasal parasite of cattle Ostertagia ostertagi (Duncan, Armour, Bairden, Jennings

Fig. 5 (1) The structures and trade-names of the benzimidazole anthelmintics and the species for which they are marketed in the United Kingdom

BASIC BENZIMIDAZOLE STRUCTURE



	Position 2	Position 5	Trade Names	Dose (mg/kg) for >95% Control of Sheep/cattle Gut Parasites	Marketed for Species
Thiabendazole		H-	Thibenzole Equizole Thiprazole	50-100	S,C,H,P
Cambendazole	"	$(CH_3)_2CHCO_2NH$-	Porcam	25	P
Parbendazole	$CH_3C(=O)-O-NH$-	$CH_3CH_2CH_2CH_2$-	Helmatac	20-30	S,C,P
Oxibendazole	"	$CH_3CH_2CH_2O$-	Rycovet Widespec	10-20	S,C
Mebendazole	"		Equivurm Telmin	15	H,D, Cats
Flubendazole	"		Flubenol	Not tested	P
Fenbendazole	"		Panacur	5	S,C,H
Oxfendazole	"		Systamex Synanthic	5	S,C
Albendazole	"	$CH_3CH_2CH_2S$-	Valbazen	5	S,C

and Urquhart, 1976). It is also extremely effective against lungworms in cattle (Düwel, 1974) and sheep (Ross, 1975) and has recently been shown to have some activity against liver flukes and tapeworms (Corba, Lietava, Düwel and Reisenleiter, 1979).

Oxfendazole, marketed soon after fenbendazole, has a similarly broad spectrum of activity (Downey, 1976; Chalmers, 1977, 1978; Corwin, 1977; Lyons, Drudge and Tolliver, 1977) and albendazole, the most recent of the three, in addition has good flukicidal activity at only a slightly increased dose rate (Theodorides, Gyurik, Kingsbury and Parish, 1976). Recently marketed (1980) in the United Kingdom only for pigs is flubendazole, a very close analogue of mebendazole.

Several of the benzimidazoles have been shown to inhibit the fumarate reductase system of helminths (Prichard, 1970, 1973, 1978; Malkin and Camacho, 1972; Romanowski, Rhoads, Colglazier and Kates, 1975) which interferes with energy production by the parasite. Borgers and De Nollin (1975); De Brabander and Thienpont (1975) have demonstrated the effects of mebendazole on the ultrastructure of helminths. These authors reported disruption of the microtubules in the cytoplasm of the parasite cells, particularly the absorptive cells of the gut. The loss of microtubules, which occurs within a few hours of exposure, leads to an accumulation of secretory granules, eventual lysis of cells, and consequent loss of absorptive function of the parasite.

The same authors reported a marked decrease in glycogen content of the parasites after a few hours of exposure to the benzimidazole, and reduced uptake of low molecular weight nutrients, including glucose, has been demonstrated previously in nematodes

treated with mebendazole (Van den Bossche and De Nollin, 1973).

It is not known which mechanism (enzyme inhibition or microtubular disruption) is the cause of death in the parasites, but it is probable that failure of glucose uptake would in itself eventually cause loss of fumarate reductase activity. Either of these possible mechanisms of anthelmintic activity would require the duration of exposure of the parasite to the drug, as well as the drug concentration achieved, to be important in determining efficacy.

It has been suggested that the benzimidazoles all have a similar mode of action and that differences in potency and spectrum of activity between members of the group may be due to differences in the pharmacokinetics of the drugs (Prichard, Hennessy and Steel, 1978). This is supported by the fact that the later, more potent benzimidazole drugs have much slower absorption and elimination than the earlier members of the group.

Maximum plasma concentrations of thiabendazole are reached 4 hours after oral administration to sheep (Tocco, Buhs, Brown, Matzuk, Mertel, Harman and Trenner, 1964), and of oxibendazole and parbendazole by 6 hours (Theodorides et al, 1973; Dicuollo, Miller, Mendelson and Pagano, 1974). In contrast, maximum plasma concentrations of fenbendazole and oxfendazole occur at around 24 hours (Düwel et al, 1975; Prichard et al, 1978) and of albendazole at 15 hours (Dicuollo, Miller, Colman,Kraeer and Wong, 1977) after oral administration to sheep. Prichard et al (1978) have also shown that even thiabendazole is effective against inhibited ostertagia larvae in cattle if drug concentrations are maintained by

continuous or frequently repeated administration. Previously thiabendazole has been shown to be ineffective against inhibited larvae (Armour, 1969, 1975; Anderson, 1977).

It appeared, therefore, that a study of the kinetics of the benzimidazoles might be of use in further understanding of the action of these drugs and also allow a more rational approach to the divided dose regimes which are required for good activity in monogastric animals, and which give improved activity in ruminant animals. (Gaenssler, Wilkins and O'Donnovan, 1978; Thienpont, Vanparijs, Niemegeers and Marsboom, 1978).

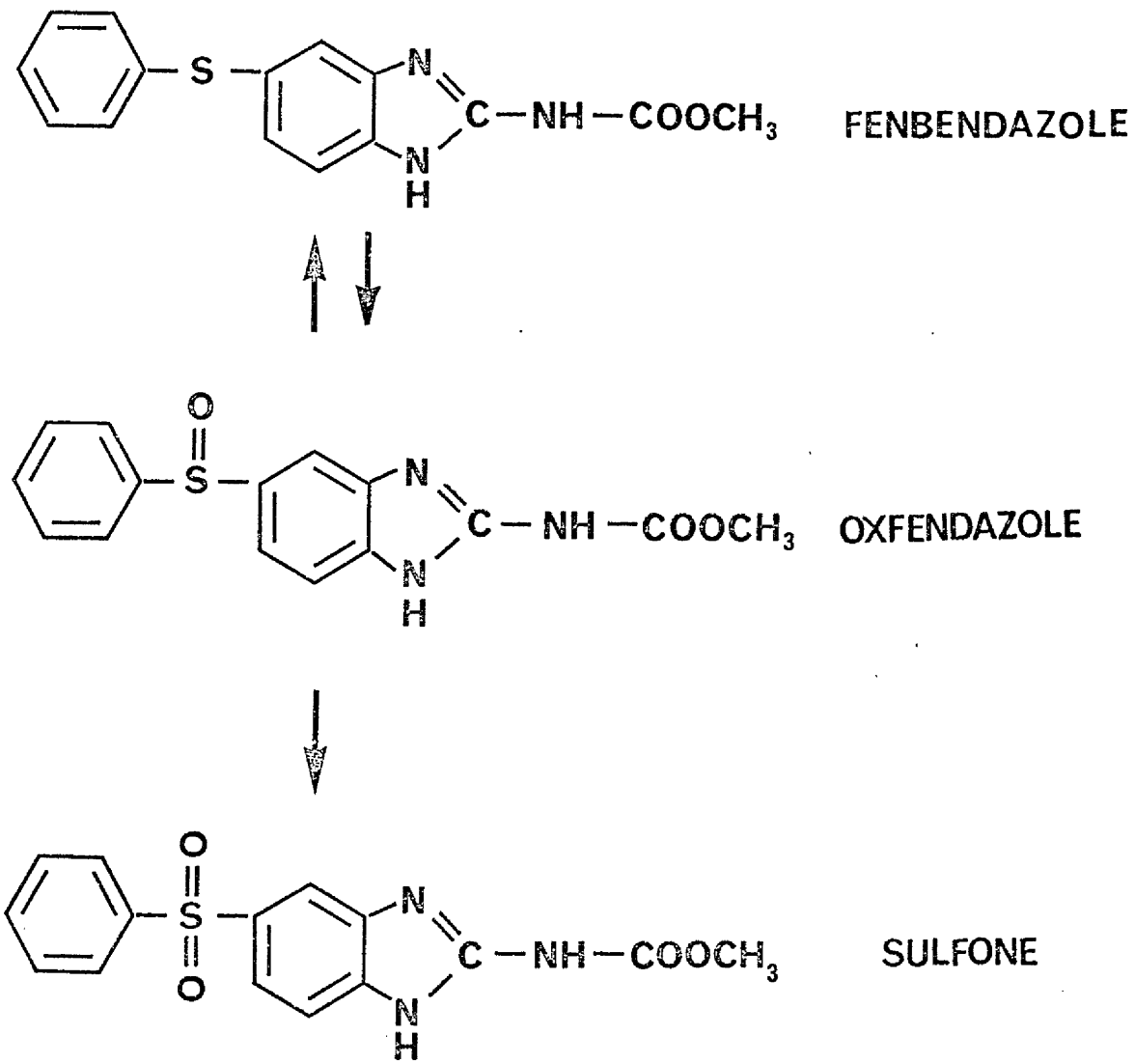
EXPERIMENTS WITH FENBENDAZOLE

Fenbendazole (5 phenylthio-benzimidazole-2-methyl carbamate) is marketed in the United Kingdom for use in cattle, sheep, pigs and horses. It is formulated as a suspension, as a powder and as granules "Panacur", Hoechst) for oral use only.

Fenbendazole has been measured by Düwel et al, (1975) in the plasma of sheep, cattle and other species. Using their fluorescence method, these authors reported maximum plasma concentrations of between 0.2 and 0.4 µg/ml occurring 6-24 hours after administration at a dose rate of 5 mg/kg to sheep. In cattle at this dose rate plasma concentrations were slightly higher and reached a maximum at about 30 hours in most animals. No drug was detected by 96 hours after treatment. Prichard et al (1978) have reported plasma and abomasal concentrations of ^{14}C labelled fenbendazole in cattle after intra-ruminal administration. In their studies maximum drug concentrations of 0.6 and 6.0 µg/ml were found in plasma and abomasal fluid respectively by about 24 hours after administration of 5 mg/kg.

Düwel (1977) has also measured the terminal metabolites of fenbendazole ^{14}C in urine and faeces of sheep, but the plasma concentrations of metabolites were not reported. None of these studies, therefore, has provided reliable data on plasma concentrations of fenbendazole since, because of the methods used for estimation of the drug, they do not distinguish parent drug and metabolites.

Fig. 5 (2) The structures of fenbendazole and its major metabolites


S
N
C—NH—COOCH3
N
H
FENBENDAZOLE
O
S
N
C—NH—COOCH3
N
H
OXFENDAZOLE
O
S
O
N
C—NH—COOCH3
N
H
SULFONE

In this study plasma, abomasal fluid and ruminal fluid concentrations of fenbendazole and its two major metabolites (Fig. 5 (2)) were measured in six sheep after oral administration (Experiment 1). In three of these sheep plasma concentrations were also measured after intra-abomasal administration (Experiment 2) and after intra-ruminal administration (Experiment 3).

MATERIALS AND METHODS

Fenbendazole ("Panacur", Hoechst) was administered in all cases as a 2.5% suspension at a dose rate of 10 mg/kg. The oral dose was given using a syringe placed on the back of the animal's tongue and intra-ruminal and intra-abomasal doses were administered via the respective cannulae.

The oral administration was made to three sheep on two occasions. The first experiment was designed on the basis of the data published by Güwel et al (1975). Three sheep were used and sampling was carried out before drug administration and at 4, 8, 12, 16, 20, 24, 30, 36, 48, 72, 96 and 120 hours (5 days) after administration. An additional abomasal fluid sample was taken at 10 minutes. When the samples were assayed and fenbendazole found to be present in the 120 hour sample, it was decided to repeat the experiment with less frequent sampling but over a longer period of time. This was done several months later using different sheep.

The original three sheep were used for the intra-abomasal experiment, and for the intra-ruminal administration. Unfortunately one of these animals lost its ruminal cannula during the course of this experiment and had to be destroyed.

The taking of samples, the procedure for storage and method of assay for fenbendazole and its metabolites were all as described earlier in Chapter 2.

RESULTS

When samples of plasma and abomasal fluid were subjected to HPLC analysis for fenbendazole estimation, an additional chromatographic peak with a retention time shorter than that of fenbendazole was observed in both plasma and abomasal fluid extracts. At that time the identity of the metabolites of fenbendazole was not known, and the concentration could not be measured directly. However, it was clear that this metabolite was quantitatively important since in many of the samples the peak height of the metabolite was greater than that of fenbendazole. Later, when by analogy with the concurrent albendazole study, it was suspected that the probable metabolic route for fenbendazole was via the sulfoxide of fenbendazole (which is itself marketed as oxfendazole), and the sulfone, samples of these compounds were obtained by gift from the manufacturers of oxfendazole (Syntex). When these authentic samples of sulfoxide and sulfone were subjected to HPLC they both had retention times equal to that of the metabolite peak seen in the fenbendazole samples. Using other HPLC solvents, as outlined in the Methods section, the sulfoxide and sulfone could be separated.

Further identification of the sulfoxide was obtained by collecting the appropriate fraction of the HPLC eluent from a number of injections, evaporating these and subjecting the residue to infra-red spectroscopy. This residue was found to have an absorption

band at 1037 cm^{-1}, typical of a sulfoxide grouping. Comparison of the infra-red spectrum of this compound with that of authentic fenbendazole sulfoxide confirmed the identity of this HPLC peak. The "sulfone" peak identity was not unequivocally identified but had similar HPLC characteristics to that of authentic sulfone. Further samples of plasma were, therefore, analysed for the quantitation of the sulfoxide and sulfone separately.

Table 5 (1) Plasma and abomasal fluid concentrations of fenbendazole, oxfendazole and oxfendazole sulfone after oral administration of fenbendazole (10 mg/kg) as a suspension

Time	Fenbendazole							Oxfendazole							Sulfone						
	Sheep Name							Sheep Name							Sheep Name						
	M	S	B	E	R	G	Mean ± SEM	M	S	B	E	R	G	Mean ± SEM	M	S	B	E	R	G	Mean ± SEM
PLASMA CONCENTRATION (μg/ml)																					
Baseline	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30 min	-	-	-	0.02	0	0.02	0.01±0.01	-	-	-	0	0	0	0	-	-	-	0	0	0	0
4 hours	0.04	0.05	0.02	-	-	-	0.04±0.01	0.02	0.07	0	-	-	-	0.03±0.02	0	0	0	-	-	-	0
8	0.04	0.12	0.05	0.18	0.07	0.09	0.08±0.02	0.05	0.16	0.05	0.12	0.02	0.05	0.07±0.02	0	0	0	0.01	0	0	0.002±0.001
12	0.12	0.12	0.08	0.20	0.14	0.21	0.15±0.02	0.07	0.24	0.09	0.25	0.22	0.28	0.19±0.06	0	0.03	0.01	0.07	0.05	0	0.03 ±0.01
16	0.13	0.07	0.07	0.22	0.26	0.21	0.12±0.01	0.09	0.30	0.18	0.26	0.38	0.26	0.24±0.04	0	0.03	0.04	0.11	0.11	0.06	0.06 ±0.02
20	0.11	0.13	0.13	-	-	-	0.12±0.01	0.11	0.38	0.20	-	-	-	0.23±0.08	0	0.05	0.02	-	-	-	0.02 ±0.01
24	0.08	0.16	0.13	0.18	0.23	0.12	0.15±0.02	0.12	0.47	0.25	0.27	0.40	0.20	0.28±0.05	0.01	0.07	0	0.13	0.17	0.09	0.08 ±0.02
30	0.13	0.15	0.	-	-	-	0.13±0.01	0.26	0.35	0.25	-	-	-	0.29±0.03	0.05	0.10	0.08	-	-	-	0.07 ±0.01
36	0.06	0.12	0.14	0.11	0.18	0.10	0.12±0.02	0.30	0.27	0.27	0.33	0.29	0.25	0.28±0.01	0.14	0.16	0.10	0.24	0.21	0.19	0.17 ±0.02
48	0.07	0.10	0.17	0.04	0.11	0.08	0.10±0.03	0.31	0.26	0.33	0.12	0.21	0.15	0.23±0.03	0.17	0.18	0.10	0.11	0.19	0.16	0.15 ±0.01
72	0.08	0.03	0.24	0.03	0.03	0.03	0.07±0.03	0.20	0.07	0.30	0.04	0.11	0.06	0.13±0.04	0.18	0.08	0.11	0.15	0.18	0.06	0.13 ±0.02
96	0.09	0	0.13	0	0	0.02	0.04±0.02	0.12	0.03	0.59	0	0.27	0.01	0.17±0.09	0.23	0	0.23	0.04	0.08	0.02	0.10 ±0.04
5 days	0.06	0	0.04	0	0	0	0.02±0.01	0.05	0.01	0.40	0	0	0	0.08±0.06	0.16	0	0.30	0.02	0.03	0	0.08 ±0.05
7	-	-	-	0	0	0	0	-	-	-	0	0	0	0	-	-	-	0	0.01	0	0.003±0.003
8	-	-	-	0	0	0	0	-	-	-	0	0	0	0	-	-	-	0	0	0	0
9	-	-	-	0	0	0	0	-	-	-	0	0	0	0	-	-	-	0	0	0	0
11	-	-	-	0	0	0	0	-	-	-	0	0	0	0	-	-	-	0	0	0	0
14	-	-	-	0	0	0	0	-	-	-	0	0	0	0	-	-	-	0	0	0	0
ABOMASAL FLUID CONCENTRATION (μg/ml)																					
Baseline	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10 min	0.16	0.16	0.39	0.10	0.04	0.07	0.15±0.05	0	0	0	0	0	0	0	-	-	-	0	0	0	0
4 hours	1.26	0.22	0.35	0.60	0.56	0.31	0.55±0.15	0	0.06	0.08	0.22	0	0.17	0.09±0.04	0	0	0	0	0	0	0
8	1.99	0.18	1.42	-	-	-	1.20±0.53	0.07	0.12	0.24	-	-	-	0.14±0.05	0	0	0	-	-	-	0
12	1.91	0.22	1.84	0.57	0.50	0.30	0.89±0.45	0.20	0.18	0.48	0.99	0.38	0.35	0.43±0.11	0	0	0	0	0	0	0
16	1.98	0.22	2.48	-	-	-	1.56±0.69	0.13	0.16	0.54	-	-	-	0.28±0.13	0	0	0	-	-	-	0
20	4.50	0.13	3.13	0.35	0.49	0.18	1.46±0.77	0.80	0.32	0.62	0.58	0.41	0.45	0.53±0.07	0	0	0	0.04	0	0	0.01±0.01
24	2.77	0.13	3.08	0.42	0.54	0.20	1.19±0.55	0.64	0.60	0.74	0.93	0.46	0.45	0.64±0.07	0	0.02	0.03	0.07	0.03	0.01	0.03±0.01
30	2.84	0.09	2.54	-	-	-	1.82±0.87	0.62	0.10	1.05	-	-	-	0.59±0.27	0	0	0.04	-	-	-	0.01±0.01
36	2.50	0.13	2.79	0.18	0.26	0.17	1.01±0.52	0.80	0.08	1.35	0.68	0.45	0.53	0.65±0.17	0.03	0	0.06	0.07	0.04	0.02	0.04±0.01
48	1.16	0.07	3.16	0.07	0.26	0.11	0.81±0.50	0.47	0.20	1.72	0.41	0.68	0.46	0.66±0.22	0.04	0	0.10	0.08	0.08	0.04	0.06±0.03
72	0.88	0	2.96	0.02	0.15	0.03	0.67±0.48	0.38	0.08	2.33	0.09	0.28	0.13	0.55±0.36	0.10	0	0.18	0.05	0.05	0.03	0.07±0.03
96	1.56	0	2.18	0.01	0.03	0.03	0.64±0.40	0.80	0.06	2.50	0	0	0	0.56±0.41	0.20	0	0.04	0	0	0.01	0.04±0.03
5 days	1.83	0	1.03	0	0	0.02	0.48±0.32	0.20	0	1.40	0	0	0	0.27±0.23	0.15	0	0	0	0	0	0.03±0.03
7	-	-	-	0	0	0	0	-	-	-	0	0	0	0	-	-	-	0	0	0	0
8	-	-	-	0	0	0	0	-	-	-	0	0	0	0	-	-	-	0	0	0	0
9	-	-	-	0	0	0	0	-	-	-	0	0	0	0	-	-	-	0	0	0	0
11	-	-	-	0	0	0	0	-	-	-	0	0	0	0	-	-	-	0	0	0	0
14	-	-	-	0	0	0	0	-	-	-	0	0	0	0	-	-	-	0	0	0	0

