STUDIES ON EQUINE INTESTINAL PERMEABILITY TO ^{51}Cr-EDTA

by

JUAN ESCALA L.M.C.V., M.R.C.V.S.

Thesis submitted for the degree of Master of Veterinary Medicine in the Faculty of Veterinary Medicine, University of Glasgow

Department of Veterinary Medicine
University of Glasgow
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SUMMARY.

The aim of the studies reported in this thesis was to evaluate the suitability of ^{51}Chromium-labelled ethylenediaminetetraacetate (^{51}Cr-EDTA) as a marker for the assessment of intestinal permeability in the horse.

Initial studies were performed in apparently healthy ponies. The percentages of orally administered ^{51}Cr-EDTA measured in urine were found to be consistent and not significantly affected by alterations on intestinal motility following premedication with atropine or bethanecol.

Absorption of a proportion of ^{51}Cr-EDTA from the large intestine was confirmed following intracaecal administration of test doses in two animals with permanent caecal fistulation.

The ^{51}Cr-EDTA test was used to investigate alterations in intestinal permeability in ponies following induction of enteropathy by experimental cyathostome infection and by prolonged phenylbutazone administration. Alterations of intestinal permeability were detected in cyathostome-infected animals but similar changes were not seen following phenylbutazone administration. To some extent these results confirm the reliability of the test since parasitic typhlitis/colitis was detected at post-mortem examination in two animals whereas there were no clinical or laboratory evidence of phenylbutazone toxicity in the animals studied.

Intestinal permeability was investigated by use of the ^{51}Cr-EDTA test on three clinical cases with naturally-occurring chronic enteropathy and found to be increased in one horse.

To my wee Heather

CHAPTER I. GENERAL INTRODUCTION.

INTRODUCTION.

The equine gastrointestinal tract has caused great consternation to the clinican for centuries. However, current knowledge of the conditions which affect this body system is extremely limited, particularly when it is compared to the advances achieved in other animal species. An area which has received relatively little attention is that of the equine chronic enteritides. Cases of chronic enteropathy are not uncommon in equine medicine but frequently they cannot be definitively diagnosed. It is believed that progress in the understanding of the aetiopathogenic mechanisms involved in these conditions will bring about realisation of their importance and it will create a basis for specific therapy of affected animals.

The following review highlights the main features of the aetiology, pathogenesis, clinical signs, pathological findings and therapy of those equine chronic enteropathies in which alterations of intestinal permeability are believed to be of significance in the pathogenesis. Subsequently, the currently available diagnostic techniques are discussed.

EQUINE CHRONIC ENTEROPATHIES.

ALIMENTARY LYMPHOSARCOMA.

Lymphosarcoma (LSA) is a neoplastic disease characterized by lymphatic tissue enlargement or invasion of non-lymphatic tissue by cells of lymphoblastic character (Bruere, Sutton and Davis, 1970). Lymphosarcoma in the horse is the most common neoplastic condition affecting the haemopoietic system (Jeffcott, 1977) and probably the most frequently encountered malignant neoplasia associated with death in this species (Rebhun and Bertone, 1984). Although different associations occur, it has generally been classified into 4 groups: multicentric, alimentary, thoracic and cutaneous (Van den Hoven and Franken, 1983; Madewell and Theilen, 1987). The alimentary tract, liver, spleen and associated lymph nodes appear to be involved most frequently in equine lymphosarcoma (Jeffcott, 1977).

Although a viral origin has been demonstrated in cases of feline lymphosarcoma (Theilen and Madewell, 1987) and enzootic bovine leukosis (Ferrer, 1980), similar investigations have not been undertaken in equine LSA (Neufeld, 1973; Madewell and Theilen, 1987). No age, breed, and sex predisposition have been established for equine haemopoietic neoplasms (Madewell and Theilen, 1987) and genetic resistance or susceptibility to the disease has not been studied in the horse (Neufeld, 1973).

The age of the reported cases of alimentary LSA ranges from two months to 23 years but the approximate mean is stated to be around 10 years (Neufeld, 1973; Van den Hoven and Franken, 1983). Marked weight loss, depression and inappetence are the most commonly reported signs and the clinical course is protracted with a history of illness over several weeks or even months. Affected animals may have signs of intermittent, mild colic (Neufeld, 1973; Van den Hoven and Franken, 1983) and,

usually of a chronic nature, may be the major presenting sign when the large intestine is involved (Wiseman, Petrie and Murray, 1974; Roberts and Pinsent, 1975; Wilson, Sutton, Groenendyk and Seawright, 1985). Ventral oedema may result from both hypoalbuminaemia and/or lymphatic obstruction (Roberts and Pinsent, 1975; Schalm and Carlson, 1982; Van den Hoven and Franken, 1983; Humphrey, Watson, Edwards and Wood, 1984).

Enlarged mesenteric lymph nodes may be palpated on rectal examination (Wiseman and others, 1974; Van den Hoven and Franken, 1983; Rebhun and Bertone, 1984; Wilson and others, 1985; Crawley, 1985; Madewell and Theilen, 1987) although it is possible that only thickened intestinal loops be present which render this observation quite difficult (Roberts and Pinsent, 1975). Non-specific skin lesions, probably due to an immune-mediated response, have also been associated with alimentary LSA (Wilson and others, 1985).

In cases of alimentary LSA fibrinous peritonitis (Reef, Dyson and Beech, 1984) and fibrous adhesions between intestinal loops and the omentum (Platt, 1987) have been reported. The mesenteric lymph nodes are almost invariably found to be enlarged (Neufeld, 1973; Wiseman and others, 1974; Van den Hoven and Franken, 1983; Madewell and Theilen, 1987; Platt, 1987). Patchy or diffuse thickening and mucosal ulceration of the small intestine are common findings (Platt, 1987). The large intestine may appear oedematous and reddened, with some serosal splash haemorrhages (Roberts and Pinsent, 1975) and mucosal ulcerations (Crawley, 1985).

Platt (1987) described the histopathology and cytology of the alimentary lymphomas in the horse. In all cases diffuse pleomorphic lymphoid cell infiltration of the small intestinal mucosa, particularly in the ileum, resulted in villous atrophy and in some animals it formed small crypt abscesses.

Most mesenteric lymph nodes showed extensive extracapsular spread and nodular neoplastic follicles and/or diffuse lymphoid infiltration disrupted the nodal architecture. Lymphatic obstruction and subsequent increase in the mucosal hydraulic pressure may, in addition to the alterations observed in the intestinal wall, impair normal absorption processes and enhance loss of proteins into the gastrointestinal tract (Roberts and Pinsent, 1975; Palmer, 1983; Roberts, 1984).

In Platt's case series (1987) the large intestine was rarely infiltrated but others have recorded large bowel thickening due to lymphocytic infiltration of the lamina propia and massive submucosal oedema (Wiseman and others, 1974; Roberts and Pinsent, 1975).

Equine LSA is considered to be a fatal disease and no specific successful treatment has been reported (Ward and Whitlock, 1967; Madewell and Theilen, 1987). Although treatment in other species has been widely reported (Gorman and White, 1987), the poor outlook and the expense involved preclude any attempt of treatment in the horse. Animals suffering from this disease are most commonly dealt with by euthanasia on humane grounds.

GASTRIC SQUAMOUS CELL CARCINOMA.

Squamous cell carcinoma (SCC) is the primary neoplasm involving the equine stomach although it represents a very small proportion of all equine carcinomas (Whitlock, 1980). The tumour appears to originate from the oesophageal region of the stomach, but the aetiology remains unknown although environmental and/or dietary factors have been suggested (Tennant, Keirn, White, Bentinck-Smith and King, 1982).

The approximate mean age of reported cases was 10 years, but no age, sex or breed predisposition was ascertained and the main clinical complaints described by different authors were similar (Meagher, Wheat, Tennant and Osburn, 1974; Wrigley, Gay, Lording and Haywood, 1981; Tennant and others, 1982). Affected cases were anorectic with chronic weight loss for weeks or months, and pale mucous membranes and fever were usually noticed. Mild, intermittent colic and diarrhoea were occasionally observed, dysphagia was apparent in one reported animal. Ventral oedema and/or ascites were present in some cases. On rectal examination, metastatic nodules or masses were palpated in most animals. In some cases, spread of the tumour to the thoracic cavity was responsible for signs of respiratory distress. Anaemia and neutrophilia were demonstrated by haematological analysis, and hypoalbuminaemia was a feature of the biochemical investigation but only occasionally were neoplastic cells observed in abdominal or thoracic fluid.