TABLE 5 (2) RUMINAL FLUID CONCENTRATIONS (µg/ml) OF FENBENDAZOLE AFTER ORAL ADMINISTRATION OF FENBENDAZOLE (10 mg/kg) AS A SUSPENSION

Time	Sheep name						Mean ± S.E.M.
	M	S	B	E	R	G	
Baseline	0	0	0	0	0	0	0
30 min	-	-	-	-	-	-	-
4 hours	6.12	0.80	4.50	-	-	-	3.81 ± 1.57
8	4.27	0.96	6.67	0.97	1.84	0.43	2.52 ± 1.00
12	5.52	0.57	2.79	0.30	0.28	0.19	1.61 ± 0.88
16	3.75	0.27	1.55	0.27	0.19	0.19	1.04 ± 0.58
20	2.79	0.40	2.97	-	-	-	2.05 ± 0.83
24	3.07	0.33	6.10	0.21	0.19	0.26	1.69 ± 0.99
30	2.56	0.30	2.97	-	-	-	1.94 ± 0.83
36	2.05	0.37	3.75	0.12	0.17	0.13	1.10 ± 0.61
48	1.93	0.20	2.17	0.08	0.14	0.05	0.76 ± 0.41
72	1.51	0.00	1.71	0.02	0.05	0.03	0.55 ± 0.34
96	0.84	0.10	2.64	0.01	0.02	0.01	0.60 ± 0.43
5 days	0.54	0.07	1.71	0	0	0	0.39 ± 0.28
7	-	-	-	0	0	0	0
8	-	-	-	0	0	0	0
9	-	-	-	0	0	0	0
11	-	-	-	0	0	0	0
14	-	-	-	0	0	0	0

Fig. 5 (3) Mean plasma concentrations (μg/ml) of fenbendazole, oxfendazole and oxfendazole sulfone in six sheep after oral administration of fenbendazole (10 mg/kg) as a suspension

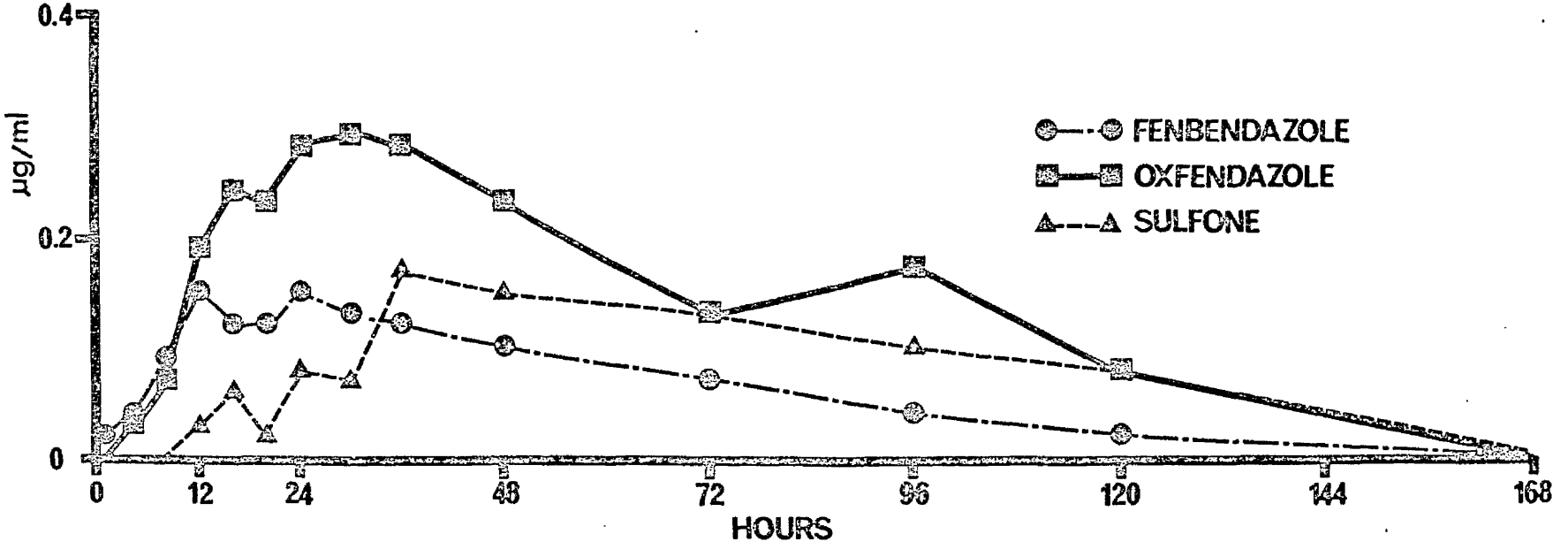
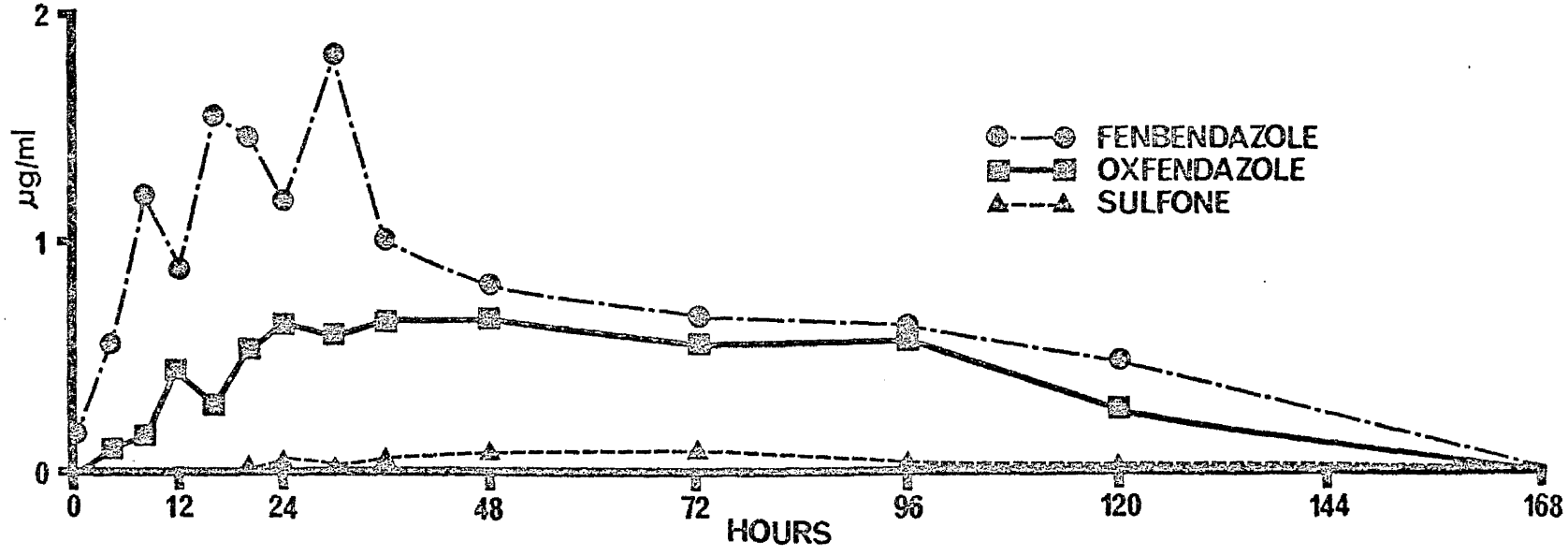

0.4
0.2
0
µg/ml
FENBENDAZOLE
OXFENDAZOLE
SULFONE
0
12
24
48
72
96
120
144
168
HOURS

Fig. 5 (4) Mean abomasal fluid concentrations (µg/ml) of fenbendazole, oxfendazole and oxfendazole sulfone in six sheep after oral administration of fenbendazole (10 mg/kg) as a suspension


2
1
0
µg/ml
FENBENDAZOLE
OXFENDAZOLE
SULFONE
0
12
24
48
72
96
120
144
168
HOURS

EXPERIMENT 1

Plasma, abomasal fluid and ruminal fluid concentrations of fenbendazole, and plasma and abomasal fluid concentrations of its major metabolites, oxfendazole and the sulfone, are shown in Tables 5 (1) and 5 (2). Figs. 5 (3) and 5 (4) show the mean concentrations of fenbendazole and its metabolites in plasma and abomasal fluid respectively.

Maximum plasma concentrations of fenbendazole were variable and low, less than 0.3 µg/ml in any sheep; but persisted for up to 5 days. The maximum concentration occurred at around 24 hours in most animals. The major metabolite, oxfendazole, was detectable in plasma by 4 hours after drug administration and reached its maximum concentrations at 30 hours. In all six sheep the maximum oxfendazole concentration was much higher than that of the parent drug.

The sulfone was detectable by 8-12 hours after administration of fenbendazole and maximum concentrations were found at 36 hours. The sulfone was present in lower amounts than the sulfoxide.

Fenbendazole was present in the abomasum by 10 minutes after administration in all animals, and was detectable for up to 5 days. In two of the three sheep dosed in the initial experiment, abomasal fluid concentrations were fairly high at 5 days, and it is almost certain that if the sampling period had been extended, fenbendazole would have been detectable for greater than 5 days in these animals. The metabolites were detected in abomasal fluid

at 4 hours (oxfendazole) and 20 hours (sulfone) and reached maximum concentrations by 48 and 72 hours respectively. Concentrations of both metabolites in the abomasum were lower than those of the parent drug. Oxfendazole reached higher concentrations in abomasal fluid than in plasma, but the sulfone did not.

Ruminal fluid concentrations of fenbendazole were extremely variable. The drug persisted for 4 days in three animals and for at least 5 days in the other three.

In some sheep it could definitely be stated that no oxfendazole or sulfone was detectable in the ruminal fluid at any time at the limit of detection for ruminal fluid (0.05 µg/ml). In others, because of difficulties already referred to (poor limit of detection for sulfoxide using methanol/perchloric acid; contamination of ruminal fluid samples) and also in some cases due to insufficient sample remaining after the initial measurement of fenbendazole, it is possible that low concentrations of these metabolites were present.

EXPERIMENT 2

The concentrations in plasma of fenbendazole and of total metabolites after intra-abomasal administration of fenbendazole are shown in Tables 5 (3) and 5 (4). Fig. 5 (5) shows mean plasma fenbendazole concentrations after intra-abomasal administration compared with those after oral administration. In only one animal (M) were the metabolites measured separately. The relative percentages of sulfoxide and sulfone present at any time were similar to those found after oral administration.

TABLE 5 (3) PLASMA CONCENTRATIONS (µg/ml) OF FENBENDAZOLE AFTER INTRA-ABOMASAL ADMINISTRATION OF FENBENDAZOLE (10 mg/kg) AS A SUSPENSION

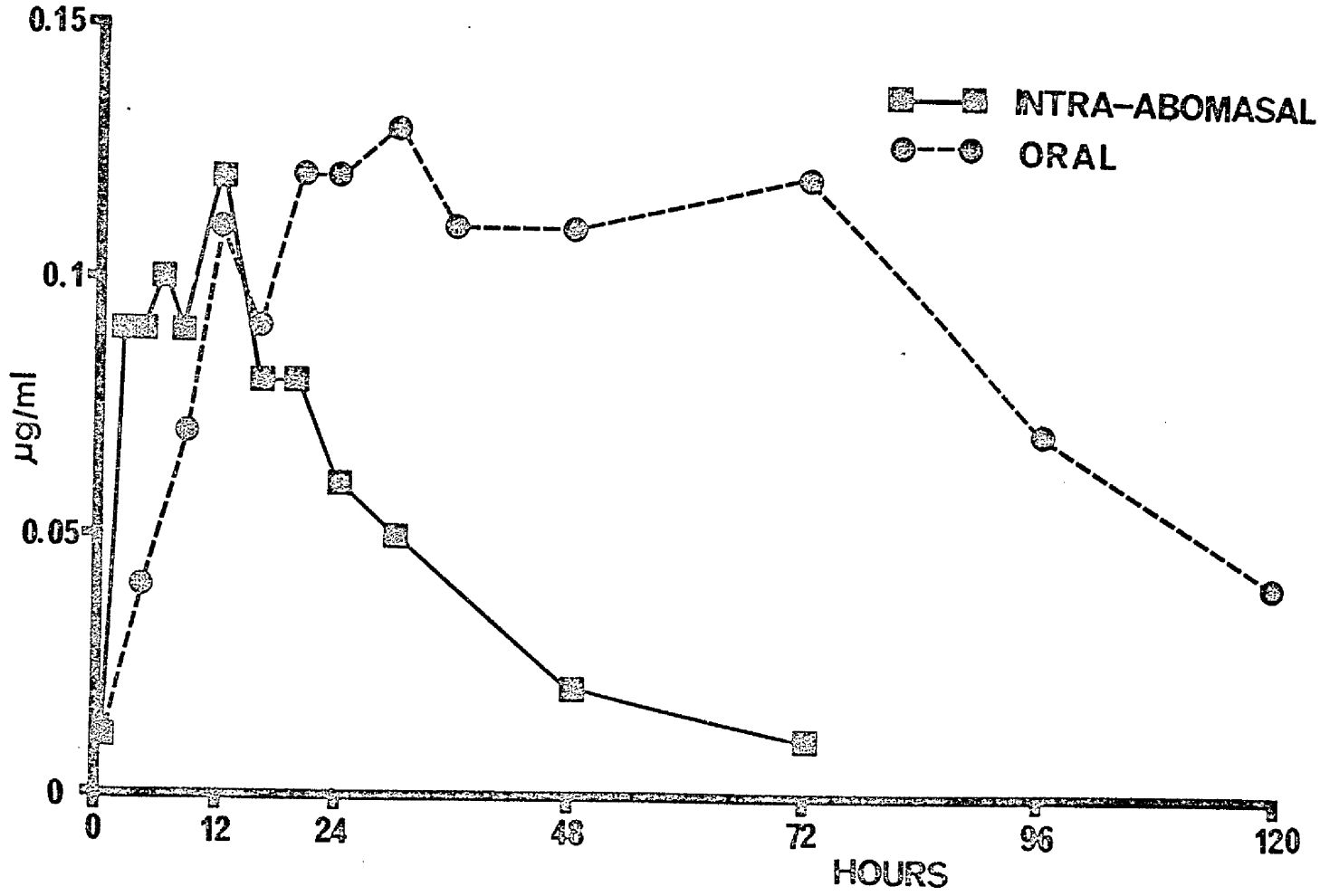
Time	Sheep name			Mean ± S.E.M.
	M	S	B	
Baseline	0	0	0	0
30 min	0.03	0.01	0	0.013 ± 0.009
2 hours	0.13	0.05	0.08	0.09 ± 0.02
4	0.10	0.09	0.08	0.09 ± 0.01
6	0.10	0.08	0.11	0.10 ± 0.01
8	0.10	0.07	0.10	0.09 ± 0.01
12	0.16	0.06	0.15	0.12 ± 0.03
16	0.14	0.02	0.08	0.08 ± 0.03
20	0.14	0.02	0.07	0.08 ± 0.03
24	0.07	0.03	0.06	0.06 ± 0.01
30	0.06	0.02	0.06	0.05 ± 0.01
48	0.03	0.01	0.03	0.02 ± 0.01
72	0.02	0.01	0.01	0.013 ± 0.003

TABLE 5 (4) PLASMA CONCENTRATION (μg/ml) OF METABOLITES AFTER INTRA-ABOMASAL ADMINISTRATION OF FENBENDAZOLE (10 mg/kg) AS A SUSPENSION

Time	Sheep name				
	M*		S	B	Mean ± S.E.M.
	Sulfoxide	Sulfone			
Baseline	0	0	0	0	0
30 minutes	0	0	0	0	0
2 hours	0.02	0	0.02	0.03	0.02 ± 0.003
4	0.04	0	0.09	0.07	0.07 ± 0.01
6	0.07	0.01	0.15	0.18	0.14 ± 0.03
8	0.12	0.01	0.17	0.21	0.17 ± 0.02
12	0.22	0.03	0.20	0.33	0.26 ± 0.04
16	0.29	0.05	0.13	0.33	0.27 ± 0.07
20	0.31	0.06	0.09	0.34	0.27 ± 0.09
24	0.28	0.06	0.08	0.36	0.26 ± 0.09
30	0.19	0.07	0.07	0.33	0.22 ± 0.08
48	0.12	0.06	0.06	0.31	0.18 ± 0.07
72	0.08	0.06	0.02	0.15	0.10 ± 0.04

* Only in Sheep M were sulfoxide and sulfone measured separately

Fig. 5 (5) Mean plasma concentrations (µg/ml) of fenbendazole after oral and intra-abomasal administration of fenbendazole (10 mg/kg) to the same three sheep


0.15
0.1
0.05
0
µg/ml
INTRA-ABOMASAL
ORAL
0
12
24
48
72
96
120
HOURS

In the initial 12 hour period after administration plasma concentrations of fenbendazole and its metabolites after intra-abomasal administration were higher than those after oral administration. At all times after this, drug and metabolite concentrations were lower, and they persisted for a shorter period than after oral administration. The area under the plasma concentration-time curve from Fig. 5 (5) after extrapolating to zero is in the ratio 3.2:1 for oral:abomasal administration.