Tennant and his colleagues (1982) gave a full pathological description of equine gastric SCC: the gross appearance of the stomach was characterized by a nodular luminal surface with a cauliflower-like appearance, mainly in the oesophageal region of the stomach, presenting fissures or ulcers and covered by necrotic debris, and clearly demarcated from the normal gastric epithelium. Multiple metastases were present on the serosal surface of the stomach and infiltrated the liver, diaphragm, spleen, intestines, peritoneum and pleurae. Adhesions between abdominal organs were not uncommon. Under the microscope, these neoplastic structures were formed by cords of polyhedral or oval epithelial cells with prominent nucleoli within pale, vesicular nuclei, and which were separated by connective tissue.

Deterioration is progressive and most horses are euthanatized on humane grounds (Meagher and others, 1974; Wrigley and others, 1981; Tennant and others, 1982).

CHRONIC INFLAMMATORY ENTEROPATHIES.

Several conditions will be discussed under this heading, all of which are characterized by severe focal or diffuse inflammatory reactions in the lamina propia, submucosa, or the entire wall of a part or the whole gastrointestinal tract (Roberts, 1984). The clinical syndrome observed with these diseases is a consequence of malabsorption and/or hypoalbuminaemia (Merritt, Cimprich and Beech, 1976; Lindberg, 1984; Platt, 1986). In the review by Roberts (1984) the chronic infiltrative enteritides listed in addition to alimentary lymphosarcoma were granulomatous enteritis, chronic eosinophilic enteritis, tuberculosis, and histoplasmosis.

GRANULOMATOUS ENTERITIS.

Granulomatous enteritis was first described in the USA (Cimprich, 1974) and since then there have been a few reports of the condition from the USA (Merritt and others, 1976; Sweeney, Sweeney, Saik and Lichtensteiger, 1986), Canada (Meuten, Butler, Thomson and Lumsden, 1978), South Africa (Bester and Coetzer, 1978), Australasia (Roberts and Kelly, 1980; Hodgson and Allen, 1982), Sweden (Lindberg, 1984), and the UK (Platt, 1986).

To date, no clear aetiology for granulomatous enteritis has been ascertained but the condition has been likened to Crohn's disease and intestinal tuberculosis in man (Cimprich, 1974). Merritt and others (1976) reported the clinical features of nine cases of granulomatous enteritis and they isolated *Mycobacterium avium* from samples of faeces and rectal mucosa of only one animal while Lindberg (1984) isolated bacteria of the group *Mycobacterium avium-intracellulare* from the bowel of one horse and from a mesenteric lymph node of another, but he concluded that the significance of this findings was unclear. Current hypotheses held on the

aetiopathogenesis of Crohn's disease implicate environmental factors such as chemicals in food and water, type of diet, or infective agents which could trigger off an aberrant immune response in the host, possibly in genetically susceptible patients (Lennard-Jones, 1980) and similar theories have been proposed for equine granulomatous enteritis (Merritt and others, 1976; Roberts and Kelly, 1980; Lindberg, 1984; Sweeney and others, 1986). The latter authors reported on three sibling standardbred horses suffering from granulomatous enteritis.

The alterations observed in granulomatous enteritis result in malabsorption and protein-losing enteropathy: increased protein loss into the gastrointestinal tract has been demonstrated by studies with radioisotopes (Merritt and others, 1976) and decreased absorption of carbohydrates and lipids has also been established (Merritt and others, 1976; Meuten and others, 1978; Roberts and Kelly, 1980). Immunologically mediated mechanisms have been involved in the origin of the skin lesions observed in some cases (Argenzio and Whitlock, 1980).

The history in all the reported cases of granulomatous enteritis was of progressive weight loss over several weeks or months and the mean age of the cases recorded on the literature was four years. Progressive anorexia or a capricious appetite was a common complaint. Pitting subcutaneous oedema and pale mucous membranes were frequently seen. Slight intermittent fever has been reported (Merritt and others, 1976; Bester and Coetzer, 1978) but did not appear to be a consistent finding although there are reports of horses with granulomatous enteritis which initially had presented with "fever of unknown origin" (Hodgson and Allen, 1982; Mair, Taylor and Pinsent, 1989). Scant but formed faeces were frequently observed (Merritt and others, 1976; Roberts and Kelly, 1980) although some cases presented with soft, unformed faeces, or occasionally watery diarrhoea (Merritt and others, 1976; Sweeney and others, 1986). Mild colic was present in some cases (Merritt and others, 1976) and diffuse thickening of intestinal loops, and firm, small masses in

the mesentery and on the wall of the bowel, and enlarged mesenteric lymph nodes could be palpated *per rectum* in many cases (Merritt and others, 1976; Roberts and Kelly, 1980; Hodgson and Allen, 1982; Sweeney and others, 1986). Reduced phagocytic activity of cells in the peritoneal fluid, and pruritus and alopecia have been reported (Merritt and others, 1976).

The pathological features of equine granulomatous enteritis have been described by Cimprich (1974), Lindberg (1984), Lindberg and Karlsson (1985) and Platt (1986). Horses appeared to be thin or emaciated and ventral oedema was commonly noticed. A great variability of features was observed macroscopically. Signs of chronic peritonitis and adhesions between omentum, intestinal loops and body wall were frequently observed. The mucosa appeared granular and thickened with raised and flattened areas and a "cobblestone" pattern. Mesenteric lymph nodes were wet, firm and mottled.

Histologically, the ileum was the most severely affected region and the large intestine the least. Many areas which appeared grossly normal were abnormal under the microscope. Marked villous atrophy and superficial erosions were frequently encountered. A few granulomas were scattered in the submucosa of the small intestine and crypt abscesses were associated with degeneration or necrosis of the basal crypt epithelium. There was a diffuse infiltration by mononuclear cells and oedema of the lamina propia and submucosa was apparent. A similar infiltrate, often enclosed by fibrous tissue, was the most marked alteration in the mesenteric lymph nodes and it was also observed in some animals in other parts of the gastrointestinal tract such as the pharynx, stomach and large intestine. *Mycobacterium* spp. were only found in the bowel and/or mesenteric lymph nodes of three reported cases.

Electron microscopic studies showed a consistent shortening of the microvilli of surface enterocytes probably associated with cellular injury and degeneration which may be responsible for intestinal permeability alterations (Lindberg and Karlsson, 1985).

In addition to symptomatic, supportive treatment therapeutic regimens similar to those used in cases of Crohn's disease, with salicylazosulfapyridine and/or corticosteroids and the use of metronidazole and azathioprine have been advocated (Merritt and others, 1976; Meuten and others, 1978). In human regional enteritis surgical removal of affected intestine is commonly necessary, but the widespread involvement in equine cases precludes this possibility (Merritt and others, 1976). The prognosis appears to be poor and most horses are euthanased on humane grounds.

CHRONIC EOSINOPHILIC GASTROENTERITIS.

Equine chronic eosinophilic gastroenteritis is a rare condition which was reported for the first time in Australia (Pass and Bolton, 1982). The aetiology remains obscure although the most popular hypothesis held considers an immediate-type hypersentivity reaction to antigens absorbed from or excreted into the gut which might originate from some diet components, parasites or enteric pathogens (Gibson and Alders, 1987).

The clinical presentation was one of chronic weight loss and/or diarrhoea but skin lesions in the form of nodules of different sizes or coronitis, with patchy areas of alopecia, thickening and mild hyperkeratosis were common (Pass and Bolton, 1982; Breider, Kiely and Edwards, 1985; Gibson and Alders, 1987). Hypoalbuminaemia was a feature in all the cases reported and carbohydrate malabsorption was demonstrated by Pass and Bolton (1982).

Post-mortem findings were, with the exception of the skin lesions, limited to the gastrointestinal tract and associated organs (Pass and Bolton, 1982). Grossly, thickening of the wall of the whole alimentary canal was characteristic. The intestinal mucosal surface appeared smooth although some ulceration was occasionally evident and multiple firm nodules were observed scattered throughout the bowel, around pancreatic ducts, throughout the hepatic parenchyma, on the serosa of the small intestine, and in mesenteric lymph nodes.

Histopathology revealed diffuse infiltration, primarily by eosinophils, of the lamina propia, submucosa, muscularis and serosa of the gastrointestinal tract accompanied by villous atrophy, fibrosis of the lamina propia and hypertrophy of the muscularis mucosae. The nodules generally consisted of masses of eosinophils. In the cutaneous lesions foci of degenerate keratinocytes, eosinophils and neutrophils were present within the epidermis and at the epidermal-dermal junction in the areas with acanthosis and hyperkeratosis (Pass and Bolton, 1982). Pancreatitis in one case was due to periductal fibrosis and diffuse eosinophilic infiltration but there was no evidence of parasites (Breider and others, 1985).

Administration of corticosteroids together with symptomatic, supportive therapy and topical medication of skin lesions with a combination of corticosteroids and antibiotics has been used successfully in two reported cases (Gibson and Alders, 1987).

TUBERCULOSIS.

Tuberculosis (TB) appears to occur very rarely in the horse and although most old reports of this condition implicated *Mycobacterium tuberculosis*, in recent years bacteria belonging to the *Mycobacterium*

avium-intracellulare-scrofulaceum (MAIS) group have been incriminated, probably due to the programme of erradication of TB in cattle (Mair, Taylor, Gibbs and Lucke, 1986; Mair and Jenkins, 1990c).

Tuberculosis may be localized or generalized and the bacteria generally enter the body via ingestion (Dungworth, 1985). The mesenteric lymph nodes may present tuberculous ulcers or uniform tubercles which resemble a sarcoma on macroscopic examination and histologically they consist of macrophages, epithelioid cells and giant cells (Dungworth, 1985). MAIS organisms have been isolated in three reported cases of granulomatous enteritis (Merritt and others, 1976; Lindberg, 1984). Clinically, the most common complaint appears to be chronic weight loss and subsequent weakness and lethargy (Beech, 1990).

Although rifampin, isoniazid and streptomycin may be used for the treatment of equine tuberculosis (Beech, 1990), the prognosis appears to be poor and the prolonged therapy required would only be warranted in very valuable horses and should be instituted under strict isolation due to the public health risks.

HISTOPLASMOSIS.

Histoplasmosis has been recorded in association with granulomatous colitis in a horse (Dade, Lickfeldt and McAllister, 1973). The authors suggested that *Histoplasma capsulatum* had gained entry orally and the signs reported were progressive weight loss, lethargy, ventral oedema and sudden onset of chronic, profuse diarrhoea.

On post-mortem examination a large amount of serous fluid was recovered from all body cavities. The mesenteric lymph nodes were enlarged and the walls of the caecum and colon were thickened and the

mucosa appeared reddened and ulcerated. Microscopically, the normal architecture of the mesenteric lymph nodes was distorted by diffuse proliferation of cells of the reticuloendothelial system within which there were many structures identical to *H. capsulatum*. The mucosa and submucosa of the caecum and colon were infiltrated by macrophages containing similar structures. Oedema of the submucosa was marked.

The therapeutic protocol was not recorded but the animal did not respond to treatment. Amphotericin B and ketoconazole are the recommended drugs for equine histoplasmosis (McCullough, 1983).

STRONGYLOSIS.

The most common and clinically significant parasites of horses associated with chronic gastrointestinal disease are the intestinal nematodes of the family Strongylidae. Until recently, *Strongylus* spp. (the large strongyles) were considered to be the most harmful equine internal parasites under normal field conditions (Drudge and Lyons, 1983; Ogbourne and Duncan, 1985) but there have been several recent reports of clinical disease in horses due to cyathostome infection (Chiejina and Mason, 1977; Jasko and Roth, 1984; Giles, Urquhart and Longstaffe, 1985; Church, Kelly and Obwolo, 1986; Love, 1990). Indeed, in a report of 66 cases of equine diarrhoea, the most commonly identified cause was larval cyathostomiasis (Mair, de Westerlaken, Cripps and Love, 1990a). Mixed populations of small and large strongyles occur under natural conditions and faecal strongyle eggs cannot be differentiated to species by microscopic examination but third-stage larvae obtained by culture of faecal samples can be differentiated to the level of subfamily i.e. Strongylinae vs. Cyathostominae (Drudge and Lyons, 1983).

Strongylus vulgaris, *S. edentatus* and *S. equinus* are the main species of the large strongyles and they all migrate (Drudge, Lyons and Tolliver, 1989) during their larval stages: *S. vulgaris* in walls of arteries, particularly in those that supply the gastrointestinal tract; *S. edentatus* in the veins to invade the liver and other abdominal organs; and *S. equinus* larvae migrate into the liver and pancreas. The approximate pre-patent periods for these species are six, eleven and nine months, respectively (Drudge and Lyons, 1983). The adult stages are attached to the mucosa of the large intestine.

The subfamily Cyathostominae is composed of over 40 species and they have been grouped into eight genera (Lichtenfels, 1975). Details of the cycles of individual species are not available but Reinemeyer (1986) summarised the presumed direct life cycle of these species: third-stage larvae are ingested by the host and exsheath to invade the large intestinal wall where they develop to the fourth larval stage within a mucosal cyst. Subsequently, fourth-stage larvae emerge into the lumen of the caecum and large colon, and develop to the fifth-stage and finally achieve full sexual maturity. Although the pre-patent period usually ranges from six to fourteen weeks, arrested or inhibited development of third- and fourth-stage larvae in the large intestinal wall has been reported (Smith, 1976; Eysker, Jansen and Mirck, 1984; Eysker, Boersema and Kooyman, 1990).

The pathogenesis of the large strongyles involves both larvae and adult parasites (Argenzio and Whitlock, 1980). Larvae migrating along mesenteric arteries may induce arteritis and thromboembolism (Slocombe, McCraw, Pennock and Llewellyn, 1981). Interference with the blood supply to the gastrointestinal tract, probably resulting from vasoconstriction as a consequence of the release of vasoactive substances by aggregating platelets (Becht, 1984; White, 1986) or by an immune response triggered off by parasitic antigens (White, 1981), has been associated with the focal intestinal ischaemia observed in infected

animals. Altered intestinal motility has been demonstrated by studies on the ileal myoelectrical activity in ponies experimentally infected with *S. vulgaris* third-stage larvae (Bueno, Ruckebusch and Dorchies, 1978; Berry, Merritt, Burrows, Campbell and Drudge, 1986; Clark, 1990).

Adult *S. vulgaris* parasites attach to the mucosa of the caecum and large colon and produce bleeding ulcerations due to their feeding by sucking blood, and secondary bacterial invasions may lead to a catarrhal enteritis and diarrhoea may result (Greatorex, 1975; Argenzio and Whitlock, 1980). Duncan and Dargie (1975) demonstrated an increased loss of albumin and red blood cells into the gastrointestinal tract of animals to which adult *S. vulgaris* had been transplanted.

The life cycle of the cyathostomes is presumed to be non-migratory and, in consequence, the pathological processes are limited to the gastrointestinal tract (Ogbourne, 1978). No attempt has been made to define the pathogenic effects of individual species and all studies carried out experimentally were based on mixed infections (Love, 1990). The cysts containing developing larvae in the large intestinal mucosa and submucosa are surrounded by a mononuclear infiltrate and/or a thick fibrous capsule, and hypertrophy and hyperplasia of goblet cells are present in the nearby mucosa (Ogbourne, 1978) which might to a certain extent impair the normal physiological functions of caecum and large colon (Blackwell, 1973; Church and others, 1986). In northern temperate climates mass emergence of inhibited larvae into the gut lumen, usually during the early months of the year, is accompanied by a severe inflammatory reaction which has been associated with the distinct clinical syndrome of larval cyathostomiasis (Ogbourne, 1978; Giles and others, 1985) which is considered to be a protein-losing enteropathy (Blackwell, 1973; Roberts, 1984; Giles and others, 1985; Church and others, 1986; Love, 1990).

A reduced motility of the gastrointestinal tract was demonstrated in ponies experimentally infected with cyathostomes (Bueno and others, 1979) although Church and others (1986) reported clinical evidence of continuous, intense muscular activity in the caecum of a pony with larval cyathostomiasis from which intestinal biopsies were being taken via laparotomy.

Adult cyathostomes are attached to the mucosa of the caecum and large colon by their buccal capsules (Ogbourne, 1978) but although ulceration of the large intestinal mucosa may occur it is thought that mature parasites do not cause clinical disease unless present in very high numbers, or a secondary bacterial infection occurs (Ogbourne, 1978; Al-Mashat and Taylor, 1986). Generally, mixed strongyle populations take place in most natural infections and therefore it is difficult to attribute specific clinical signs to adult cyathostomes (Reinemeyer, 1986).