Ruminal fluid samples were also taken and assayed in this experiment. No fenbendazole nor its metabolites were detected in any of these samples.

EXPERIMENT 3

Plasma concentrations of fenbendazole and of total metabolites after intra-ruminal administration are shown in Tables 5 (5) and 5 (6). Oxfendazole and the sulfone were not measured separately. Since figures are available from only two animals for this experiment it is difficult to make valid comparisons. Plasma concentrations of fenbendazole and total metabolites after intra-ruminal administration were generally higher and more prolonged than after intra-abomasal administration. However, the maximum fenbendazole concentration was achieved earlier (16 hours) and the drug was less persistent than in the same two sheep after oral administration.

TABLE 5 (5) PLASMA CONCENTRATIONS (µg/ml) OF FENBENDAZOLE AFTER INTRA-RUMINAL ADMINISTRATION OF FENBENDAZOLE (10 mg/kg) AS A SUSPENSION

	Sheep name		
Time	M	B	Mean
Baseline	0	0	0
4 hours	0.10	0.09	0.01
8	0.10	0.15	0.13
16	0.18	0.16	0.17
24	0.07	0.12	0.10
32	0.08	0.15	0.12
40	0.06	0.16	0.09
48	0.07	0.08	0.08
60	0.02	0.06	0.04
72	0	0.06	0.03
96	0	0	0

TABLE 5 (6) PLASMA CONCENTRATIONS (μg/ml) OF METABOLITES AFTER INTRA-RUMINAL ADMINISTRATION OF FENBENDAZOLE (10 mg/kg) AS A SUSPENSION

	Sheep name		
Time	M	B	Mean
Baseline	0	0	0
4 hours	0	0	0
8	0.03	0.08	0.06
16	0.13	0.17	0.15
24	0.13	0.19	0.16
32	0.12	0.26	0.19
40	0.04	0.28	0.16
48	0.08	0.17	0.13
60	0.04	0.16	0.10
72	0	0.15	0.08
96	0	0.03	0.02

OXFENDAZOLE

Oxfendazole (methyl 5 (6)-phenylsulfinyl-2-benzimidazole carbamate) is marketed in the United Kingdom as a suspension for oral use in cattle and sheep("Systamex", Wellcome; "Synanthic", Syntex).

Oxfendazole concentrations in the plasma of calves after oral dosage have been reported by Nerenberg, Runkel and Matin (1978), using a radioimmunoassay procedure developed by them. These authors found maximum concentrations of around 0.4 µg/ml occurring 6-12 hours after administration of an oral dose of 3 mg/kg. No details as to the age or weight of the calves, or whether or not they were pre-ruminant were given.

Prichard et al (1978), using the same assay, reported maximum plasma oxfendazole concentrations as high as 3.0 µg/ml in sheep dosed at 5 mg/kg occurring 24 hours after administration.

In this study oxfendazole was given orally at a dose rate of 10 mg/kg to six sheep in two separate experiments. The plasma, abomasal fluid and ruminal fluid concentrations of oxfendazole and the sulfone were measured at various times after dosing. In a number of plasma samples fenbendazole was also measured.

MATERIALS AND METHODS

Initially the experiment was carried out on three sheep (M, S and B) but when oxfendazole was subsequently found to be present in the final sample (taken at 5 days) it was repeated in a further three sheep. In this group fewer samples were taken to minimise the analysis required, and an additional sample of abomasal fluid was taken at 10 minutes to assess possible reticular groove function.

Oxfendazole was administered in the form of a commercial suspension "Synanthic", Syntex) containing 2.265% w/v suspension via a syringe placed on the back of the tongue.

The methods of sampling, storage and analysis have all been described in Chapter 2.

RESULTS

Plasma and abomasal fluid concentrations of oxfendazole and sulfone are shown in Table 5 (7) and the mean concentrations are also represented in Figs. 5 (6) and 5 (7) respectively.

Ruminal fluid concentrations of oxfendazole are shown in Table 5 (8).

The maximum concentrations of oxfendazole and sulfone achieved both in plasma and abomasal fluid, and the length of time for which they were detectable, were extremely variable in different sheep.

No oxfendazole was detected in plasma at 30 minutes after administration, demonstrating the relatively slow absorption of the drug. Maximum concentrations of 0.6-1.3 µg/ml occurred between

Table 5 (7) Plasma and abomasal fluid concentrations of oxfendazole and oxfendazole sulfone after oral administration of oxfendazole (10 mg/kg) as a suspension

	Oxfendazole							Sulfone						
	Sheep Name							Sheep Name						
Time	M	S	B	E	R	C	Mean ± SEM	M	S	B	E	R	S	Mean ± SEM
							PLASMA CONCENTRATION (μg/ml)							
Baseline	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30 min	0	0	0	-	-	-	0	0	0	0	-	-	-	0
4 hours	0.07	0.23	0.05	-	-	-	0.12±0.06	0	0.05	0	-	-	-	0.02±0.02
8	0.19	0.44	0.17	-	-	-	0.27±0.09	0	0.04	0.03	-	-	-	0.02±0.01
12	0.33	0.51	0.22	0.91	0.59	0.69	0.54±0.10	0.04	0.11	0.03	0.17	0.16	0.08	0.10±0.03
16	0.49	0.62	0.34	-	-	-	0.48±0.08	0.08	0.13	0.08	-	-	-	0.09±0.02
20	0.44	0.56	0.45	-	-	-	0.48±0.04	0.08	0.20	0.08	-	-	-	0.12±0.04
24	0.98	0.62	0.48	0.55	0.22	1.33	0.73±0.16	0.16	0.25	0.09	0.20	0.10	0.29	0.19±0.03
30	0.98	0.64	0.66	-	-	-	0.76±0.11	0.23	0.21	0.15	-	-	-	0.20±0.02
36	0.64	0.51	0.68	-	-	-	0.61±0.05	0.16	0.17	0.23	-	-	-	0.19±0.02
48	1.26	0.33	0.51	0.27	0.08	0.70	0.53±0.17	0.41	0.16	0.19	0.22	0.04	0.38	0.24±0.06
72	0.61	0.27	0.44	0.11	0	0.59	0.34±0.10	0.29	0.07	0.26	0.13	0.02	0.39	0.19±0.06
96	0.31	0.07	0.51	0.06	0	0.48	0.24±0.09	0.19	0.05	0.34	0.10	0	0.33	0.17±0.06
5 days	0.21	0	0.32	0.03	0	0.33	0.15±0.06	0.22	0.03	0.21	0.07	0	0.30	0.14±0.05
6	-	-	-	0.02	0	0.21	0.04±0.03	-	-	-	0.03	0	0.20	0.06±0.06
7	-	-	-	0	0	0.15	0.03±0.03	-	-	-	0	0	0.14	0.05±0.05
8	-	-	-	0	0	0	0	-	-	-	0	0	0.07	0.02±0.02
9	-	-	-	0	0	0	0	-	-	-	0	0	0.02	0.01±0.01
11	-	-	-	0	0	0	0	-	-	-	0	0	0	0
14	-	-	-	0	0	0	0	-	-	-	0	0	0	0
							ABOMASAL FLUID CONCENTRATION (μg/ml)							
Baseline	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10 min	-	-	-	1.45	3.98	0.07	1.83±1.14	-	-	-	0	0	0	0
4 hours	0.35	0.75	0.81	-	-	-	0.64±0.14	0	0	0	-	-	-	0
8	0.80	0.60	2.42	-	-	-	1.27±0.56	0	0	0	0	0	0	0
12	3.37	1.27	4.10	2.03	1.83	2.87	2.58±0.43	0	0	0	-	-	-	0
16	4.08	0.66	5.64	-	-	-	3.46±1.47	0	0.03	0	-	-	-	0.01±0.01
20	4.41	1.19	5.05	-	-	-	3.55±1.19	0.08	0	0.06	0.03	0	0	0.03±0.01
24	5.65	0.96	3.69	2.26	0.64	2.05	2.54±0.76	0.12	0.06	0.04	-	-	-	0.07±0.02
30	5.92	0.98	3.12	-	-	-	3.34±1.43	0.48	0.07	0.12	0.05	0	0	0.12±0.07
36	6.70	0.74	6.24	1.04	0.31	2.11	2.86±1.17	1.15	0.03	0.23	0.09	0	0.15	0.27±0.18
48	10.19	0.60	5.93	0.47	0.13	2.17	3.25±1.64	0.82	0	0.13	0.09	0	0.09	0.19±0.13
72	5.03	0.07	1.86	0.20	0.07	2.21	1.57±0.79	0.66	0	0.09	0.04	0	0.05	0.14±0.11
96	4.04	0.04	1.20	0.02	0	1.17	1.21±0.76	0.21	0	0.04	0	0	0	0.04±0.03
5 days	2.30	0.01	0.94	0.01	0	0.70	0.66±0.37	-	-	-	0	0	0	0
6	-	-	-	0	0	0.29	0.10±0.10	-	-	-	0	0	0	0
7	-	-	-	0	0	0.20	0.07±0.07	-	-	-	0	0	0	0
8	-	-	-	0	0	0.12	0.04±0.04	-	-	-	0	0	0	0
9	-	-	-	0	0	0.06	0.02±0.01	-	-	-	0	0	0	0
11	-	-	-	0	0	0	0	-	-	-	0	0	0	0
14	-	-	-	0	0	0	0							

Fig. 5 (6) Mean plasma concentrations (µg/ml) of oxfendazole and oxfendazole sulfone in six sheep after oral administration of oxfendazole (10 mg/kg) as a suspension

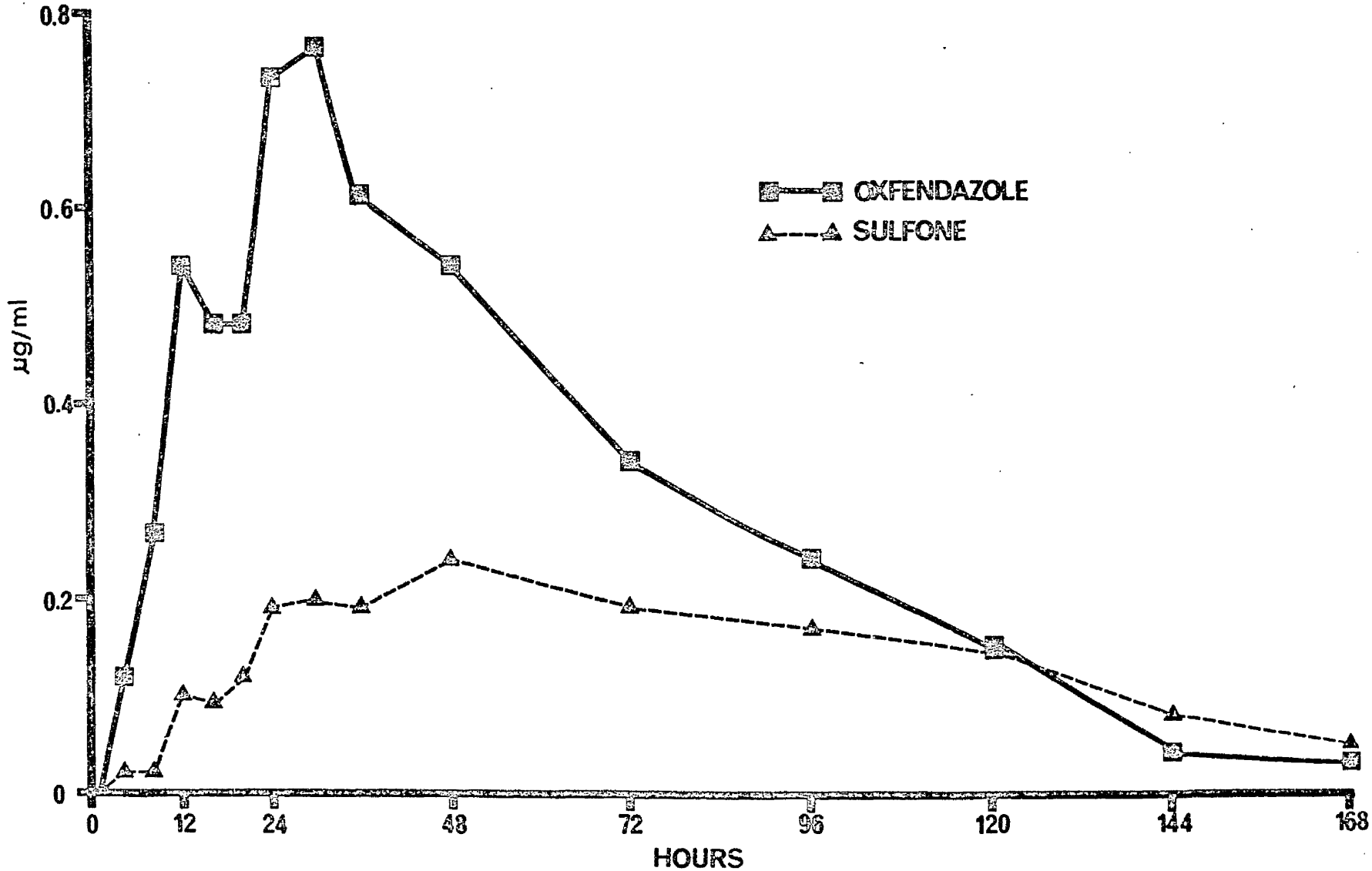

0.8
0.6
0.4
0.2
0
µg/ml
OXFENDAZOLE
SULFONE
0
12
24
48
72
96
120
144
168
HOURS

Fig. 5 (7) Mean abomasal fluid concentrations (µg/ml) of oxfendazole and oxfendazole sulfone in six sheep after oral administration of oxfendazole (10 mg/kg) as a suspension

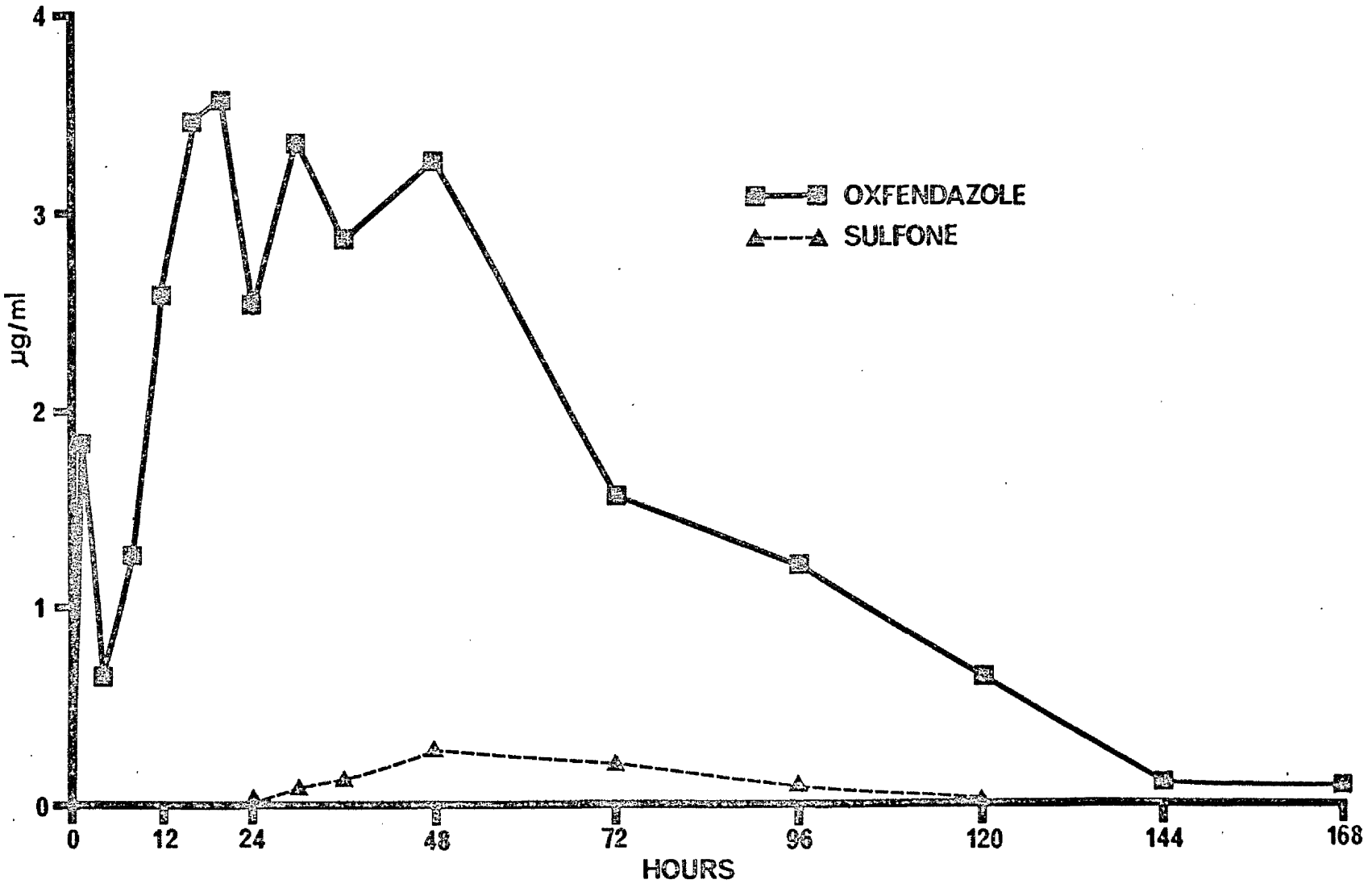

4
3
2
1
0
µg/ml
OXFENDAZOLE
SULFONE
0
12
24
48
72
96
120
144
168
HOURS

TABLE 5 (8) RUMINAL FLUID CONCENTRATIONS (µg/ml) OF OXFENDAZOLE AFTER ORAL ADMINISTRATION OF OXFENDAZOLE (10 mg/kg) AS A SUSPENSION