The clinical course of strongylosis may be protracted in horses of all ages and characterized by recurrent colic, anorexia, weight loss, diarrhoea, and chronic depression (Platt, 1986; Mair, Hillyer and Pearson, 1991; Robertson, 1990). The diarrhoea may be acute and associated with shock, or it may become chronic, either profuse and continuous or alternating between cow-pat faeces and episodes of severe diarrhoea (Argenzio and Whitlock, 1980). Anaemia, poor performance and unthriftiness have been ascribed to the presence of adult strongyle parasites in the large intestine of horses (Ogbourne and Duncan, 1985) and Roberts (1984) considered *S. vulgaris* larval infection to be a protein-losing enteropathy. Adult cyathostominae may contribute to these chronic signs but a specific entity has been reported to be caused by larval small strongyles in which a chronic diarrhoea of sudden onset in late winter with progressive but rapid weight loss, emaciation and eventually death occur if not treated promptly (Giles and others, 1985; Love, 1990). Although larval cyathostomiasis appears to occur more frequently in

young animals, there have been reports of affected horses at up to 16 years of age (Giles and others, 1985; Love, 1990). In addition to these clinical signs, ventral oedema may be a feature of the condition.

Bleeding intestinal ulcerations may be found with both large and small strongyle adult infections but the major pathological changes are due to the larval stages. Penetration of strongyle larvae into the intestinal mucosa and submucosa results in an infiltration by neutrophils, mononuclear cells and eosinophils in association with submucosal haemorrhage (Ogbourne, 1978; Ogbourne and Duncan, 1985). The larval migration of *S. vulgaris* produces considerable thickening of the wall of the anterior mesenteric artery and of its branches due to an intense inflammatory reaction and may result in thrombus formation and proliferation of the intima, and cellular infiltration of the arterial wall with plasma cells, lymphocytes, macrophages and neutrophils (Ogbourne and Duncan, 1985). Intestinal infarction and inflammation may result from the impaired blood supply (Platt, 1986; Mair and others, 1991). *Strongylus edentatus* larvae migrating through the hepatic parenchyma and peritoneum induce the formation of foci of inflammatory reaction probably related to antigens released by the larvae and scars and tags of fibrous tissue may be observed on the hepatic capsule (Barker and van Dreumel, 1985) and *S. equinus* larvae may induce similar lesions in their migration through liver and hepatic ligament into the pancreas and peritoneal cavity. Subserosal haemorrhagic plaques known as *haemomelasma ilei* composed of oedema, haemorrhage, different leucocytes, and macrophages ingesting erythrocytes are frequently associated with migrating strongyle larvae (Barker and van Dreumel, 1985).

Animals with larval cyathostomiasis may show different degrees of emaciation, with liquid large intestinal contents in which large numbers of cyathostome parasites are usually observed and the oedematous caecal and colonic walls may appear congested and containing cyathostome

nodules within the mucosa (Giles and others, 1985). On histopathology, cyathostome larvae may be seen encysted in the large intestinal mucosa deforming the Lieberkuhn glands, and surrounded by lymphocytes, eosinophils and some plasma cells. Larvae in the submucosa induce the formation of a thicker capsule and a similar cellular infiltrate (Reinemeyer, 1986). Emerging larvae cause an intense inflammatory reaction, predominantly eosinophils, as well as haemorrhagic foci and oedema, and numerous erosions may be observed (Ogbourne, 1978).

Treatment of strongylosis is by elimination of larval and adult strongyles by administration of larvicidal dosages of the modern anthelmintic agents, although one has to bear in mind the existence of anthelmintic resistance in a number of cyathostome species (Drudge, Lyons and Tolliver, 1979; Herd, 1990) and the apparent lack of efficacy of these compounds against inhibited cyathostome larval stages (Church and others, 1986). Successful management of larval cyathostomiasis has been recorded by a combined course of corticosteroids and ivermectin at the recommended dosage (Church and others, 1986).

PHENYLBUTAZONE TOXICITY.

Phenylbutazone (PBZ) is a widely used nonsteroidal antiinflammatory drug (NSAID) for the treatment of soft tissue inflammation (Tobin, Chay, Kamerling, Woods, Weckman, Blake and Lees, 1986). In recent years, its potential toxicity in the equine patient has been demonstrated both experimentally (Snow, Bogan, Douglas and Thompson, 1979; Snow, Douglas, Thompson, Parkins and Holmes, 1981; Lees, Creed, Gerring, Gould, Humphreys, Maitho, Michell and Taylor, 1983; Meschter, Maylin and Krook, 1984; Collins and Tyler, 1985; Meschter, Krook, Maylin and Corradino, 1990) and in clinical reports (Gunson and Soma, 1983; Read, 1983; Traub, Gallina, Grant, Reed, Gavin and Paulsen, 1983; Behm and Berg, 1987).

The dosage regimen generally recommended for PBZ is 8 mg/kg/day for one day; then 3.3 mg/kg/day for five days; finally 3.3 mg/kg on alternate days for four days. Phenylbutazone is generally considered a safe drug at a normal dose and toxicity in the horse is most commonly associated with excessive dosage of the compound, combination with other NSAIDs, administration to dehydrated animals or those with reduced water intake, or to those suffering from liver dysfunction (Murray, 1990b).

Phenylbutazone possesses an ulcerogenic effect in the alimentary tract which appears to result from its inhibition of prostaglandin synthesis (Tobin and others, 1986) and/or subsequent local vasoconstriction, hypoxia and necrosis (Lees and Higgins, 1985) and/or decreased production of protective mucus (Collins and Tyler, 1985). These hypotheses are supported by the results of studies by Collins and Tyler (1985) which found that concurrent administration of PBZ and synthetic PGE_2 prevented the development of ulcers and hypoproteinaemia, although there is recent evidence that other processes such as bacterial invasion might be involved in the formation and perpetuation of gastrointestinal erosions (Meschter and others, 1990). A vasculopathy has been suggested as the primary lesion in PBZ toxicity (Meschter and others, 1984; Meschter and others, 1990). Snow and others (1981) demonstrated an increased loss of proteins into the gastrointestinal tract by studies with radioisotopes and the authors concluded that this loss was most likely associated with the gastrointestinal ulcerations; however protein-losing enteropathy (PLE) was also observed in animals which lacked such lesions but were found to have a characteristic mucosal atrophy (Collins and Tyler, 1985). Other toxic effects of PBZ in the horse include nephrotoxicity (Gunson and Soma, 1983; Read, 1983; Behm and Berg, 1987) and hepatotoxicity (Lees and others, 1983).

The first signs of PBZ toxicity are depression, inappetence and weight loss (Snow and others, 1981; Tobin and others, 1986). Oral ulcers may be present and diarrhoea may be a feature of the condition (Snow and others, 1981; Collins and Tyler, 1985). Moderate to severe abdominal discomfort has been reported (Snow and others, 1981; Byars, 1990; Murray, 1990b; Meschter and others, 1990) and ventral oedema may be present (Murray, 1990b). Some cases present in hypovolaemic shock or with endotoxaemia (Snow and others, 1981; Collins and Tyler, 1985) and septicaemia may occur (Murray, 1990b). Oliguria or haematuria may indicate renal disease (Read, 1983; Behm and Berg, 1987; Byars, 1990).

At post-mortem examination ulcers, erosions and areas of necrosis may be seen along the gastrointestinal tract, and submucosal oedema may be evident (Snow and others, 1981; Collins and Tyler, 1985; Meschter and others, 1990). On histopathology, oral ulcers generally appear well demarcated whereas lesions in other portions of the alimentary tract may range from mucosal atrophy to erosions or ulcerations with cellular infiltrates, and a reduced number of cells may be apparent (Collins and Tyler, 1985). Sloughed epithelium may be separated from the lamina propia by subepithelial clefts containing fibrin strands and cellular debris (Meschter and others, 1990) and alterations in the submucosa include oedema and congestion of veins and lymphatics (Collins and Tyler, 1985).

In his review Murray (1990b) gives guidelines for treatment of cases with PBZ toxicity: administration of the drug should be stopped immediately. Symptomatic therapy is required for animals presenting in shock but care should be taken where crystaloid fluids are administered - plasma or colloids may be necessary to correct the hypoproteinaemia; if septicaemia is present, broad-spectrum antibiotics should be administered, and narcotic analgesics may be used to alleviate pain; healing of ulcers may be promoted by histamine H_2 antagonist administration, and synthetic prostaglandins might be helpful, although currently they are at a experimental stage.

OTHER CONDITIONS OF THE ALIMENTARY SYSTEM RESULTING IN CHRONIC WEIGHT LOSS AND/OR DIARRHOEA.

Other chronic enteropathies described in equine medicine may result in chronic weight loss and/or diarrhoea. However it is unlikely that the pathogenesis of such conditions involve alterations of intestinal permeability.