Time	Sheep name						Mean ± S.E.M.
	M	S	B	E	R	G	
Baseline	0	0	0	0	0	0	0
30 min	-	-	-	-	-	-	-
4 hours	0.61	0.26	0.15	-	-	-	0.34 ± 0.14
8	0.55	0.22	0.28	-	-	-	0.35 ± 0.10
12	0.24	0.16	0.46	0.05	0.00	0.51	0.24 ± 0.09
16	0.43	0.08	0.39	-	-	-	0.30 ± 0.11
20	0.43	0.08	0.22	-	-	-	0.24 ± 0.10
24	0.49	0.17	0.24	0.14	0.00	0.16	0.20 ± 0.07
30	0.34	0.08	0.25	-	-	-	0.22 ± 0.08
36	0.27	0.07	0.24	-	-	-	0.19 ± 0.06
48	0.61	0.03	0.15	0.12	0.00	0.25	0.19 ± 0.09
72	0.24	0.07	0.16	0.08	0.00	0.18	0.12 ± 0.04
96	0.06	0.05	0.12	0.08	0.08	0.18	0.10 ± 0.02
5 days	0.21	0.05	0.09	0.53	0.00	0.09	0.16 ± 0.08
6	-	-	-	0.16	0.00	0.04	0.07 ± 0.05
7	-	-	-	0.08	0.04	0.07	0.06 ± 0.01
8	-	-	-	0.18	0.00	0.08	0.09 ± 0.05
9	-	-	-	0.82	0.00	0.23	0.35 ± 0.24
11	-	-	-	0.24	0.00	0.08	0.11 ± 0.07
14	-	-	-	0.00	0.00	0.18	0.06 ± 0.06

24 and 48 hours after dosing, and drug was detectable for up to 7 days. Sulfone was present by 4-8 hours after administration and reached maximum concentrations by 48-72 hours. It was present in much lower amounts than oxfendazole. Oxfendazole was present in the abomasal fluid by 10 minutes after dosing, and persisted for up to 9 days. Sulfone was detectable by 16-20 hours and was below the limit of detection by 5 days.

Ruminal fluid concentrations of oxfendazole were remarkably low, the maximum in any of the six sheep being 0.6 µg/ml. The sulfone was not found in any of the ruminal fluid samples.

During the course of these experiments Nerenberg et al (1978) described the sulfide of oxfendazole (fenbendazole) as being one of the metabolites of oxfendazole found in the plasma of sheep treated with oxfendazole. (Evidence for this observation was not given).

Under the solvent conditions used routinely to measure oxfendazole, fenbendazole would have been detectable only at relatively high concentrations. A number of plasma samples were therefore re-assayed and the presence of fenbendazole confirmed. Subsequently two different sheep were dosed with oxfendazole at 10 mg/kg as in the previous experiments, and plasma oxfendazole and fenbendazole concentrations determined simultaneously. The results are given in Table 5 (9).

The concentrations of fenbendazole found at any time varied between 0 and 20% of those of oxfendazole present at that time.

TABLE 5 (9) PLASMA CONCENTRATIONS (μg/ml) OF OXFENDAZOLE AND FENBENDAZOLE AFTER ORAL ADMINISTRATION OF OXFENDAZOLE (10 mg/kg) AS A SUSPENSION

	OXFENDAZOLE			FENBENDAZOLE		
	Sheep name			Sheep name		
Time	L	N	Mean	L	N	Mean
Baseline	0	0	0	0	0	0
6 hours	0.43	0.80	0.62	0.04	0.07	0.06
12	0.79	1.37	1.08	0.18	0.18	0.18
16	0.80	1.24	1.02	0.18	0.18	0.18
20	0.97	1.18	1.08	0.20	0.19	0.20
24	0.86	0.96	0.91	0.17	0.14	0.16
36	0.68	0.67	0.68	0.13	0.08	0.11
48	0.59	0.27	0.43	0.10	0.03	0.07
86	0.12	0.05	0.09	0.00	0	0.00

ALBENDAZOLE

Albendazole, methyl (5-(propylthio)-1H-benzimidazol-2-yl) carbamate, is available in the United Kingdom for use in sheep and cattle as an oral suspension ("Valbazen", Smith Kline Animal Health). In other European countries it is also marketed as a paste formulation.

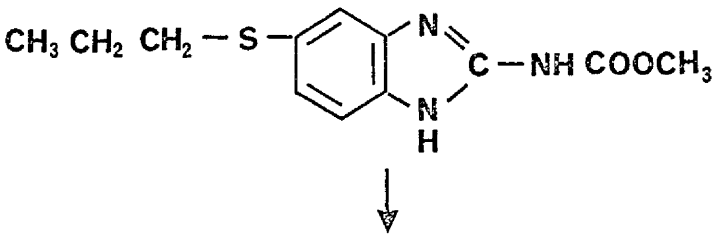
Kinetic studies with albendazole have been made using radio-labelled ^{14}C albendazole (Dicuollo et al, 1977). In sheep given a single oral dose of 16 mg/kg, maximum plasma concentrations equivalent to 3.7 µg/ml of albendazole or related products (not distinguished) were found 15 hours after administration. In calves given 20 mg/kg, the peak concentrations were 5.5 µg/ml 15-24 hours after treatment.

During the early part of this study plasma samples from ten lambs being used in an efficacy study of albendazole were assayed for the drug. No albendazole was detected in the plasma of any of these lambs dosed with 5 mg/kg, but high concentrations of a metabolite were present. Samples of the major metabolites of albendazole in sheep, the sulfoxide, the sulfone and the 2-amino sulfone (Fig. 5 (8)) were obtained by gift from Smith Kline Animal Health Products and concentrations of albendazole and these metabolites investigated in the experimental sheep.

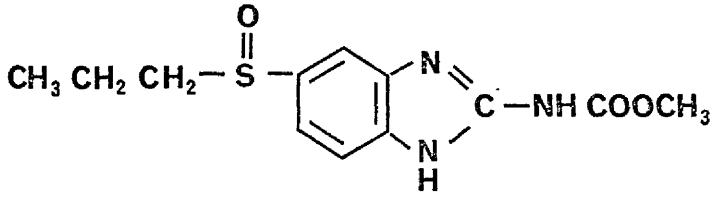
Initially the study was performed using commercial suspension "Valbazen" containing 2.5% w/v of albendazole.

On completion of the study using albendazole suspension,

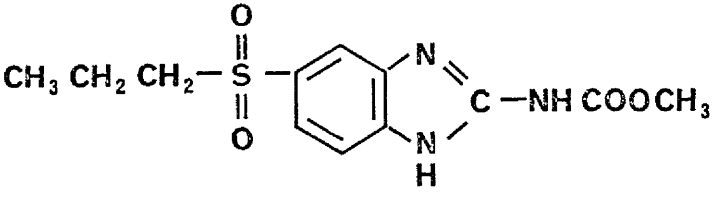
Fig. 5 (8) The structures of albendazole and its major metabolites



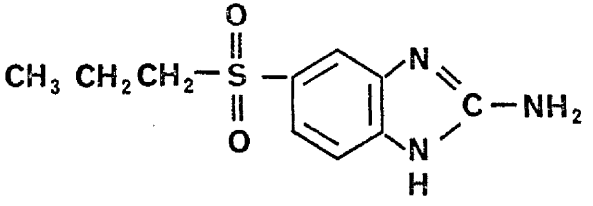
ALBENDAZOLE



SULFOXIDE



SULFONE



2—AMINO—SULFONE

the question arose as to whether the kinetics of albendazole when delivered as a paste formulation were similar to those after the suspension. This was of considerable commercial interest to Smith Kline, since regulatory authorities in other E.E.C. countries were questioning whether the considerable volume of efficacy data obtained using the suspension formulation could be translated to the paste. To investigate this problem the study was repeated using the same experimental design, with the drug being given as a paste.

MATERIALS AND METHODS

The same sheep B, S, G, were used for both the paste and the suspension trials which were carried out 2 months apart. In both cases the dose rate was 10 mg/kg, the drug being given via a syringe placed on the back of the tongue. The paste was supplied by Smith Kline Animal Health (Lot No. 12/78 ko3) and contained 30% w/v albendazole.

Samples of plasma, abomasal fluid and ruminal fluid were taken prior to drug administration and at 4, 8, 12, 16, 20, 24, 32, 48, 72, 96 hours and 5, 7, 8 and 11 days after administration. Additional abomasal fluid samples were taken at 10 minutes (suspension) and 10 minutes and 30 minutes (paste).

Samples were taken, stored and analysed for albendazole, albendazole sulfoxide and albendazole sulfone as described in Chapter 2.

RESULTS

The concentrations of albendazole, sulfoxide and sulfone in plasma and abomasal fluid after the administration of albendazole suspension are shown in Tables 5 (10) and 5 (11). Ruminal fluid concentrations of albendazole are given in Table 5 (12). The sulfoxide and sulfone could not be detected in rumen fluid following either paste or suspension treatment.

Tables 5 (13), 5 (14) and 5 (15) show plasma and abomasal fluid concentrations of albendazole, the sulfoxide and sulfone, and ruminal fluid concentrations of albendazole, after albendazole paste administration.

TABLE 5 (10) PLASMA CONCENTRATIONS (µg/ml) OF ALBENDAZOLE, ALBENDAZOLE SULFOXIDE AND ALBENDAZOLE SULFONE AFTER ORAL ADMINISTRATION OF ALBENDAZOLE 10 mg/kg AS A SUSPENSION

	Albendazole			Sulfoxide			Sulfone			Mean concentration ± S.E.M.		
Sheep Name	B	S	G	B	S	G	B	S	G	Albendazole	Sulfoxide	Sulfone
Time												
Baseline	0	0	0	0	0	0	0	0	0	0	0	0
4 hours	0	0.02	0.02	1.49	1.39	1.42	0.09	0.11	0	0.01 ± 0.01	1.43 ± 0.03	0.07 ± 0.03
8	0	0.01	0.05	2.22	1.49	2.67	0.28	0.08	0.19	0.02 ± 0.01	2.13 ± 0.34	0.18 ± 0.06
12	0	0	0.01	2.39	2.23	2.37	0.51	0.28	0.18	0	2.33 ± 0.05	0.32 ± 0.10
16	0	0	0.01	2.03	2.67	4.02	0.69	0.42	0.49	0	2.91 ± 0.59	0.53 ± 0.08
20	0	0	0	1.71	3.08	4.74	0.82	0.72	0.64	0	3.18 ± 0.88	0.73 ± 0.05
24	0	0	0	1.30	2.64	4 56	0.83	0.79	0.72	0	2.83 ± 0.95	0.77 ± 0.03
32	0	0	0	0.72	2.31	4.18	0.83	0.80	1.17	0	2.40 ± 1.00	0.93 ± 0.12
48	0	0	0	0	1.05	1.62	0.04	0.59	1.80	0	0.89 ± 0.47	0.81 ± 0.52
72	0	0	0	0	0	0	0	0	0.47	0	0	0.16 ± 0.16
96	0	0	0	0	0	0	0	0	0	0	0	0
5 days	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 5 (11) ABOMASAL FLUID CONCENTRATIONS (μg/ml) OF ALBENDAZOLE, ALBENDAZOLE SULFOXIDE AND ALBENDAZOLE SULFONE AFTER ORAL ADMINISTRATION OF ALBENDAZOLE 10 mg/kg AS A SUSPENSION

	Albendazole			Sulfoxide			Sulfone			Mean concentration ± S.E.M.		
Sheep Name	B	S	G	B	S	G	B	S	G	Albendazole	Sulfoxide	Sulfone
Time												
Baseline	0	0	0	0	0	0	0	0	0	0	0	0
10 min	0.36	0.03	4.13	0	0	0	0	0	0	1.51 ± 1.32	0	0
4 hours	2.73	1.44	8.69	12.70	5.29	1.01	0.38	0.81	0	4.29 ± 2.24	6.33 ± 3.41	0.40 ± 0.23
8	2.10	4.10	11.76	21.82	18.28	9.41	1.37	1.60	0.43	5.99 ± 2.95	16.50 ± 3.69	1.13 ± 0.36
12	1.96	5.13	11.25	29.37	25.49	14.14	2.92	0.50	0.38	6.11 ± 2.73	23.00 ± 4.57	1.27 ± 0.83
16	0.87	2.71	9.06	20.42	32.80	19.88	3.61	1.29	0.61	4.21 ± 2.48	24.37 ± 4.22	1.84 ± 0.91
20	0.50	0.71	4.99	14.13	35.19	29.42	3.52	1.50	0.48	2.07 ± 1.46	26.25 ± 6.28	1.83 ± 0.89
24	0.57	2.76	4.16	10.62	39.87	18 .31	3.30	2.64	1.00	2.50 ± 1.05	22.93 ± 8.75	2.31 ± 0.68
32	0.21	1.41	2.47	8.78	62.56	18.58	4.02	5.27	1.19	1.36 ± 0.65	29.97 ± 16.54	3.49 ± 1.21
48	0.07	0.30	0.18	3.37	24.21	9.42	2.73	9.46	1.29	0.18 ± 0.07	12.33 ± 6.19	4.49 ± 2.52
72	0.03	0.06	0	0	0	3.82	0	1.31	2.49	0.03 ± 0.02	1.27 ± 1.28	1.27 ± 0.72
96	0.02	0.02	0	0	0	2.19	0	0	2.10	0.01 ± 0.01	0.73 ± 0.73	0.70 ± 0.70
5 days	0	0.01	0	0	0	0	0	0	0	0	0	0
7	0	0.01	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 5 (12) RUMEN CONCENTRATIONS (µg/ml) OF ALBENDAZOLE AFTER ORAL ADMINISTRATION OF ALBENDAZOLE (10 mg/kg) AS A SUSPENSION

Time	Sheep Name			Mean ± S.E.M.
	B	S	G	
Baseline	0	0	0	0
4 hours	0.72	1.31	1.77	1.27 ± 0.31
8	0.69	1.21	N. S.	0.95 ± 0.26
12	0.69	1.76	1.19	1.21 ± 0.31
16	0.51	1.10	1.68	1.10 ± 0.33
20	0.66	0.53	1.50	0.90 ± 0.31
24	0.38	0.50	1.07	0.65 ± 0.21
32	0.17	0.24	0.66	0.36 ± 0.16
48	0.05	0.05	0.19	0.10 ± 0.05
72	0.02	0	0.04	0.02 ± 0.01
96	0	0	0	0
5 days	0	0	0	0
7	0	0	0	0
8	0	0	0	0
11	0	0	0	0

TABLE 5 (13) PLASMA CONCENTRATIONS (μg/ml) OF ALBENDAZOLE, ALBENDAZOLE SULFOXIDE AND ALBENDAZOLE SULFONE AFTER ORAL ADMINISTRATION OF ALBENDAZOLE 10 mg/kg AS A PASTE

	Albendazole			Sulfoxide			Sulfone			Mean concentrations ± S.E.M.		
										Albendazole	Sulfoxide	Sulfone
Sheep Name	B	S	G	B	S	G	B	S	G			
Time												
Baseline	0	0	0	0	0	0	0	0	0	0	0	0
4 hours	0	0.02	0.03	1.71	1.08	2.21	0.21	0.03	0.07	0.02 ± 0.01	1.67 ± 0.33	0.10 ± 0.05
8	0	0	0.03	2.68	2.39	2.77	0.39	0.20	0.22	0.01 ± 0.01	2.61 ± 0.11	0.27 ± 0.06
12	0	0	0.01	2.23	3.27	4.20	0.41	0.51	0.48	0	3.23 ± 0.57	0.47 ± 0.03
16	0	0	0	2.39	3.82	5.59	0.89	0.62	0.79	0	3.93 ± 0.93	0.77 ± 0.08
20	0	0	0	2.21	3.83	4.77	1.02	0.90	0.58	0	3.60 ± 0.75	0.83 ± 0.13
24	0	0	0	1.90	3.41	2 58	1.29	1.12	0.39	0	2.63 ± 0.44	0.93 ± 0.28
32	0	0	0	1.19	2.03	2.19	1.38	0.71	0.61	0	1.80 ± 0.31	0.90 ± 0.24
48	0	0	0	0	0.99	0.51	1.84	0.68	0.48	0	0.50 ± 0.29	1.00 ± 0.42
72	0	0	0	0	0	0	0	0	0.20	0	0	0.10 ± 0.10
96	0	0	0	0	0	0	0	0	0	0	0	0
5 days	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 5 (14) ABOMASAL FLUID CONCENTRATIONS (µg/ml) OF ALBENDAZOLE, ALBENDAZOLE SULFOXIDE AND ALBENDAZOLE SULFONE AFTER ORAL ADMINISTRATION OF ALBENDAZOLE 10 mg/kg AS A PASTE

	Albendazole			Sulfoxide			Sulfone			Mean concentration ± S.E.M.		
Sheep Name	B	S	G	B	S	G	B	S	G	Albendazole	Sulfoxide	Sulfone
Time												
Baseline	0	0	0	0	0	0	0	0	0	0	0	0
10 min	0.78	0.19	3.95	0	0	0	0	0	0	1.64 ± 1.17	0	0
30	2.79	2.04	16.42	0.21	0	0.39	0	0	0	7.08 ± 4.67	0.20 ± 0.11	
4 hours	5.17	4.85	16.93	7.81	3.10	19.59	0.21	0	0.29	8.98 ± 3.97	10.17 ± 4.90	0.17 ± 0.08
8	3.93	8.06	6.44	12.42	13.88	37.00	0.81	1.43	1.56	6.14 ± 1.20	21.10 ± 7.96	1.27 ± 0.23
12	3.06	7.72	2.55	14.41	19.62	30.07	1.30	1.74	1.03	4.44 ± 1.64	21.37 ± 4.60	1.33 ± 0.17
16	1.92	4.39	1.77	16.13	28.17	40.81	1.97	3.03	2.61	2.69 ± 0.85	28.37 ± 7.12	2.54 ± 0.31
20	2.46	3.99	2.44	19.87	28.57	35.02	3.19	4.02	2.62	2.97 ± 0.51	27.82 ± 4.39	3.28 ± 0.40
24	0.59	2.94	2.58	6.09	26.20	31.11	1.22	5.00	1.68	2.04 ± 0.73	21.13 ± 7.66	2.63 ± 1.19
32	1.61	1.43	1.68	17.21	21.38	29.51	4.78	5.52	2.80	1.57 ± 0.07	22.70 ± 3.61	4.37 ± 0.81
48	0.35	0.49	0.79	6.69	7.64	10.37	5.09	5.11	2.41	0.54 ± 0.13	8.23 ± 1.10	4.20 ± 0.89
72	0.14	0.04	0.26	0.41	0	0.79	0.29	0.10	0.81	0.15 ± 0.05	0.40 ± 0.23	0.40 ± 0.21
96	0.02	0.02	0.02	0	0	0	0	0	0	0.02 ± 0	0	
5 days	0.02	0.02	0.01	0	0	0	0	0	0	0.02 ± 0.01	0	
7	0.01	0	0.02	0	0	0	0	0	0	0.01 ± 0.01	0	
8	0.04	0	0	0	0	0	0	0	0	0.01 ± 0.01	0	
11	0	0	0	0	0	0	0	0	0	0	0	

TABLE 5 (15) RUMEN CONCENTRATIONS (μg/ml) OF ALBENDAZOLE AFTER ORAL ADMINISTRATION (10 mg/kg) OF ALBENDAZOLE AS A PASTE

Time	Sheep Name			Mean ± S.E.M.
	B	S	G	
Baseline	0	0	0	0
4 hours	1.11	1.07	1.04	1.07 ± 0.02
8	0.70	0.92	1.15	0.92 ± 0.13
12	0.65	0.59	0.81	0.68 ± 0.06
16	0.60	0.51	0.61	0.57 ± 0.03
20	0.39	0.51	0.58	0.49 ± 0.05
24	0.76	0.26	0.36	0.46 ± 0.15
32	0.20	0.17	0.33	0.23 ± 0.04
48	0.06	0.09	0.17	0.11 ± 0.03
72	0.03	0.02	0.04	0.03 ± 0.01
96	0.03	0	0.03	0.02 ± 0.01
5 days	0	0	0	0
7	0	0	0	0
8	0	0	0	0
11	0	0	0	0

Mean plasma and abomasal fluid concentrations of albendazole, sulfoxide and sulfone after suspension and paste formulations are also represented in Figs. 5 (9) and 5 (10) and 5 (11) and 5 (12) respectively.

Plasma albendazole concentrations were below the limit of detection at all times in one animal and the drug was present only at very low concentration and only for a short time in the other two sheep. Similar results were obtained with both formulations of the drug.

Albendazole sulfoxide was detectable in plasma by 4 hours after treatment, and maximum concentrations were achieved by 16-20 hours in most cases. The sulfone was also detectable by 4 hours but maximum plasma concentrations of this metabolite occurred somewhat later, at around 32-48 hours after drug administration.

Albendazole was present in the abomasal fluid of all three sheep by 10 minutes after administration of both suspension and paste formulations, with maximum concentrations occurring at 4-12 hours.

The sulfoxide was not present in any of the 10 minute samples, but by 4 hours had reached high concentration in abomasal fluid. In all animals with both formulations of albendazole, the peak sulfoxide concentrations in abomasal fluid were greater than those of the parent drug. Maximum sulfoxide concentrations were measured at 16-20 hours, and the metabolite was below the detectable limit by 3-5 days.

Albendazole sulfone was present in abomasal fluid by 4-8

hours. Maximum concentrations were lower than those of albendazole and the sulfoxide and, as in plasma, occurred later than those of sulfoxide, at 32-48 hours.

Rumen fluid concentrations of albendazole were generally below 1 µg/ml, the highest concentration found at any time being 1.8 µg/ml. Drug persisted for 3-4 days.

The concentrations of albendazole, the sulfoxide and the sulfone in plasma, ruminal fluid and abomasal fluid after administration of paste and suspension formulations were tested for significant differences, using the Student t-test for paired samples. Only at one time were the albendazole concentrations significantly different at the 5% level (48 hours in the abomasum), and at no time the sulfoxide or sulfone concentrations.

Since values were compared at sixteen different times, it may be that this significant difference at 48 hours in the abomasum was the result of chance.

Fig. 5 (9) Mean plasma concentrations (µg/ml) of albendazole, albendazole sulfoxide and albendazole sulfone in three sheep after oral administration of albendazole (10 mg/kg) as a suspension

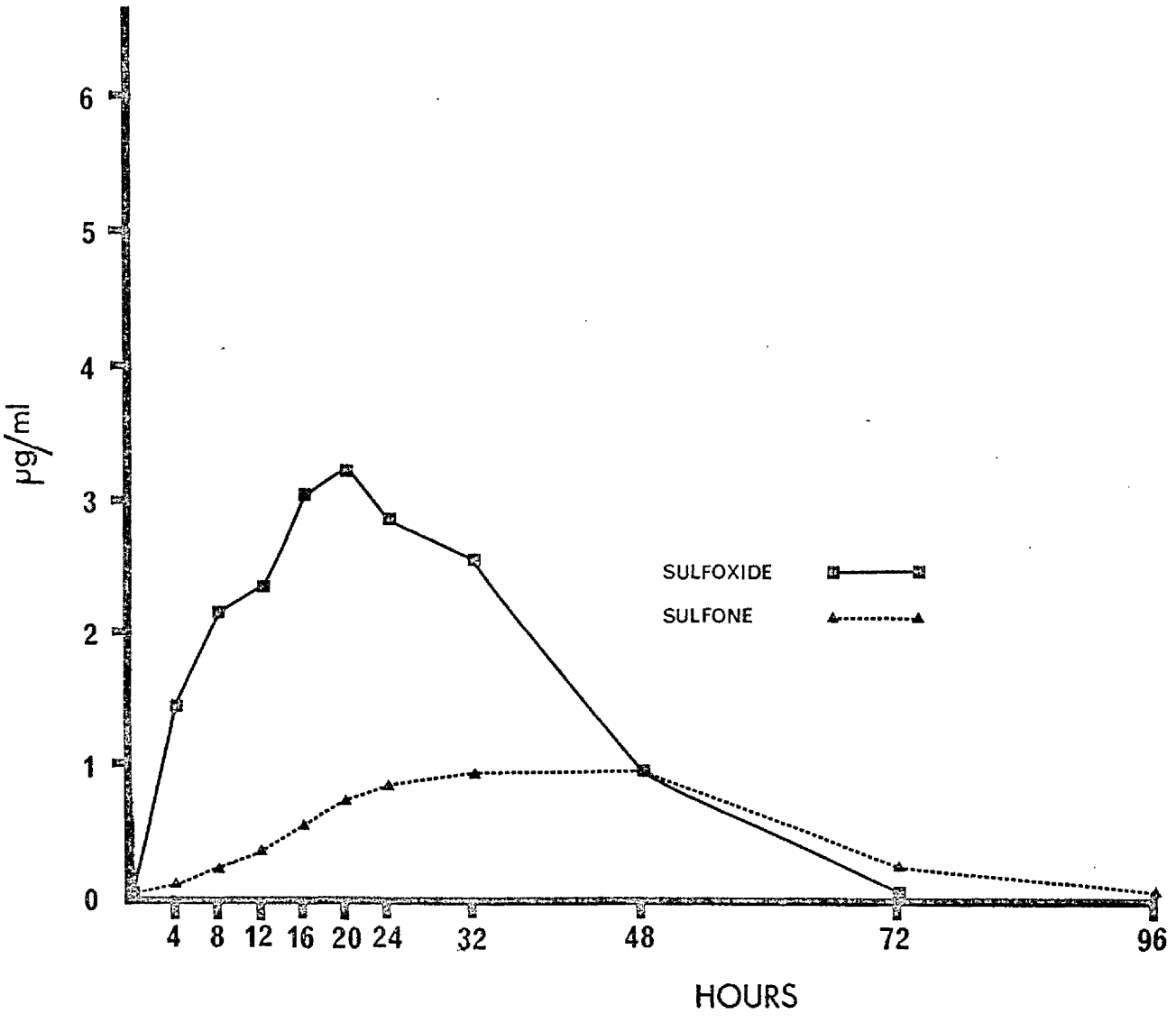

6
5
4
3
2
1
0
µg/ml
SULFOXIDE
SULFONE
4
8
12
16
20
24
32
48
72
96
HOURS

Fig. 5 (10) Mean abomasal fluid concentrations (µg/ml) of albendazole, albendazole sulfoxide and albendazole sulfone in three sheep after oral administration of albendazole (10 mg/kg) as a suspension

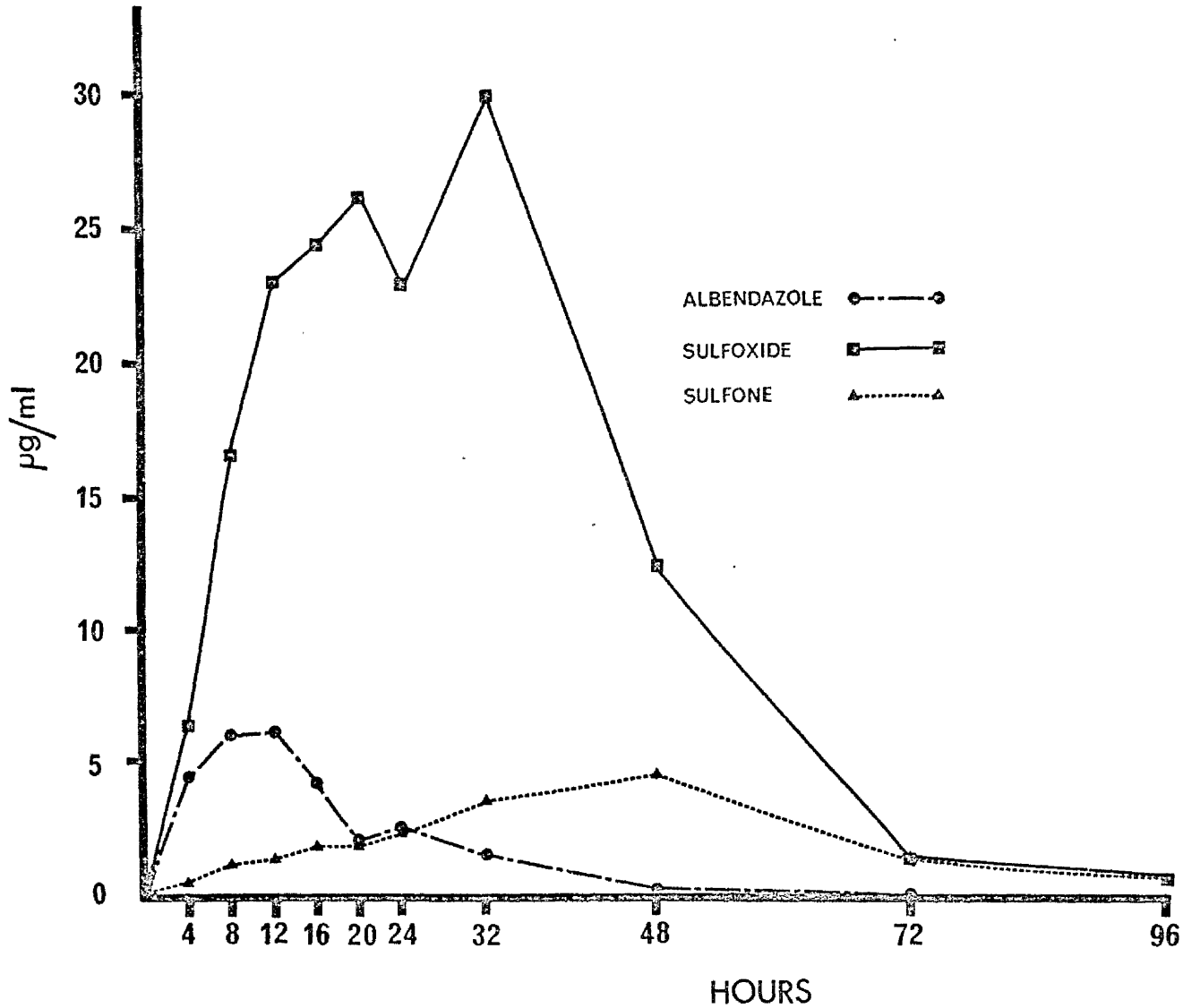

30
25
20
15
10
5
0
µg/ml
ALBENDAZOLE
SULFOXIDE
SULFONE
4
8
12
16
20
24
32
48
72
96
HOURS

Fig. 5 (11) Mean plasma concentrations (µg/ml) of albendazole, albendazole sulfoxide and albendazole sulfone in three sheep after oral administration of albendazole (10 mg/kg) as a paste