Several bacterial pathogens have been suggested to be involved in the origin of enteric lesions in the horse (reviewed by Al-Mashat and Taylor, 1986). Abdominal abscesses and other causes of chronic peritonitis have been associated in horses with chronic weight loss, chronic or intermittent colic, depression, anorexia, intermittent fever, ventral oedema, decreased borborygmi and diarrhoea (Dyson, 1983; Rumbaugh, 1983; Spier, Carlson, Nyland, Snyder and Fischer, 1986; Mair, Hillyer and Taylor, 1990b; Semrad, 1990). *Rhodococcus equi* infection may produce a chronic progressive inflammation of the gastrointestinal tract and associated lymph nodes which may result in chronic diarrhoea (Merritt, Bolton and Cimprich, 1975; Hillidge, 1986).

Protozoal organisms such as *Globidium* (*Eimeria*) *leuckarti* (Wheeldon and Greig, 1977) or *Tritrichomonas* spp. (Laufenstein-Duffy, 1969) have been associated with cases of equine chronic diarrhoea.

Chronic gastritis (Whitlock, 1980), chronic pancreatitis (Pass and Bolton, 1982; Morris, 1983; Barker and van Dreumel, 1985) and exocrine pancreatic adenocarcinoma (Church, West and Baker, 1987) may result in horses in chronic weight loss, depression, mild intermittent colic and diarrhoea. Similar abnormalities may be observed in cases of chronic liver disease (Byars, 1987; Milne, 1990), chronic sand enteropathy (Colahan, 1987; Scrutchfield, 1987; Murray, 1990a) and chronic grass sickness (Edwards, 1987).

APPROACH TO THE DIAGNOSIS OF EQUINE CHRONIC ENTEROPATHIES.

Chronic enteropathies in the horse present a considerable diagnostic challenge to clinicians due to the similarity in their clinical presentation and extensive and expensive investigative procedures may be required in order to reach a definitive diagnosis.

CASE HISTORY.

To some extent the case history and clinical presentation may enable the clinician to focus attention to the gastrointestinal tract. For example, inadequate parasite prophylaxis may suggest parasitism as the cause of the problem and a history of recent administration of phenylbutazone may be ascertained, but in many instances nonspecific history of chronic weight loss and/or diarrhoea, and possibly anorexia, depression and oedema may be all that can be obtained.

PHYSICAL EXAMINATION.

On physical examination of a case of equine chronic enteropathy, the findings are rarely pathognomonic of a specific condition. However, some clinical features may be indicative of certain diseases. For example, enlarged peripheral lymph nodes may occasionally be present in cases of alimentary lymphosarcoma (Rebhun and Bertone, 1984; Madewell and Theilen, 1987) and enlarged lymph nodes, abnormal nodules or masses and diffuse thickening of intestinal loops may also be palpated *per rectum* in horses with alimentary LSA (Wiseman and others, 1974; Roberts and Pinsent, 1975; Van den Hoven and Franken, 1983; Crawley, 1985; Madewell and Theilen, 1987). Similar abnormalities may be present in other conditions including granulomatous enteritis (Merritt and others,

1976; Roberts and Kelly, 1980; Hodgson and Allen, 1982; Sweeney and others, 1986), intra-abdominal abscesses (Spier and others, 1986) or gastric SCC (Meagher and others, 1974; Wrigley and others, 1981; Tennant and others, 1982) such that they have low specificity. A useful clinical finding is the presence of small strongyle parasites on the rectal sleeve or in faeces which is a diagnostic feature of larval cyathostomiasis (Giles and others, 1985; Love, 1990). Although appreciation *per rectum* of cranial mesenteric arteritis may be indicative of migrating strongyle larvae, reliability and actual significance of this finding is doubtful (Dyson, 1983). Skin lesions have been described in horses with granulomatous enteritis (Merritt and others, 1976), chronic eosinophilic gastroenteritis (Pass and Bolton, 1982; Breider and others, 1985; Gibson and Alders, 1987), strongylosis (Thomsett, 1985) and alimentary LSA (Wilson and others, 1985). Oral ulcers may be present in animals with PBZ toxicity (Snow and others, 1981; Collins and Tyler, 1985). Dysphagia has been reported in a horse with gastric squamous cell carcinoma (Tennant and others, 1982).

As a result of the difficulty of making a specific diagnosis on the basis of case history and physical findings it is common practice to further investigate these cases by analyses of samples of blood and/or faeces and/or peritoneal fluid. In some animals additional investigative procedures such as intestinal function tests, radiography or endoscopy may be undertaken.

HAEMATOLOGY.

Anaemia is a nonspecific finding in many chronic equine enteropathies, but immune-mediated haemolytic anaemia positive to a Coombs' test has been described in horses with LSA (Farrelly, Collins and Collins, 1966; Reef and others, 1984; Mair, Taylor and Hillyer, 1990d).

Thrombocytopaenia, probably immune-mediated, has also been reported in equine LSA (Reef and others, 1984; Rebhun and Bertone, 1984; Platt, 1987).

Neutrophilia is a common feature in many chronic enteropathies in horses including LSA (Wiseman and others, 1974; Roberts and Pinsent, 1975; Reef and others, 1984; Humphrey and others, 1984; Wilson and others, 1985; Sheeran, 1987), chronic eosinophilic gastroenteritis (Gibson and Alders, 1987), large or small strongyle infections (Ogbourne and Duncan, 1985; Giles and others, 1985), PBZ toxicity (Lees and others, 1983) and gastric SCC (Wrigley and others, 1981; Tennant and others, 1982).

Eosinophilia is said to occur in parasitised animals, although this finding is inconsistent (Ogbourne and Duncan, 1985; Giles and others, 1985). Eosinophilia associated with chronic eosinophilic gastroenteritis has been recorded (Gibson and Alders, 1987).

Lymphocytopaenia was reported in only one animal with granulomatous enteritis (Roberts and Kelly, 1980). Leukaemia may occasionally be encountered in equine LSA (Theilen and Fowler, 1962; Madewell, Carlson, MacLachlan and Feldman, 1982) but lymphopaenia has been reported in alimentary LSA (Wilson and others, 1985).

Plasma fibrinogen levels were found to be increased in inflammatory processes such as internal abscesses (Spier and others, 1986), LSA (Schalm and Carlson, 1982) or peritonitis of various origins (Mair and others, 1990b), but fibrinogen is a nonspecific acute phase protein and it might be more useful to monitor recovery rather than as a diagnostic tool (Allen and Kold, 1988; Taylor, 1990).

BLOOD BIOCHEMISTRY.

By far the most common biochemical abnormality found in horses with chronic enteropathies is low plasma albumin levels as recorded in clinical reports of LSA (Wiseman and others, 1974; Roberts and Pinsent, 1975; Van den Hoven and Franken 1983; Humphrey and others, 1984; Crawley, 1985; Sheeran, 1987), granulomatous enteritis (Merritt and others, 1976; Meuten and others, 1978; Roberts and Kelly, 1980; Hodgson and Allen, 1982; Sweeney and others, 1986), chronic eosinophilic gastroenteritis (Pass and Bolton, 1982; Breider and others, 1985; Gibson and Alders, 1987), tuberculosis and histoplasmosis (Roberts, 1984), strongylosis (Round, 1970; Patton, Mock, Drudge and Morgan, 1978; Giles and others, 1985; Church and others, 1986; Love, 1990), PBZ toxicity (Snow and others, 1981; Lees and others, 1983; Collins and Tyler, 1985), gastric SCC (Wrigley and others, 1981; Tennant and others, 1982) and pancreatic adenocarcinoma (Church and others, 1987). However, since hypoalbuminaemia may occur in so many different intestinal diseases it clearly has no diagnostic specificity. In gastrointestinal disorders low plasma albumin levels may arise as a result of a variety of mechanisms including: increased losses of protein into the gastrointestinal tract due to mucosal cell erosion; mucosal ulceration; active secretion or altered metabolism; passive intercellular diffusion due to lymphatic obstruction; increased capillary pressure and permeability; or ultrastructural separation of junctional complexes (Roberts, 1984; Clark, Morris, Allen and Tyler, 1988), but it may be exacerbated by concurrent decreased absorption due to inappetence and/or malabsorption of amino acid precursors (Coffman, 1979; Dargie, 1975; Pearson, 1990).