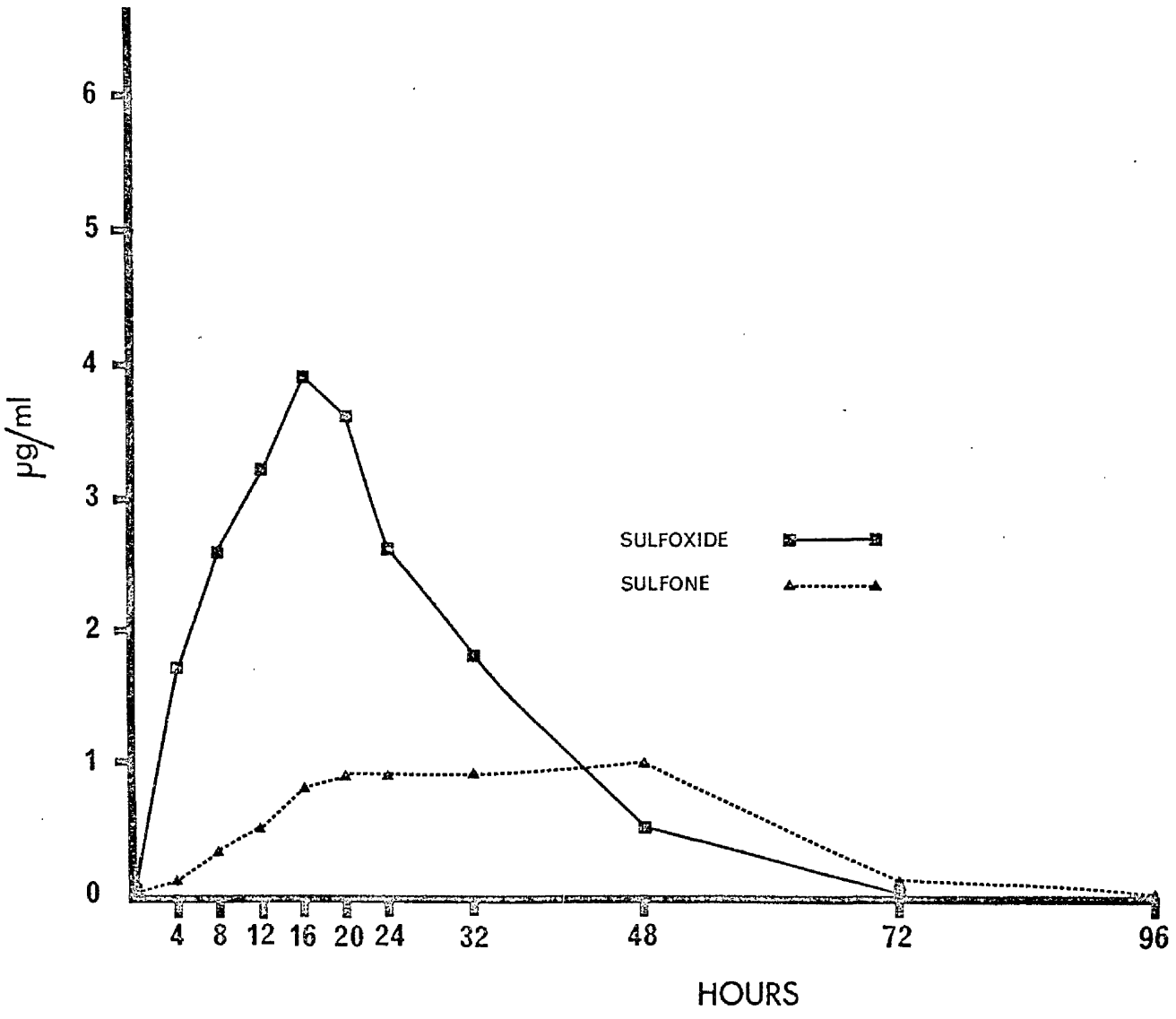
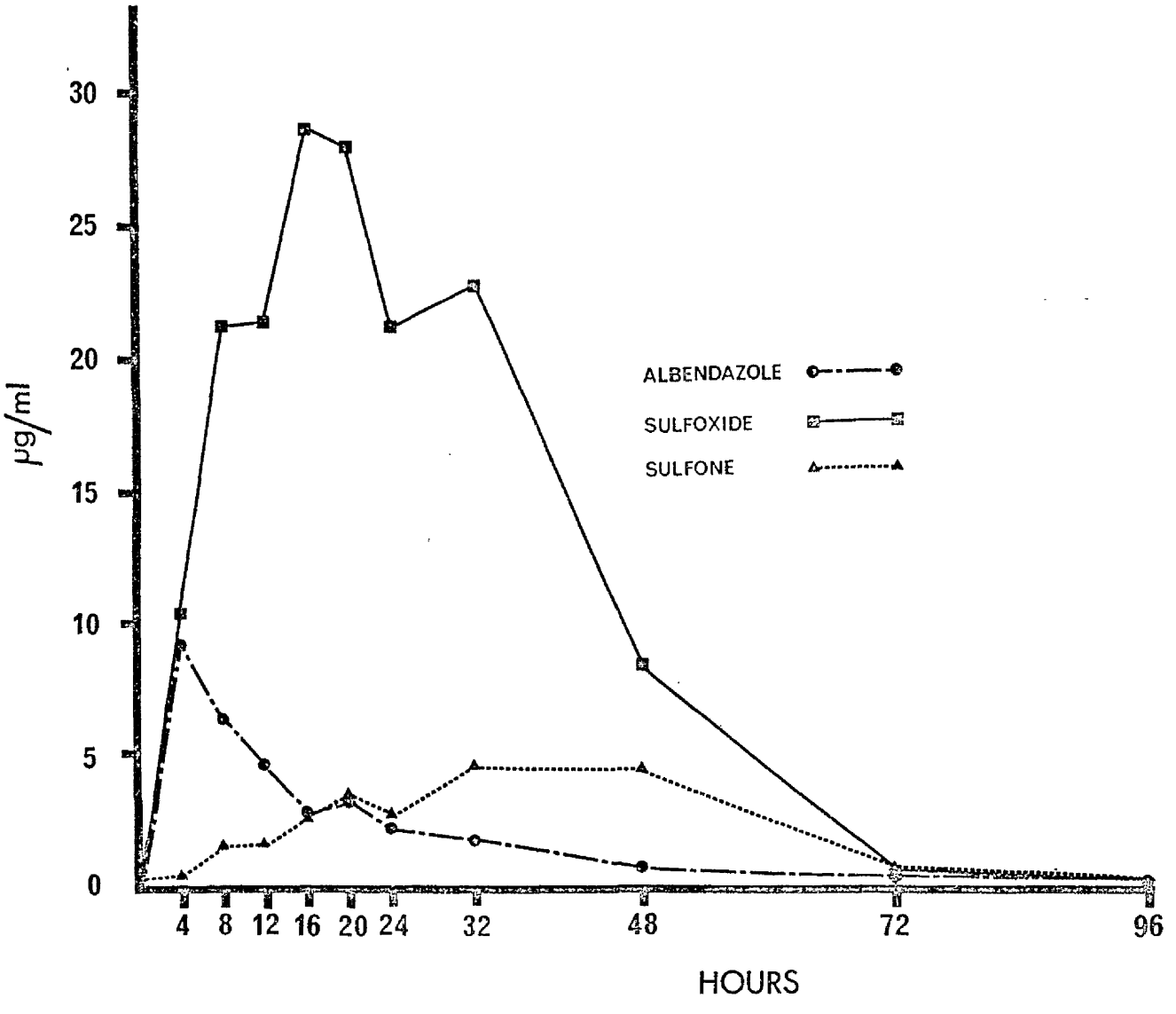

µg/ml
6
5
4
3
2
1
0
SULFOXIDE
SULFONE
4
8
12
16
20
24
32
48
72
96
HOURS

Fig. 5 (12) Mean abomasal fluid concentrations (µg/ml) of albendazole, albendazole sulfoxide and albendazole sulfone in three sheep after oral administration of albendazole (10 mg/kg) as a paste


30
25
20
15
10
5
0
µg/ml
ALBENDAZOLE
SULFOXIDE
SULFONE
4
8
12
16
20
24
32
48
72
96
HOURS

INVESTIGATION OF THE SITE OF METABOLISM OF BENZIMIDAZOLE DRUGS

The site of metabolism of the benzimidazoles was of obvious importance because of the high concentration of metabolites found in these experiments.

Most drug metabolism in mammalian species is carried out by the hepatic microsomal enzyme systems, one of the most important groups of enzymes in this respect being the mixed function oxidases. These enzymes catalyse many drug transformations, including sulfoxide formation (Goodman and Gilman, 1975). Such drug transformations involve the reduction of cytochrome P-450, a component of the mixed function oxidase system, by NADPH. The reduced form of cytochrome P-450 together with molecular oxygen reacts with the substrate (drug) to form oxidised drug with regeneration of the oxidised form of cytochrome P-450.

Drug metabolism also occurs in the gastro-intestinal tract; microflora of the gut including those of the rumen are capable of certain drug transformations. Chloramphenicol, for example, is reduced by ruminal microflora (Theodorides et al, 1968; De Corte Baeten and Debackere, 1978), and Nielson et al (1978) have shown trimethoprim to be extensively degraded in the functional rumen.

Circumstantial evidence from the concentrations of metabolites in the various body compartments suggested that the liver rather than ruminal micro-organisms was involved in benzimidazole metabolism. Although high concentrations of metabolites, particularly sulfoxides, were found in plasma (and abomasal fluid) after oral administration of parent drug, these

metabolites were not detected in ruminal fluid. The presence of oxfendazole in plasma after intra-abomasal administration of fenbendazole also suggested that the rumen was not the site of conversion. For this to be the case fenbendazole would require to be absorbed from the abomasum/small intestine, diffuse back into the rumen, undergo metabolism and the oxfendazole be reabsorbed. Neither fenbendazole or oxfendazole were detected in the rumen fluid of these animals.

One further consideration is that conditions within the normal reticulo-rumen strongly favour reduction reactions (Broberg, 1957). Sulfide conversion to sulfoxide and sulfone is in both cases an oxidation, which is more likely to be liver associated.

In order to investigate the site of metabolism of these drugs more closely, albendazole, albendazole sulfoxide, fenbendazole and oxfendazole were incubated separately with sheep ruminal fluid and abomasal fluid and with a bovine hepatic microsomal preparation. Samples were taken at intervals and assayed for drug and metabolites.

MATERIALS AND METHODS

Ruminal and abomasal fluid

The procedure was similar for each drug. Fresh ruminal and abomasal fluid was collected from three sheep, centrifuged, and the supernatant fluid filtered through coarse filter paper (Whatman No. 4). The samples of each fluid were pooled and divided into 25 ml aliquots (measuring cylinder). To these were added 15 µl (Eppendorf) of the appropriate commercial suspension "Panacur" 2.5%" (Hoechst U.K. Ltd), "Valbazen 2.5%" (Smith Kline Animal Health Ltd) and "Synanthic" (Syntex) containing 2.265% w/v oxfendazole, to give a concentration of about 15 µg/ml. In the absence of a commercial preparation of albendazole sulfoxide, the pure substance as supplied by Smith Kline Animal Health Products was used. Because of the insolubility of this compound in aqueous fluids, and the difficulty of producing a homogeneous aqueous suspension the required final concentration of 15 µg/ml was achieved by adding 3.75 ml of a solution of albendazole sulfoxide(100 µg/ml)in absolute alcohol to a 50 ml beaker. The alcohol was evaporated to dryness, 1 ml of water added to the residue and the beaker sonicated for 5 minutes. 24 ml of blank rumen fluid or abomasal fluid were then added. 25 ml aliquots of blank ruminal fluid and abomasal fluid and of the appropriate drug in phosphate buffer (pH 6) were prepared at the same time as controls.

A 5 ml aliquot of each fortified sample and of each control sample was removed and immediately deep frozen (-20^{o}C). The remainder was transferred to 50 ml glass conical flasks connected to

a source of carbon dioxide. The flasks were incubated in a shaking water bath for 24 hours at a constant temperature of 39^{o}C under a continuous flow of carbon dioxide.

At intervals the flasks were removed and, after correcting the volume for evaporation with warm distilled water, 5 ml aliquots were removed and frozen.

The samples were later thawed, extracted and assayed for parent drug and metabolites.

Bovine hepatic microsomes

Preparation of bovine hepatic microsomes for incubation was carried out with the assistance of Mr. Hanafy, Department of Veterinary Pharmacology, by the following method:
Liver tissue from three freshly killed 6-12 month old Ayrshire calves was chopped with scissors and excess connective tissue removed. The tissue (50 g) was homogenised in four volumes of ice-cold media composed of 0.154M Kcl and 0.05M Tris-Hcl buffer (pH 7.4) in an Atomix blender (MSE Ltd) for 2 minutes. The homogenised material was strained through gauze and centrifuged at 16,000 xg for 20 minutes. The 16,000 xg supernatant was decanted into ultracentrifuge tubes and centrifuged at 105,000 xg for 60 minutes (Beckman L2-65B ultracentrifuge with type 50.1 rotor). The resultant microsomal pellets were re-homogenised in phosphate buffer pH 7.4.

Each incubated sample contained, in a volume of 1.5 ml phosphate buffer (pH 7.4), 4 mg of microsomal protein, 8.5 mg of NADP, 63 mg of glucose-6-phosphate and 2 units of glucose-6-phosphate

dehydrogenase as an NADPH generating system. To this, the appropriate drug was added as substrate.

A similar preparation with boiled (10 minutes) microsomes was used as a control, and each drug was also incubated in the equivalent volume of phosphate buffer (pH 7.4) with cofactors.

Because of the impossibility of preparing an aqueous solution, or a homogeneous aqueous suspension of these drugs, no attempt was made at quantitative measurement of conversion. The drugs were added as 0.5 ml of a suspension of pure drug in pH 7.4 buffer.

The fresh and control preparations were then incubated in open test tubes in a shaking water bath at a constant temperature of 39^{o}C. A 1 ml aliquot of each was removed after 3 hours and the remainder after 6 hours. These were deep frozen and assayed the following day after thawing for parent drug and metabolites.

RESULTS

Ruminal and abomasal fluid

No metabolites were detected in samples assayed after incubation of albendazole and fenbendazole with either ruminal or abomasal fluid, nor was there any decrease in concentration of the parent drug over the 24 hour incubation period.

Incubation of oxfendazole with ruminal fluid resulted in the appearance of a chromatographic peak which had a retention time exactly similar to that of fenbendazole. The relative concentrations of oxfendazole and fenbendazole are shown in Table 5 (16). This metabolite was not found after incubation with abomasal fluid.

Similarly incubation of albendazole sulfoxide with ruminal fluid but not abomasal fluid resulted in the appearance of a peak on the chromatogram with a retention time exactly similar to that of albendazole. The relative concentrations of albendazole and its sulfoxide are shown in Table 5 (17).

None of the control incubations gave rise to any detectable metabolite formation.

TABLE 5 (16) CONCENTRATION OF OXFENDAZOLE AND FENBENDAZOLE AFTER INCUBATION OF OXFENDAZOLE WITH RUMINAL FLUID

	Concentration µg/ml)	
Time	Oxfendazole	Fenbendazole
Pre-incubation	14.40 (100)	0 (0)
4 hours	14.23 (98.2)	0.26 (1.8)
8 hours	13.97 (97.2)	0.40 (2.8)
24 hours	13.24 (91.2)	1.27 (8.8)

Figures in parenthesis represent concentration as a percentage of total.

TABLE 5 (17) CONCENTRATION OF ALBENDAZOLE SULFOXIDE AND ALBENDAZOLE AFTER INCUBATION OF ALBENDAZOLE SULFOXIDE WITH RUMINAL FLUID

Time	Concentration µg/ml	
	Albendazole sulfoxide	Albendazole
Pre-incubation	14.80 (1))	0 (0)
4 hours	14.61 (100)	0 (0)
8 hours	14.79 (99.9)	0.02 (0.1)
24 hours	14.41 (88.9)	1.80 (11.1)

Figures in parenthesis represent concentration as a percentage of total.

Bovine hepatic microsomes

Incubation of both albendazole and fenbendazole with fresh bovine hepatic microsomes resulted in the production of a metabolite with an exactly similar retention time under HPLC to that of the respective sulfoxide and sulfone. When the extracts were re-injected under the methanol/perchloric acid solvent routinely used to separate these metabolites both metabolites were found to be present.

The relative concentrations of fenbendazole, oxfendazole and sulfone, and of albendazole, sulfoxide and sulfone are shown in Tables 5 (18) and 5 (19).

Sulfoxide incubation with microsome preparations in both cases resulted in formation of small amounts of albendazole and fenbendazole respectively as well as albendazole and fenbendazole sulfones. (Tables 5 (20) and 5 (21)).

There was slight conversion with the boiled microsomal preparations from sulfides to sulfoxides and sulfones, but no apparent reduction of sulfoxides to sulfides. No conversion occurred when the drugs were incubated with buffer + co-factors.

The results show that the in vivo metabolism of the three benzimidazole drugs studied was due to the activity of liver microsomes and not to gastro-intestinal micro-organisms. Metabolism by similar oxidation enzyme systems might also have occurred to some extent in other organs, particularly the lung and kidneys, but almost certainly the contribution of these sites to the overall conversion would be small compared with that of the liver.

Because of the difficulties caused by the aqueous

insolubility of these drugs and also because a detailed study of their metabolism was outwith the main theme of this thesis, no further attempt was made to measure rates of conversion or to identify properly the enzyme system involved.

The apparent reduction of sulfoxide to sulfide was surprising; reduction reactions of this type are uncommon in the liver and, since from these studies it appeared that the oxidation reaction was occurring at a much faster rate than the reduction, it was difficult to understand why the sulfide was detectable at all. The sulfide was not formed during the extraction procedure as was evidenced by failure to detect sulfide in those incubations with all the components present, with the exception of microsomes. The sulfide metabolite was identified purely on the basis of its chromatographic characteristics under two HPLC solvent systems, and therefore could not be regarded as beyond dispute.

The occurrence of both oxidation and reduction reactions simultaneously was confirmed, however, by a subsecuent report describing the incubation of both fenbendazole and albendazole with mouse liver enzymes (Douch and Buchanan, 1979). These authors found sulfoxide formation and sulfoxide reduction to occur in the microsomal fraction of mouse liver with both fenbendazole and albendazole and determined the optimal conditions for each enzyme activity. Non-emzymic oxidation of fenbendazole, but not albendazole, was also reported; this non-enzymic oxidation may explain the sulfoxide formation found in this study during incubation with boiled microsomes. This had previously been thought to be due to failure to denature

completely the microsomal enzymes by the boiling procedure. (The 'boiled' samples were placed in test tubes in a boiling water bath and in the 10 minute boiling period may not have attained 100^{o}C). A further demonstration of the reversibility of the sulfide-sulfoxide reaction has recently been found with the unrelated drug sulindac, a non-steroidal anti-inflammatory drug (Duggan, Hooke and Hwang, 1980). Irreversible oxidation of sulfoxide to sulfone also occurs. When different radio-active labels were attached to sulfoxide (^{14}C-label) and sulfide (^{3}H-label), and both drugs administered simultaneously by intravenous injection to rats and guinea pigs, these authors found an extremely rapid equilibration of both labels among the three forms of the drug.

TABLE 5 (18) CONCENTRATION OF FENBENDAZOLE, OXFENDAZOLE AND SULFONE AFTER INCUBATION OF FENBENDAZOLE WITH BOVINE HEPATIC MICROSOMES

	Concentration (µg/ml)					
	Fresh microsomes			Boiled microsomes		
Time	Fenbendazole	Oxfendazole	Sulfone	Fenbendazole	Oxfendazole	Sulfone
3 hours	10.76(76.9)	2.95(21.1)	0.28(2.0)	19.76(97.1)	0.60(2.9)	0
6 hours	10.37(74.1)	3.57(25.5)	0.34(2.4)	18.29(99.3)	0.13(0.7)	0

Figures in parenthesis represent concentration as a percentage of total.