Increased total plasma globulin level is a nonspecific finding in many disease processes including enteropathies (Coffman, 1979) and may accompany hypoalbuminaemia if there is concurrent chronic inflammation, hepatic dysfunction or during the prepatent period in strongylosis (Roberts, 1984). Serum protein electrophoresis may be more

informative (Matthews, 1982) and it is the method of choice to assess protein levels since the chemical assays by dye methods may be inaccurate when albumin levels are low, and the concentration of other proteins is elevated (Blackmore, Henley and Mapp, 1983). Hyperalpha-globulinaemia is a common feature of chronic equine enteropathies, although it is often found in animals with severe inflammation of any type (Coffman, 1984), and may appear together with hyperbetaglobulinaemia. Increased plasma betaglobulin levels have been commonly reported in animals infected with strongyles (Round, 1970; Ogbourne and Duncan, 1985; Giles and others, 1985; Church and others, 1986; Reinemeyer, 1986) which is generally due to raised levels of gammaglobulin G (T) which migrate in the betaglobulin fraction. Hypergammaglobulinaemia may be caused by concurrent infection or liver disease (Coffman, 1979; Schalm and Carlson, 1982; McLaughlin, 1990).

Electrolyte alterations are generally nonspecific and the hyponatraemia, hypochloraemia and hypokalaemia frequently noticed in horses with chronic diarrhoea (Murray, 1990a) are of more use as a base for the symptomatic management of affected animals rather than to clarify their aetiologic diagnoses. Hypocalcaemia may result from inappetence (Murray, 1990a), but it may be associated with protein loss into the gastrointestinal tract (Collins and Tyler, 1985; Crawley, 1985). Hypomagnesaemia was recorded in animals with PBZ toxicity (Lees and others, 1983).

Analyses of plasma enzyme levels are useful to rule out involvement of other organs such as liver or pancreas (Coffman, 1984; Humphrey and others, 1984; Church and others, 1987; Taylor, 1990). Increased alkaline phosphatase activity, particularly the intestinal phosphatase, may indicate enteric damage (Taylor, 1990) but otherwise plasma enzymes do not add more specific information.

Measurements of plasma levels of urea and creatinine may be helpful in detecting renal disease from PBZ nephrotoxicity or other causes (Read, 1983) but they might also indicate pre-renal azotaemia (Taylor, 1990).

PERITONEAL FLUID ANALYSIS.

Abdominal paracentesis is a straightforward and generally safe procedure which is useful as an aid to the diagnosis of abdominal disease in horses (Hamilton and Hardenbrook, 1973; Bach and Rickets, 1974; Cowell, Tyler, Clinkenbeard and MacCallister, 1987). Peritonitis is considered when the total white blood cell count in the peritoneal fluid (PF) is at least 10^{10}/litre and the predominant cell type is the neutrophil (Dyson, 1983; Mair and others, 1990b). Increased numbers of normal or abnormal lymphocytes may be observed in peritoneal fluid from cases of equine LSA (Bach and Ricketts, 1974; Schalm and Carlson, 1982; Sweeney, 1987). Increased numbers of PF eosinophils have been recorded in chronic eosinophilic gastroenteritis (Gibson and Alders, 1987) and they might also occur as a result of strongyle larvae migrating in the intestinal wall (Bach and Ricketts, 1974; Merritt, 1983). A reduced phagocytic activity of mesothelial cells in PF was considered to be a significant finding for the diagnosis of equine granulomatous enteritis (Merritt and others, 1976). Other tumour cells may be evident on examination of PF (Bach and Ricketts, 1974; Tennant and others, 1982; Fulton, Brown and Yamini, 1990).

FAECAL ANALYSIS.

Various analyses of faeces from cases with chronic enteric disease may be helpful to diagnosis.

Bacteriological culture of faeces should be carried out on several occasions to increase the chances of isolating *Salmonella* spp., which may be intermittently shed (Murray, 1990a) although the significance of positive cultures is doubtful (Smith, 1983). Other bacteria which might be of possible significance if isolated in faecal samples include beta-haemolytic *Escherichia coli*, *Clostridium perfringens A*, *Actinobacillus equuli*, *Campylobacter coli*, *C. jejuni*, *Aeromonas hydrophila*, *Pseudomonas* spp., *Bacteroides vulgatus*, *Mycobacterium paratuberculosis* and other *Clostridium* spp. (Al-Mashat and Taylor, 1986). Occult blood may be a feature in some cases of ulcerated LSA (Reef and others, 1984; Rebhun and Bertone, 1984; Crawley, 1985), PBZ toxicity (Byars, 1990) or gastric SCC (Whitlock, 1980). An alteration in numbers of protozoa - either increased or decreased - observed in direct faecal smears may indicate an altered gastrointestinal environment, but they are not diagnostic *per se* of any specific condition (Merritt, 1983). The presence of neutrophils in faeces may be associated with bacterial colitis (Roberts, 1987).

Small strongyle parasites may be observed directly in faeces from heavily parasitized animals (Giles and others, 1985; Love, 1990). Strongyle faecal worm egg counts should always be carried out in horses with suspected chronic enteropathies, although the most important clinical conditions are induced by larval parasitic stages such that a negative or very low egg count in faeces does not rule out a diagnosis of parasitism (Giles and others, 1985; Church and others, 1986; Reinemeyer, 1986; Love, 1990). Although faecal worm egg counts may not closely correlate to size of worm burdens they may give some indication of the efficacy of parasite control programmes (O'Brien, 1985; Herd, 1986; Wescott, 1986).

HISTOPATHOLOGY.

Histopathology of biopsy specimens of abdominal organs obtained at laparotomy will be diagnostic in many cases. Chronically ill and hypoalbuminaemic horses are at high risk for post-operative complications and consequently this method of investigation is frequently avoided (Roberts, 1984; Sweeney, 1987). As equine medical and anaesthetic techniques continue to improve it is likely that clinicians will be less reluctant to use this investigative procedure since it offers the most reliable method of reaching a definitive diagnosis.

Rectal mucosal biopsies may be obtained relatively safely by means of uterine biopsy punch (Palmer, 1987) or rectal biopsy forceps (Traver and Thacker, 1978). Although the rectum may frequently show no changes in animals with chronic enteropathies, histopathology of specimens taken by this technique has proved of value in cases of granulomatous enteritis (Merritt and others, 1976; Hodgson and Allen, 1982; Sweeney and others, 1986), chronic eosinophilic gastroenteritis (Gibson and Alders, 1987) and larval cyathostomiasis (Church and others, 1986). Acid-fast organisms were observed in rectal biopsy material obtained from a horse with granulomatous enteritis (Merritt and others, 1976). Various authors also recommend that biopsy specimens should be cultured for bacteria (Traver and Thacker, 1978; Palmer, 1987).

Skin biopsies may be useful in cases of chronic eosinophilic gastroenteritis (Breider and others, 1985; Gibson and Alders, 1987).

ENDOSCOPY.

Endoscopy may be used to examine the equine stomach for gastric SCC (Tennant and others, 1982).

RADIOLOGY.

Radiology is of very limited use in the investigation of gastrointestinal diseases of adult horses, but it may contribute to the diagnosis of TB (Mair and others, 1986) or gastric SCC (Wrigley and others, 1981). Fluoroscopy may be routinely used for the assessment of oesophageal function in suspected cases of grass sickness (Greet and Whitwell, 1986).

INTESTINAL FUNCTION TESTS.

Routine laboratory analyses may be normal in some cases of malabsorption and specific carbohydrate or lipid absorption tests may be necessary to demonstrate intestinal disease in such animals (Sweeney, 1987). Radioisotope tracer studies are required for the assessment of intestinal permeability and protein loss into the gastrointestinal tract (Roberts, 1984).

ORAL MONOSACCHARIDE TOLERANCE TESTS.

Tests to assess small intestinal function have been described in the horse by using either glucose (Roberts and Hill, 1973) or D(+) xylose (Roberts, 1974; Roberts and Norman, 1979). These tests have been used successfully to demonstrate carbohydrate malabsorption due to villous atrophy, oedema or necrosis of the small intestinal lamina propia (Bolton, Merritt, Cimprich, Ramberg and Streett, 1976) in cases of alimentary LSA (Roberts and Pinsent, 1975; Wilson and others, 1985), granulomatous enteritis (Merritt and others, 1976; Meuten and others, 1978; Roberts and Kelly, 1980; Hodgson and Allen, 1982; Sweeney and others, 1986), chronic eosinophilic gastroenteritis (Pass and Bolton, 1982; Gibson and Alders,

1987) and in two horses with exocrine pancreatic adenocarcinoma and associated diffuse lymphocytic infiltration of the small intestine (Church and others, 1987).

Recently the specificity of oral glucose tolerance tests to small intestinal dysfunction have been studied by Mair and others (1991). These authors found the test to have good specificity for detecting small intestinal disease but they emphasized the need to interpret results of monosaccharide absorption tests with caution since it has been suggested that these tests may be influenced, in addition to actual disease processes, by gastric emptying, intestinal transit time, mucosal blood flow (Roberts and Norman, 1979), bacterial population in the small intestine, diet, and liver and kidney function (Jacobs, Norman, Hodgson and Cymbaluk, 1982; Sweeney, 1987) and, when glucose is used, insulin levels, stress and length of fasting should be taken into account (Bolton and others, 1976).