TABLE 5 (19) CONCENTRATION OF ALBENDAZOLE, ALBENDAZOLE SULFOXIDE AND ALBENDAZOLE SULFONE AFTER INCUBATION OF ALBENDAZOLE WITH BOVINE HEPATIC MICROSOMES

	Concentration (µg/ml)					
	Fresh microsomes			Boiled microsomes		
Time	Albendazole	Sulfoxide	Sulfone	Albendazole	Sulfoxide	Sulfone
3 hours	29.38(44.4)	33.55(50.7)	3.20(4.6)	27.05(98.4)	0.45(1.6)	0
6 hours	25.74(41.2)	33.55(53.7)	3.20(5.1)	25.04(98.7)	0.32(1.3)	0

Figures in parenthesis represent concentration as a percentage of total.

TABLE 5 (20) CONCENTRATION OF FENBENDAZOLE, OXFENDAZOLE AND SULFONE AFTER INCUBATION OF OXFENDAZOLE WITH BOVINE HEPATIC MICROSOMES

	Concentration (µg/ml)					
	Fresh microsomes			Boiled microsomes		
Time	Fenbendazole	Oxfendazole	Sulfone	Fenbendazole	Oxfendazole	Sulfone
3 hours	1.67(4.2)	36.66(92.1)	1.48(3.7)	0	23.05(96.6)	0.8(3.4)
6 hours	0.51(1.27)	38.01(94.7)	1.60(4.0)	0	25.20(96.9)	0.8(3.1)

Figures in parenthesis represent concentration as percentage of total.

TABLE 5 (21) CONCENTRATION OF ALBENDAZOLE, ALBENDAZOLE SULFOXIDE AND ALBENDAZOLE SULFONE AFTER INCUBATION OF ALBENDAZOLE SULFOXIDE WITH BOVINE HEPATIC MICROSOMES

	Concentration (µg/ml)					
	Fresh microsomes			Boiled microsomes		
Time	Albendazole	Sulfoxide	Sulfone	Albendazole	Sulfoxide	Sulfone
3 hours	0.16(5.6)	2.61(90.9)	0.10(.35)	NS	NS	NS
6 hours	0.04(1.5)	2.47(95.4)	0.08(3.1)	0	1.69(100)	0

Figures in parenthesis represent concentration as a percentage of total.

THE EFFICACY OF ALBENDAZOLE AND ITS TWO MAJOR METABOLITES AGAINST *NIPPOSTRONGYLUS ERAZILIENSIS* IN THE RAT

The aim of this experiment was to compare the anthelmintic activity of albendazole sulfoxide and albendazole sulfone with that of albendazole itself. The three compounds were administered in feed to rats experimentally infected with third stage larvae of *Nippostrongylus braziliensis* and the worm burdens compared at slaughter.

Since *in vivo* metabolism of albendazole to the sulfoxide and sulfone might be expected to occur in rats as in sheep, making it impossible to assess the anthelmintic activity of albendazole *per se*, it was decided also, in one group of rats, to inhibit this metabolism by the use of a hepatic microsomal enzyme inhibitor. The compound chosen for this purpose was piperonyl butoxide which has been shown to inhibit hepatic microsomal drug metabolism *in vivo* and *in vitro* (Anders, 1968).

MATERIALS AND METHODS

Thirty male rats (hooded Wistar strain) weighing between 160 and 210 g were each inoculated subcutaneously with 4,000 third stage *N. braziliensis* larvae on day 0. The rats were randomly divided into five groups of six rats. The groups were treated as follows:

Group 1) - Control group, fed normal ration.

Group 2) - Treated with albendazole in feed at 100 ppm from day 6 to day 9.

Group 3) - Treated with albendazole sulfoxide in feed at 100 ppm from day 6 to day 9.

Group 4) - Treated with albendazole sulfone in feed at 100 ppm from day 6 to day 9.

Group 5) - Treated with albendazole in feed at 100 ppm from day 6 to day 10, and were injected intra-peritoneally with piperonyl butoxide 500 mg/kg at 12 hour intervals beginning 2 hours before the medicated feed was offered.

All the rats were killed on day 10. Blood was taken into heparinised syringes at killing; the plasma was separated and frozen (-20^{o}C). Analysis for albendazole and its metabolites was performed later.

Preparation of the feed

Commercial rat pelleted diet was ground to a fine powder in an automatic grinder. 0.04 g of pure test compound was accurately weighed and well mixed with a small quantity of ground feed. Sufficient feed was added to bring the total weight to 400 g,

and the feed mixed thoroughly. Sufficient water was added to form a damp mash and the mixture was re-pelleted using a 5 ml syringe. The pellets were dried on top of a warm oven and stored at -20°C until required.

It would seem reasonable to assume that the heavier rats in each group would have a greater food intake than the smaller ones, and would therefore receive a proportionately greater amount of drug. The average dose then would be 6.67 mg per rat (33 mg/kg).

Estimation of worm burden

The counts of the worm burden of the rats were kindly undertaken by Mr. K. Bairden of the Department of Veterinary Parasitology.

RESULTS

When the experiment was designed it was calculated that the medicated feed would be sufficient feed from day 6 to killing on day 10. However groups 2, 3 and 4 had consumed all the medicated feed by the beginning of day 9, and normal ration was then fed. By day 8 after infection it was apparent that the rats receiving the piperonyl butoxide injections had a lower feed intake than the non-injected groups, and also were diarrhoeic. Because of this the injections were discontinued after the morning of day 8. Partial recovery of appetite occurred but these rats consumed only 157 g of feed over 4 days compared with 400 g over 3 days taken by the other treated groups (mean dose 12.6 mg/kg or 3.2 mg/kg/day for 4 days compared with 33 mg/kg or 11 mg/kg/day for 3 days).

The worm burden of each rat, the group mean and the percentage reduction compared with controls are shown in Table 5 (22).

The results demonstrate that albendazole sulfoxide has similar anthelmintic activity to albendazole and that albendazole sulfone has little or no anthelmintic activity. The low doses received by the liver enzyme inhibited group, together with the wide variation in worm burden within the group, makes it impossible to draw any conclusions as to whether metabolism of albendazole to the sulfoxide is necessary for good anthelmintic activity.

The volume of plasma obtained per rat varied from 0.5 ml to 4 ml. The majority, however, yielded >3 ml.

No albendazole or its sulfoxide or sulfone was detected in any of the samples. This was probably due to the fact that 30 hours elapsed from the ingestion of the last of the drug until killing, except in the case of Group 5 which had a very low feed intake at this time.

In order to try and overcome the problems encountered with the repeated injection of piperonyl butoxide this part of the experiment was repeated using chloramphenicol as the microsomal enzyme inhibitor.

The effect of a single oral dose of 33.3 mg/kg albendazole was also studied.

MATERIALS AND METHODS

Twenty-five rats were infected and divided into four groups of six as in the previous experiment. The groups were treated as follows:

TABLE 5 (22) RAT WORM BURDENS, GROUP MEAN AND REDUCTION AS PERCENTAGE OF CONTROLS AFTER TREATMENT

Treatment	Rat worm burden 1	2	3	4	5	6	Mean	%age Reduction
None-controls	1650	950	1190	1790	780	1660	1337	
Albendazole 100ppm	0	0	0	0	0	0	0	100
Sulfoxide 100ppm	10	0	0	0	0	10	3	98.8
Sulfone 100ppm	1190	1740	1430	1630	1480	1020	1415	0
Albendazole 100ppm + piperonyl butoxide	0	1820	70	1880	2360	30	1026	23.3

Group 1) - Controls, fed normal ration.

Group 2) - Albendazole in feed at 100 ppm from day 6 to day 9.

Group 3) - Albendazole at 100 ppm + chloramphenicol at 360 ppm in feed from day 6 to day 9. This group received feed containing chloramphenicol only for 12 hours preceding that incorporating albendazole.

Group 4) - A single oral dose of albendazole (33 mg/kg) given to each rat.

The preparation of feed, infection and killing of rats and counting of the worms were all undertaken as in the previous experiment. The single dose was administered as pure drug in a gelatin capsule, and the rat held until the drug was swallowed. Plasma samples were not taken.

RESULTS

The results are shown in Table 5 (23).

The poor larval 'take' with resultant low worm burdens in the control animals make the results difficult to interpret. It can be seen, however, that five out of six rats given the microsomal enzyme inhibitor had some adult worms at killing whereas albendazole was 100% effective in the non-inhibited group. It can also be seen that the divided dose was more efficacious than a single oral dose of the drug.

TABLE 5 (23) RAT WORM BURDENS, GROUP MEAN AND REDUCTION AS PERCENTAGE OF CONTROLS AFTER TREATMENT

Treatment	Rat worm burden 1	2	3	4	5	6	Mean	%age Reduction
None-controls	310	610	730	440	90	450	438	
Albendazole 100ppm	0	0	0	0	0	0	0	100
Albendazole 100ppm + chloramphenicol 360ppm	10	0	20	10	20	10	12	97.3
Single oral dose 33 mg/kg	30	0	40	0	0	20	15	96.6

One further experiment was undertaken in rats, namely the administration of a single oral dose of albendazole, with sequential blood samples to establish whether absorption and metabolism of albendazole in rats was similar to that seen in sheep.

MATERIALS AND METHODS

Twelve adult rats were weighed and dosed with Valbazen 2.5% suspension at a dose rate of 33 mg/kg (0.13 ml per 100 g). At times 1, 4, 8 and 24 hours post dosing groups of three rats were killed, and bled. (These times were chosen after examination of the results of an earlier study in another monogastric animal, the dog (unpublished), in which it was found that maximum plasma concentrations of albendazole and its metabolites were achieved 2 hours after oral administration of albendazole). The plasma from the three rats was pooled and assayed for albendazole and its metabolites.

RESULTS

The plasma concentrations of albendazole, sulfoxide and sulfone are as shown in Table 5 (24). These results demonstrate that the metabolic pathway in rats in similar to that in sheep. One interesting difference is that plasma concentrations of albendazole itself are higher in rats than in sheep.

TABLE 5 (24) MEAN PLASMA CONCENTRATION (µg/ml) OF ALBENDAZOLE, ALBENDAZOLE SULFOXIDE AND ALBENDAZOLE SULFONE IN 3 RATS GIVEN ALBENDAZOLE AT A DOSE RATE OF 33 mg/kg

Time of killing	Albendazole (µg/ml)	Albendazole sulfoxide (µg/ml)	Albendazole sulfone (µg/ml)
1 hour	0.31	0.53	0.54
4 hours	0.42	2.53	0.70
8 hours	0.16	1.69	0.73
24 hours	0.01	0	0

DISCUSSION

One of the most striking features of the kinetics of the three benzimidazole drugs studied is the length of time for which they and their metabolites were detected both in plasma and in the gut after a single oral dose. Oxfendazole, for example, was present in abomasal fluid for 9 days and in plasma for 7 days in one animal after oral administration of the drug.

If duration of activity is important in determining the efficacy of these drugs, then the rumen may play a crucial role in explaining the greater potency and efficacy seen in ruminant animals compared with monogastric animals. The studies in rats, and also work in the dog (unpublished) demonstrate that plasma concentrations of albendazole and its metabolites in these species are relatively very low by 24 hours after administration of doses 2-3 times greater than those given to the sheep in these experiments.

Concentrations of the three benzimidazoles and their metabolites in plasma and gastro-intestinal fluid varied greatly in individual animals and this was especially true of ruminal fluid concentrations. Dissolved ruminal fluid concentrations of oxfendazole were markedly lower than those of albendazole or fenbendazole, perhaps reflecting a lower aqueous solubility of oxfendazole.

One obvious feature of all three drugs is that the concentrations in ruminal fluid did not approach the theoretical maximum (circa 50 µg/ml) expected if the total dose was delivered to the rumen and dissolved. It is almost certain that the slow kinetics of these drugs demonstrated in this study are a result of their extremely low solubility in aqueous fluids. Even albendazole

which, of the three drugs investigated, had the most rapid kinetics, persisted in ruminal fluid for 3 days, confirming the view that the rumen acts as a "reservoir" of slowly dissolving drug which then becomes available for absorption. No data are available on the solubility of these three drugs. The closely related benzimidazole, mebendazole, has a quoted solubility of <50 µg/ml in water (Kelly, Chevis and Goodman, 1975).

Since abomasal fluid concentrations of these drugs, particularly oxfendazole and albendazole, were higher than those of ruminal fluid at the equivalent time, it is probable that some undissolved drug reached the abomasum. The lower pH of this part of the gut would be expected to result in higher dissolved concentrations of these basic drugs. Passive diffusion of benzimidazole from the systemic compartment in theory may also have contributed to the higher dissolved abomasal fluid concentrations. However the low plasma albendazole concentrations seen in all animals make this mechanism unlikely to be significant for this drug. Careful examination of the results also reveals that the ratio of concentration of oxfendazole in abomasal fluid to that in plasma after fenbendazole administration (where abomasal fluid concentrations must have resulted entirely from passive diffusion from the plasma) was much lower than that seen after administration of oxfendazole itself.

Dissolved drug may have passed on from the rumen during the process of normal rumen emptying or may have been deposited in the omasum by the action of the reticular groove during dosing, and

subsequently reached the abomasum. On each occasion when sheep were dosed, irrespective of which drug or formulation was given, all animals were found to have drug in the abomasum by 10 minutes after oral administration. The concentrations found were highly variable and their significance is difficult to assess. It was not always the case that animals which had high concentrations of drug in the abomasal fluid at 10 minutes had lower or less persistent plasma or abomasal fluid concentrations of drug or metabolites.

The current view that the rumen provides a slow release reservoir of benzimidazole has led to the suggestion that ruminal by-pass of these drugs via the reticular groove at dosing may be one reason for the occasional anthelmintic failures which have been reported (Lancaster and Hong, 1977).

These results do not actively support this hypothesis. In this small number of animals rumen by-pass after oral administration occurred,but to a small extent. The delivery of fenbendazole directly into the abomasum did, by contrast, result in markedly lower plasma concentrations of both fenbendazole and its metabolites. However, as discussed earlier in Chapter 3, the experimental administration of drugs into the abomasum via a cannula is probably not a good model for studying the fate of drugs diverted there via the reticular groove.

The metabolic route of the two sulfide drugs, fenbendazole and albendazole, to a sulfoxide and a sulfone appears to be similar. It is almost certain that there are several other metabolites, but careful comparison of the results presented here with studies using

^{14}C labelled benzimidazole leads to the conclusion that the greater part of the metabolites has been accounted for. Smith Kline have also confirmed that the major metabolites of albendazole in sheep (and in most other species) are those measured in this study (Theodorides - personal communication).

The in vitro investigation of the site of benzimidazole metabolism demonstrated clearly that no oxidation of fenbendazole or albendazole to their respective sulfoxides or sulfones occurred in either ruminal fluid or abomasal fluid. Substantial metabolism, however, occurred on incubation with hepatic microsomal enzymes. This confirms the site of metabolism as being the liver, and is in accordance with the sheep studies in vivo, where the presence of sulfoxides was not confirmed in ruminal fluid. Although sulfoxide may have been present in low concentrations in ruminal fluid, below the limit of detection of the method, it was certainly not present in the concentrations one would expect if the rumen was the site of metabolism.

There are differences in the rate and extent of metabolism of fenbendazole and albendazole. The virtual absence of albendazole from the plasma of sheep after dosing suggests an extremely rapid and almost total conversion of the parent drug. Fenbendazole was present in plasma for a similar period as the sulfoxide, suggesting a slower rate of metabolism.

This was borne out by the results of the in vitro incubations with bovine hepatic microsomal enzymes when both drugs were incubated with microsomes from the same batch, the conversion

of albendazole to its sulfoxide was greater and more rapid than that of fenbendazole.

Plasma concentrations of albendazole sulfoxide after albendazole administration, and of oxfendazole after fenbendazole administration, were in both cases much greater than those of the parent drug. Sulfone concentrations were substantially lower than those of sulfoxide for most of the time for which drug was present. Only in the later samples, 48 hours and after, did sulfone concentrations approximate to those of sulfoxide.

In the abomasum also high concentrations of sulfoxides were found; in the case of albendazole these were also higher than those of parent drug. Since metabolite formation was outwith the gastro-intestinal tract, passive diffusion across the wall of the abomasum must have been the mechanism involved. Since equivalent concentrations were not found in ruminal fluid, secretion via saliva is unlikely to have been implicated; and as benzimidazoles are basic drugs they are probably not secreted in saliva (pH 8.2) to any great extent.

It is unfortunate that the problems encountered in the analysis of rumen samples were never overcome sufficiently to be able to detect the low concentrations of metabolites which could be accurately measured in plasma and abomasal fluid. The only conclusions to be drawn are that sulfoxides did not reach concentrations greater than 0.2 µg/ml and sulfones not greater than 0.05 µg/ml. It is not possible to state categorically that diffusion of low amounts of sulfoxides back into the rumen from the

systemic compartment did not occur, but certainly the diffusion is relatively unimportant.

Passive diffusion of the sulfone metabolites into abomasal fluid was noted with both fenbendazole and albendazole, although to a lesser extent than the sulfoxides; the ratio of sulfoxide to sulfone concentrations in abomasal fluid was greater than that seen in plasma.

The appearance of low concentrations of fenbendazole in the plasma of sheep dosed with oxfendazole was at first thought to arise from the reduction of oxfendazole by ruminal micro-organisms which was seen during the in vitro incubation of rumen fluid with the drug. When oxfendazole was later incubated with bovine hepatic microsomes, however, there also appeared to be some conversion to fenbendazole. This rather surprising finding was confirmed by the subsequent publication by Douch and Buchanan (1979) who had found the same reaction to occur in mouse liver microsomes. The relative contributions of liver and ruminal reductions of oxfendazole to the measured concentration of fenbendazole in plasma cannot be determined from the limited results presented here.