RADIOISOTOPE TRACER STUDIES.

Standard laboratory methods may show no abnormalities in the chemical composition of blood and tissues of horses with chronic enteropathy and only in a few instances can the mechanisms responsible for any changes observed be ascertained. Currently the most informative approach to the understanding of the pathophysiological mechanisms of intestinal disease is brought about by radioisotope tracer techniques.

Intravenous administration of radioisotope-labelled albumin (with ^{125}Iodine or ^{51}Chromium) or ^{51}Chromium-chloride and subsequent measurement of radioactivity in plasma, faeces and urine have been used in the horse to investigate altered protein metabolism and/or protein loss into the gastrointestinal lumen in cases of granulomatous enteritis (Merritt and others, 1976; Merritt, Kohn, Ramberg, Cimprich, Reid and Bolton, 1977; Meuten and others, 1978) and PBZ toxicity (Snow and

others, 1981) and this method has also been used to study naturally occurring and experimental strongyle infections (Duncan and Dargie, 1975; Dietz and Nielsen, 1980; Love, 1990).

Loss of red blood cells into the gastrointestinal tract has been confirmed in foals experimentally infected with adult *Strongylus vulgaris* and in naturally infected ponies, in which faecal excretion of radioisotope was found to be higher than in control animals after the intravenous injection of ^{51}Cr-labelled red blood cells (Duncan and Dargie, 1975).

Decreased intestinal absorption of fat has been demonstrated in a horse with granulomatous enteritis following administration by stomach tube of a capsule containing tritiated oleic acid and measuring radioactivity counts in plasma (Meuten and others, 1978).

INTESTINAL PERMEABILITY STUDIES.

With such similarity of many of the clinical, pathological and laboratory aspects of many cases of chronic enteropathy in the horse it is frequently difficult to diagnose a specific condition.

The difficulties of reaching a definitive diagnosis in chronic alimentary diseases of horses and ponies have important implications to the treatment or management of affected animals in that these are often made on empirical judgements. In part, the difficulty of making definitive diagnoses in such cases is due to a lack of detailed knowledge of the pathogenesis of most chronic enteropathies. These diseases often manifest as an apparent abnormal absorption or increased secretion of metabolites across the bowel wall although the mechanisms of these dysfunctions are not well defined.

In human patients with chronic enteropathy it is thought that alterations of intestinal permeability may be important in the pathogenesis of various conditions. Consequently, attention has been given to developing techniques of assessing intestinal permeability using marker substances such as mannitol, lactulose and ^{51}Chromium-labelled ethylenediaminetetraacetate (^{51}Cr-EDTA). Various properties of ^{51}Cr-EDTA make it suitable as a marker of transmucosal diffusion: it has been confirmed to be stable and physiologically inert (Stacy and Thorburn, 1966); it is confined to an extracellular location and is not bound by plasma proteins; it is not taken up or metabolised by erythrocytes or organs other than the kidneys (Garnett, Parsons and Veall, 1967; Aurell, 1968; Favre and Wing, 1968; Chantler, Garnett, Parsons and Veall, 1969). Within 24 hours following intravenous administration of ^{51}Cr-EDTA about 98% of the dose is excreted via the kidneys (Garnett and others, 1967; Favre and Wing, 1968; Aurell, 1968) such that urinary excretion of ^{51}Cr-EDTA provides a method of assessing glomerular filtration rate (Chantler and others, 1969; Granerus and Aurell, 1981)

In addition to its use for investigating renal function, ^{51}Cr-EDTA has been successfully applied to the investigation of chronic enteropathies in man (Bjarnason, Peters and Veall, 1983a; Bjarnason, O'Morain, Levi and Peters, 1983b; O'Morain, Abelow, Chervu, Fleischnen and Das, 1986; Maxton, Bjarnason, Reynolds, Catt, Peters and Menzies, 1986; Behrens, Szaz, Northrop, Elia and Neale, 1987; Jenkins, Jones, Goodacre, Collins, Coates, Hunt and Bienenstock, 1987; Elia, Behrens, Northrop, Wraight and Neale, 1987; Jenkins, Ramage, Jones, Collins, Goodacre and Hunt, 1988), dogs (Hall, Batt and Brown, 1989; Hall and Batt, 1990), gerbils and lambs (Sinski, Maclean and Holmes, 1987; Maclean, Sinski and Holmes, 1989), rats (Bjarnason, Smethurst, Levi and Peters, 1985; Ramage, Stanisz, Scicchitano, Hunt and Perdue, 1988) and ponies (Love, 1990). The technique involves the administration to fasted patients of a solution of ^{51}Cr-EDTA and the amount of tracer detected in urine is used to

calculate the percentage of the administered dose absorbed from the bowel which would be an estimation of intestinal permeability. Such a technique may be of use to detect intestinal mucosal damage in, to study the pathophysiology of, and to monitor the response to treatment of, equine chronic enteropathies.

The objectives of the studies reported in this thesis were to validate ^{51}Chromium-labelled ethylenediaminetetraacetate as a marker for the assessment of intestinal permeability in normal ponies and to study alterations in intestinal permeability in ponies with either experimentally-induced or naturally occurring chronic enteropathy.

CHAPTER II. GENERAL MATERIALS AND METHODS.

CHAPTER II. GENERAL MATERIALS AND METHODS.

EXPERIMENTAL ANIMALS.

Four British native-breed female pony yearlings were used in Experiments 1, 2, 3, 5 and 6. These ponies were reared indoors with their dams which received repeated doses of ivermectin. They were considered to be helminth-naive and they were between 10 and 14 months old at the time the studies were carried out.

Before being weaned at four or five months of age, the ponies suckled their dams and were fed on hay and a coarse mix. Following weaning, animals received a commercially-produced diet (Complete Cubes, Spillers). Both food and water were available *ad libitum*. They were kept in individual stalls on woodshavings which were cleaned out daily and they were regularly exercised on concrete. For two days prior to, and during the time at which the experiments were carried out, the foals were tethered and kept on rubber matting. The animals were allowed two days to acclimatise to the collection apparatus and they were fasted for ten hours previous to and then for two hours after the administration of the radioisotope.

Two British native-breed five year-old ponies with permanent caecal fistulation were used in Experiment 4 and, in Experiment 7, the test was applied to three horses with suspected chronic enteropathy referred to the Department of Veterinary Medicine of the University of Glasgow Veterinary School. A detailed account of the history and management of the fistulated ponies, and of the history, clinical, laboratory and pathological findings and management of the clinical cases is given in the appropriate sections of Experiments 4 and 7 in Chapters III and IV, respectively.

RADIOISOTOPE TECHNIQUE.

51CHROMIUM-ETHYLENEDIAMINETETRAACETATE (51Cr-EDTA) PREPARATION.

An injectable commercial preparation of ^{51}Cr-EDTA (Chromium (^{51}Cr)-EDTA Injection, Amersham International, Amersham), commonly utilised for the assessment of glomerular filtration rate, was used in each of the experiments. The product is available in 10 ml (37 MBq $\pm$ 10%) multidose vials containing 1% benzyl alcohol as bacteriostat. According to the data sheet, each ml of Chromium (^{51}Cr)-EDTA Injection contains 0.3-0.7 mg of chromium EDTA and 0.9-2.4 mg of sodium edetate, depending on the specific activity and radioactive concentration of the batch.

ADMINISTRATION OF 51Cr-EDTA.

Four vials containing 10 ml of Chromium (^{51})-EDTA were used on each occasion. The contents of the four vials were emptied into a beaker and thoroughly mixed. After taking a sample of 1 ml which subsequently was used to make up the standard solution (*vide infra*), four equal volumes were dispensed into syringes. These syringes were accurately weighed when they were containing the ^{51}Cr-EDTA solution and again once they had been emptied.

These doses of ^{51}Cr-EDTA were administered via nasogastric intubation by means of a two metre long, one centimetre diameter, stomach tube. The ^{51}Cr-EDTA solution was then flushed through the tube with 300 ml of water.

SAMPLES.

Urine.

The design described by Harris (1988) was modified to construct urine collection funnels from rubber, aluminium strips and metal rivets. The funnels and harnesses are shown in Figures 1.1 and 1.2. (Chapter III). Urine was collected directly into polythene bags after flowing down the funnel. These bags were fixed to the neck of the funnel by rubber bands and held within canvas bags which were hanging from the harnesses. Movement of the harnesses or leakage from the funnels were checked for regularly and re-adjustments made when required. Total urine collections were performed for 72 hours: the urine bags were changed every six hours and urine weights were recorded for 6-hour periods on the first day and 12-hour periods thereafter. The bulked total collection for each interval was thoroughly mixed before four 1-ml samples were taken.