Albendazole sulfoxide was also metabolised to its parent drug during in vitro incubation with ruminal fluid. It is not known whether albendazole is present in the plasma of animals dosed with the sulfoxide, but in view of the apparently rapid conversion of albendazole to its sulfoxide seen in vivo and in vitro this is thought to be extremely unlikely. Incubation of albendazole sulfoxide with bovine hepatic microsomal enzymes similarly yielded

albendazole. It is likely that the apparent difference in the overall rates of conversion of albendazole and fenbendazole to their respective metabolites are due to differences in the relative rates of the oxidation/reduction reactions.

Species differences in rates of reaction may also explain the higher plasma concentrations of albendazole found in rats compared with those in sheep during the present study. Alternatively this may have been due to faster absorption of albendazole in this species.

The kinetics of oxfendazole when given to sheep as oxfendazole were similar to those of oxfendazole formed metabolically from fenbendazole. Oxfendazole concentrations in plasma after administration of fenbendazole were between 30% and 50% of those found after administration of oxfendazole itself at the same dose rate and in the same six sheep.

The ratio of the concentration of oxfendazole to sulfone in plasma was similar after administration of either drug, suggesting that fenbendazole was converted to sulfone only via the sulfoxide and not directly.

The question as to which of these drugs or metabolites, if any, is anthelmintically active is of great interest. The virtual absence of circulating albendazole in sheep plasma together with its known activity against lungworm suggests that one or more of the metabolites may be the agent active in the lungs. Averkin, Beard, Dvorak, Edwards, Fried, Kilian, Schiltz, Kistner, Drudge, Lyons, Sharp ar Corwin (1975) have shown the sulfoxide of the analogous drug fenbendazole

to be more potent than fenbendazole itself; and numerous parasitological trials have proved the efficacy of oxfendazole in ruminants.

The results of the studies performed here in rats show that albendazole sulfoxide is as anthelmintically active as albendazole itself. Unfortunately the attempts at inhibiting the in vivo metabolism of albendazole were unsuccessful. In the first trial the repeated injection of piperonyl butoxide resulted in poor and variable intake of the drug. In the second trial the larval 'take' as evidenced by low adult worm burdens in the control animals was probably insufficient to be able to draw any valid conclusions as to the relative activities of the two compounds. The sulfone of albendazole was found to be non-active and this is in agreement with the study by Averkin et al (1975) in which the sulfone of fenbendazole was reported to be poorly active.

Consideration of the relative concentrations of parent drug and metabolites found in vivo suggest that much of the anthelmintic activity is related to the sulfoxides. In both plasma and abomasal fluid albendazole sulfoxide was quantitatively more important than the parent drug, and in plasma although not in abomasal fluid, oxfendazole was present in higher concentrations than fenbendazole.

The possibility exists that neither the sulfide nor the sulfoxide is the 'active' metabolite. There is increasing evidence that many of the metabolites which are cytotoxic are highly reactive intermediates such as epoxides or free radicals with very

short half-lives and it has been suggested that hydroxylation of oxfendazole occurs via an epoxide intermediate (Bell and Tomlinson, 1976). Should the 'active' metabolite of the benzimidazole drugs prove to be such a highly reactive intermediate it will probably be formed by the helminth locally since it is unlikely that the required concentrations would be found at the many diverse predilection sites of parasites known to be killed by the benzimidazoles. Oxidation of fenbendazole to oxfendazole, and of albendazole to its sulfoxide and, in this case, sulfone has been shown to occur during incubation of the parent drugs with nematodal and cestodal enzymes (Douch and Buchanan, 1979). It is interesting that the same authors report that sulfoxidases of helminths have some properties similar to those of guinea pig liver (a requirement of NADPH and oxygen), whereas sulfoxide reductases of helminths are unlike those of mammalian systems. It is possible that the remarkable selectivity of benzimidazoles for helminths may be related to differing metabolic routes possibly involving different intermediates. Although the sulfides or sulfoxides may not prove to be the compounds ultimately toxic to the helminths, it is nevertheless clear that they will be associated with concentrations of such a metabolite and that kinetic studies such as these would still be appropriate.

CHAPTER 6

GENERAL DISCUSSION

The major part of this study became, in effect, a detailed investigation of the pharmacokinetics of the more recent benzimidazole anthelmintics, drugs which are unusual in both their physical properties and pharmcokinetic behaviour; however there are a few general observations to be made from the results as a whole. One of the original aims of the project was to assess the importance of the ruminal epithelium as a possible site of drug absorption in the normal animal. The evidence from the available literature suggested that absorption from the rumen could occur by passive diffusion, but none of the experimental models used allowed any conclusions to be drawn as to the extent of this process under the normal conditions of the ruminant gut.

The evidence presented in this thesis is conflicting; the meclofenamate experiments, particularly the tied-off rumen study, demonstrated that no significant absorption of this drug occurred from the rumen, although since meclofenamic acid is an acidic drug, in the acidic environment of the rumen conditions would have been thought to be favourable for absorption. By contrast, levamisole administered intra-ruminally was detected in plasma before it was detected in abomasal fluid, suggesting that some absorption occurred prior to drug reaching the abomasum/small intestine. This is rather surprising as being a basic drug levamisole would have been expected to be less likely to be absorbed at this site than meclofenamate.

One possible explanation for the failure of absorption of meclofenamic acid is that at the pH of rumen contents (approximately 6.6), the degree of ionization of the drug is so high (>99.8%) that the

large ratio of ionized drug:unionized drug limits the rate of absorption. The lowest pk_a value for a weak acid which is compatible with rapid absorption from the small intestine (the contents of which also have a pH of around 6.6) is pk_a 3 (Hogben et al, 1959). For acids with a lower pk_a value, the degree of dissociation appears to be the limiting factor in their rate of absorption. However, if there is a more acidic zone (pH 5.3) at the site of absorption from the intestine, as has been suggested (Hogben et al, (1959), and if no such acidic zone exists in the rumen, then the pk_a values at which the degree of dissociation becomes limiting for absorption will be different for these two parts of the gut.

With an absorbing pH of 5.3 the maximum ratio of ionized: unionized drug for both acids and bases which is compatible with rapid absorption appears to be 200:1. For meclofenamic acid (pk_a 3.76) in ruminal fluid the ratio of ionized:unionized drug would be almost 700:1, which is well outside this limit. For levamisole by contrast, although it is a weak base, the ratio of ionized:unionized drug would be approximately 25:1.

All the results presented, however, are in agreement with the hypothesis that absorption from the rumen, if it occurs, is greatly slower than that from the lower parts of the gastro-intestinal tract. With all of the drugs studied, plasma concentrations rose more rapidly following oral or intra-abomasal administration than after intra-ruminal administration.

This rapid rise in plasma concentrations of drug after oral administration compared with intra-ruminal administration is almost

certainly due to a proportion of orally administered drug being diverted directly to the abomasum/small intestine by the action of the reticular groove. With all the drugs studied, on almost every occasion on which oral administration was made to any of the sheep, drug was detected in the abomasal fluid by 10 minutes after administration. In sheep with duodenal as well as abomasal cannulae, it was found that drug by-passing the rumen via the groove also reached the small intestine. This is in agreement with the findings of earlier workers who used dye administration and slaughter methods to investigate reticular groove function (Clunies Ross, 1931).

The extent of ruminal by-pass was, on the evidence of abomasal fluid concentrations, extremely variable and apparently not consistent for any individual sheep; nor did it appear to increase with repeated administration. Since the closure of the groove has been shown to be due to a conditioned reflex associated with the desire to take milk in the suckling animal, and as such diminishes as the animal assumes adult feeding babits (Ørskov et al, 1970) it would not be expected that the groove would function efficiently during forced drenching. These experimental findings probably approach the field situation for anthelmintic drugs, in which animals are drenched at intervals, and it can be concluded that in these animals also, a variable, usually small, proportion of the drench is probably diverted by the groove. The importance of groove function in relation to loss of anthelmintic efficiency of the benzimidazole drugs has therefore been over-emphasized. The results presented in this thesis demonstrate very clearly that administration of drugs into the abomasum via a cannula does not mimic

the natural functioning of the reticular groove during dosing. This is probably due to deposition of drug in the omasum during closure of the groove. This organ has received little attention as regards its effect on orally administered drugs but it is likely that insoluble drugs especially may be deposited in the laminar folds and provide a further reservoir of drug supplying the abomasum/small intestine. Similarly, differences between the kinetics of drugs following the reticular groove and those administered by the technique of intra-abomasal injection are likely to occur, and the limitations of such studies should be recognized.

The concept of the rumen providing a 'reservoir' of drug is well supported by the findings of this study. Even drugs administered as solutions were generally still detectable in ruminal fluid by 22-24 hours after oral or intra-ruminal administration, and the highly insoluble drugs remained for several days. If the overall effect of the rumen on orally administered drugs is one of dilution and slow delivery of drug to the distal parts of the gastro-intestinal tract, it is probable that any condition causing depression of ruminal motility will also affect the rate of absorption and therefore possible therapeutic effect.

One recurrent finding throughout this study was the large individual variation in drug concentrations in plasma, and in the various gut compartments after a similar dose. A general observation was that animals which were in poor bodily condition had much higher gut and plasma concentrations than those in good condition. This was presumably a result of the small volume of rumen contents associated

with low feed intake in these animals. The relatively lower proportion of muscle and fat tissue would account for the higher plasma concentrations, since redistribution of drug in plasma into muscle and fat is for many drugs an important influence on plasma concentrations.

This large variation seen in individual animals has obvious implications in that an effective dose in one animal will be ineffective in another and an overdose in yet another. This is probably of greater importance for drugs administered orally for a systemic effect, than those aimed at the gastro-intestinal tract, where minimum effective concentrations for most drugs are probably more readily achieved by this route.

Another aspect of ruminant pharmacology which was of interest in this project was that of passive diffusion of drugs from the systemic compartment into the rumen, and their possible accumulation there by the process of ion trapping. There are two possible routes by which drug present in plasma can reach the rumen; either by diffusion across the rumen wall, or by diffusion into saliva which is subsequently swallowed into the rumen. For drugs which diffuse readily into saliva, the amounts reaching the rumen in this way could theroetically be significant when it is considered that the volume of saliva produced per day in sheep can be up to six times the plasma volume. Drugs which are unaffected by passage through the rumen will be available for re-absorption from the gut; any that are susceptible to microbial degradation will not, and for such drugs salivary excretion may be an important route of elimination.

Levamisole and meclofenamate were detected in rumen fluid

after subcutaneous and intra-abomasal administration respectively. It would be expected that meclofenamate being an acidic drug would be more likely to reach the rumen via accumulation in the alkaline (pH 8.2) saliva, and that levamisole being a weak base would be trapped in ruminal fluid by diffusion acorss the rumen wall. However it is possible that the predicted high ratio of ionized to unionized meclofenamate in plasma would prevent ready diffusion of the drug into saliva, and it is also possible that levamisole is secreted in saliva to a certain extent since, although the equilibrium ratio would be 3:1 in favour of plasma, the constant secretion of saliva would ensure that equilibrium would not be achieved.

Unfortunately, since salivary excretion of drugs was not measured during this study, it is impossible to assess the relative contributions of the two possible routes by which these drugs entered the rumen after intra-abomasal or subcutaneous administration. It is interesting that ruminal fluid concentrations of levamisole in this situation did not nearly approach the theoretical equilibrium distribution concentrations (calculated ratio approximately 5:1 in favour of ruminal fluid). Concentrations of meclofenamate in the rumen did achieve the expected ratio, but only in a very few samples, and in some animals the drug was not detectable in ruminal fluid for much of the sampling period.

Accumulation in the rumen was not a feature of the benzimidazole drugs; neither intra-abomasally administered fenbendazole nor any of the systemically formed metabolites reached detectable concentrations in this compartment. In the abomasum, by contrast,

sulfoxides attained concentrations of up to ten times those present in plasma, and albendazole sulfone and subcutaneously administered levamisole were also found to be concentrated there, presumably due to ionization of these drugs in the acidic environment.

The question of accumulation of systemically formed compounds and parenterally administered drugs in the abomasum is of interest, particularly with regard to anthelmintics administered for the treatment of gastro-intestinal helminthiasis. It is generally assumed that the important concentration for such an anthelmintic is that achieved in the gut. However, larvae buried within gastric glands may well be more accessible to anthelmintic in plasma than to drug in the lumen of the gut. Even adult worms have a fairly close association with the mucosal surface which, particularly in the small intestine, has a very extensive capillary network closely underlying the surface epithelium, and it is possible that even if these drugs did not accumulate in the gut fluid they would still be anthelmintically effective. In heavy parasitic infection of the abomasum, notably that of ostertagia spp, the pH of abomasal fluid may rise considerably, and it would be expected that the concentrations of systemically administered or metabolically formed drug found in the abomasal fluid of such animals would be lower than those found in the experimental animals.

Minimum effective concentrations of anthelmintics are difficult to establish in vivo, and parasites may have different susceptibilities to anthelmintic during in vitro incubations from those found for the same worm in the natural environment of the host. However

a log dose response relationship has been shown for the efficacy of levamisole against adult N. braziliensis in vitro (Coles et al, 1975). These authors found inactivation of 100% of the worms after three hours of incubation with levamisole at a concentration of 1 µg/ml, and after six hours of incubation with 0.5 µg/ml. These concentrations are of the same order as those achieved in sheep plasma during the present study with levamisole administered by all three routes, at the normal therapeutic dose. The abomasal fluid concentrations were several times greater than this, even after subcutaneous administration of the drug. Coles et al (1975) also found recovery of motility of adult worms on continued incubation with the drug, and it would appear that prolonged exposure of the parasites to the drug would not increase its efficacy indefinitely. Should the parasites not be expelled by the host during the paralysed phase, clinical cure might well not be achieved.

Minimum effective concentrations of the benzimidazole drugs are, for several reasons, even more difficult to establish. Their mode of action seems to be via their effects on the microtubular system of the parasite cell, deterioration of which causes blockage of the movement of secretory granules and impaired digestion and absorption of nutrients. This sequence of events would be expected to occur over a period of time and the stage at which the changes become irreversible is unknown. Ultrastructural changes in intestinal cells of Ascaris suum have been demonstrated after 6-9 hours of exposure to mebendazole (Borgers et al, 1975). Significant inhibition of glucose uptake by the same parasite has been found in vitro after

incubation for 24 hours with 0.05 µg/ml of mebendazole (Van den Bossche and De Nollin, 1973).

The results of the present investigation make determination of the minimum effective concentration even more complex. Assuming that the more recent benzimidazoles have a similar mode of action to that of mebendazole, the findings suggest that it is not unchanged benzimidazole but a sulfoxide or some further intermediate metabolite which binds to the nematode tubulin. If an intermediate metabolite is involved, it is likely to be formed by the helminth itself, possibly using mammalian-formed sulfoxide as the substrate. In this case the availability of substrate and the rate of its metabolism by the helminth will determine the efficacy of the drug.

No recovery of worms incubated with mebendazole has been found, although development of resistance over several generations of parasites is a well established phenomenon with benzimidazole drugs, and cross resistance between members of the group also occurs (Hogarth-Scott, Kelly, Whitlock, Thompson, James and Mears, 1976).

The failure of most anthelmintics to kill inhibited larval stages of nematodes has long been a stumbling block in the control of parasitic infection of cattle. The reason for their resistance to anthelmintic treatment is thought to be the extremely low metabolic rate of the larvae, with resultant low drug/parasite interaction. The greater duration of exposure of these parasites to the more recent benzimidazole anthelmintics, even after a single oral dose of the drugs, is the probable explanation of their greater efficacy against these inhibited larvae. Prolonged administration

of these drugs by the use of feedblock preparations or slow release rumen bullets will probably increase the larval kill even further. Why inhibited larval stages of sheep nematodes are apparently much more susceptible to anthelmintics, even thiabendazole and levamisole, than those of cattle is not known.

The differences in kinetics and modes of action of the two major groups of anthelmintics are also responsible for their differing toxicities in the host species. Levamisole acts on mammalian ganglia as it does on those of the helminth, and its therapeutic index is relatively low. Mebendazole is reported to have no effect on the microtubular system of the host cell (Borgers, De Nollin, Verheyen, De Brabander and Thienpont, 1975), although a related drug methyl(5-2-thienyl-carbonyl-H benzimidazol-zyl) carbamate with antitumoural activity appears to act by interfering with microtubules in mammalian cells (De Brabander, Van de Veire, Aerts, Gevens, Borgers, Desplenter and De Crée, 1975). It is possible that the extremely low toxicity of these compounds is entirely due to their kinetics. Because of their limited solubility, gastro-intestinal fluid will saturate with dissolved drug. Thus further increases in oral dosage will not result in increased absorption and toxicity. An LD_{50} is not measurable for fenbendazole and is high for albendazole, 100 mg/kg in sheep and >1320 mg/kg in rats. This may well be a result of the higher plasma concentrations of albendazole metabolites compared with those of fenbendazole and its metabolites, as found in this study. The lower toxicity in monogastric animals is also in agreement with this theory, lower concentrations of albendazole + metabolites being found in rats

and in dogs (unpublished) than in sheep.

Some of the benzimidazole drugs are much more toxic to the developing embryo. Cambendazole, parbendazole, mebendazole, albendazole and oxfendazole have all been shown to be teratogenic at doses far below the maximum tolerated dose in the adult (Delatour, 1980). Usually the period of risk is quite limited. Interestingly, fenbendazole has not been found to have teratogenic effects although its major metabolite oxfendazole has, possibly the rate and degree of absorption of fenbendazole is insufficient to produce embryotoxic concentrations of oxfendazole.

The ideal approach to chemotherapy of both bacterial and parasitic infections is the synthesis of compounds which are selectively toxic to some structure or physiological mechanism possessed by the foreign agent and not the host, and this has long been recognized. Once such a compound is synthesized it must also reach an effective concentration at its site of action. Studies such as this serve to emphasize the complexity of the processes involved in achieving this apparently simple goal in the ruminant species.

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