Faeces.

The faeces produced by the ponies in 72 hours were collected at intervals of six hours on the first day and of 12 hours on the second and third days. The weight of the collections at each interval was recorded. Collection was directly into polythene bags suspended within canvas bags which were held over the anus of the ponies by attachments to the harnesses. Five faecal samples of approximately 10 mg were taken following thorough mixing of the bulked total from each collection period.

Blood.

In Experiment 5, blood samples were collected 6-hourly to look for the presence of the radioisotope in the peripheral circulation. Blood was taken from left jugular veins into heparinised, vacuum tubes via 1", 21G needles. The samples were centrifuged and 4 ml of plasma taken into counting vials.

RADIOACTIVITY MEASUREMENTS.

STANDARD SOLUTIONS.

Standard solutions were made up by adding water to accurately weighed samples of approximately 1 ml of the isotope-labelled preparation to give a total volume of 1000 ml. Ten 1-ml samples of the standard solution were then pipetted into separate counting vials and 9 ml dilute NaOH were added to each one. The standard solution samples were used to calculate the injected doses of isotope once the exact weight of Chromium (^{51}Cr)-EDTA administered to each animal was known, and to detect variations in counting sensitivity of the gamma counter. They also served as correction factors for radioactive decay.

FAECES AND URINE SAMPLES.

Each of the four 1-ml urine samples was accurately weighed and then made up to 10 ml with dilute NaOH. Five samples of approximately 10 g from each faecal collection were packed into a volume of 10 ml and then accurately weighed.

RADIOACTIVITY COUNTING.

Radioactivity counting of faeces and urine samples was carried out in an automatic gamma counter (Minaxi Auto-Gamma Counter 5000 Series, Canberra-Packard). Each sample was measured for one minute and the result was expressed as counts per minute (c.p.m.).

CALCULATION OF INTESTINAL PERMEABILITY.

The percentages of administered ^{51}Cr-EDTA recovered in urine and faeces collected during 6-hour and 12-hour periods were calculated by dividing the ^{51}Cr activities within these periodic collections by the total administered activity, and multiplying the result by a factor of 100.

BIOCHEMICAL TECHNIQUES.

Serial blood samples were taken into vacutainers containing lithium heparin and all assays were performed by the laboratory of the Department of Clinical Biochemistry. Continuous flow analysis (Technicon Auto Analyser) by standard biuret and bromcresol green methods was used for measurement of total plasma protein and plasma albumin respectively. The level of serum globulin was calculated as the difference between the values of total protein and albumin.

As a degree of renal toxicity was expected in Experiment 5, blood samples were obtained during the period of phenylbutazone administration and the levels of blood urea nitrogen, creatinine and plasma electrolytes were determined by standard methods. Renal clearance and fractional excretion of sodium, potassium, chloride, calcium and phosphate (Morris, Divers and Whitlock, 1984; Kohn and Chew, 1987; Harris and Colles, 1988) were calculated to evaluate the influence of renal dysfunction on the excretion of the radioisotope tracer.

HAEMATOLOGICAL TECHNIQUES.

Vacuum tubes containing ethylenediaminetetraacetic acid were used to collect the blood samples by jugular puncture. The haematological investigations were carried out in the Department of Veterinary

Pathology. Total red and white blood cell counts were obtained by means of an automated Coulter-counter (Coulter ZF6 plus C100 Channelyzer, Coulter Electronics Ltd.) and differential cell counts were performed on blood films stained by the May-Grunwald Giemsa staining technique.

PARASITOLOGICAL AND PATHOLOGICAL TECHNIQUES.

FAECAL WORM EGG COUNTING.

The modified McMaster technique described by Gordon and Whitlock (1939) was used to examine the faecal samples. Forty-two ml of water were added to three grams of faeces which were homogenized to form a suspension that was passed through a 250 micron sieve and the filtrate collected into a plastic bowl. A 15 ml sample of the filtrate was taken into a flat bottomed tube and centrifuged at 2000 revolutions per minute for three minutes after which the supernatant which had formed was poured off. The pellet of faeces within the tube was agitated (Vortex Junior Mixer, Scientific Industries Inc., New York) and then resuspended in a saturated solution of sodium chloride. A plastic pipette was used to fill both chambers of a McMaster worm egg counting slide (Gelman Hawksley Ltd., Northampton) with this final suspension. The worm eggs under both grids of the slide were counted by means of a dissecting microscope (Wild M5 stereomicroscope) and the number multiplied by 50 to give the number of eggs per gram (epg).

LARVAL CULTURE.

Cyathostome larvae for the experimental infection were obtained from a naturally infected mare. Freshly voided faeces were collected and placed into plastic pots to occupy two thirds of their 500 ml volume. The pots were covered with loosely fitting lids and incubated at 23°C for 10-14

days. Recovery of larvae was performed in four stages. First, the cultures pots were filled with lukewarm water and left for 2-3 hours. Secondly, the fluid from the pots was passed through a coarse sieve and collected as a pooled sample. Thirdly, the pooled sample was passed through two milk filter pads (Maxa Milk Filters, A. McCaskie Ltd., Stirling) in a Buchner funnel. Finally, the filters containing the larvae, were placed onto a Baermann apparatus which was filled with lukewarm tap water. Larvae migrated out of the filter papers and down into the clamped neck of the funnel from where they were collected after six hours.

The suspension was examined microscopically in order to confirm that only larvae of cyathostome species were present and to calculate the larval concentration. A total of 1 ml of the suspension was examined, in 0.025 ml aliquots, and the number of larvae per ml was then used to calculate the volume required for the individual larval doses. Immediately prior to administration, individual doses were pipetted from the well-mixed larval suspension into glass universal bottles.

NECROPSY AND SAMPLING.

Two animals were examined post-mortem: Pony number 64 was found dead one night and examined the following morning; Pony number 63 was shot using a free bullet from a pistol. After death the carcases and viscera were examined for gross pathological changes, which were recorded, prior to separation of the gastrointestinal tract. In each animal the caecum, ventral colon and dorsal colon were collected separately along with their contents, which were washed with water into separate buckets and then made up to standardised volumes. The diluted contents were thoroughly mixed before samples of 10% volume were taken into plastic containers to which formalin was added as a preservative. The caecum, ventral colon and dorsal colon were weighed separately and

samples of 10% by weight were cut as longitudinal strips from the haustra between taenial bands; taking approximately equal amounts from proximal, middle and distal parts of each organ.

WORM COUNTING.

In order to count lumenal worms 5 ml aliquots of the intestinal contents were pipetted into Petri dishes. Each aliquot was stained for a few minutes with a few drops of 45% iodine solution, then decolourised with 5% sodium thiosulphate solution and the worms counted under a dissecting microscope. For each animal, 10% subsamples of the 10% samples of the contents of the separate intestinal segments were examined i.e. the worms in a one per cent of the intestinal contents were counted and the total values obtained by multiplying by a factor of one hundred.

A method of mural transillumination similar to that described by Reinemeyer and Herd (1986) was used to count mucosal cyathostome larval stages. When possible, mucosa of the 10% samples of caeca, ventral and dorsal colons was manually stripped from the serosa and placed in small pieces into grid-marked Petri dishes. The tissue was examined at x15 magnification with a dissecting microscope and the mucosal cyathostome larvae within the whole sample were counted.

STATISTICAL METHODS.

The cumulative percentages of ^{51}Cr-EDTA measured in urine in each experiment at 24 and 72 hours were tested statistically by one-way analysis of variance and differences were investigated by use of the Newman-Keuls Range Test. The level of significance was chosen to be probability values of $p < 0.05$.

CHAPTER III. VALIDATION OF ^{51}CHROMIUM-ETHYLENE DIAMINE TETRAACETATE (^{51}Cr-EDTA) AS A TEST TO STUDY INTESTINAL PERMEABILITY IN THE HORSE.

CHAPTER III. VALIDATION OF ^{51}CHROMIUM-ETHYLENE DIAMINE TETRAACETATE (^{51}Cr-EDTA) AS A TEST TO STUDY INTESTINAL PERMEABILITY IN THE HORSE.

INTRODUCTION.

Intestinal function tests have been widely used in horses for the investigation of chronic enteropathies. Those presently available are based on the absorption of sugars i.e. oral glucose (Roberts and Hill, 1973) and oral D(+)-xylose tolerance tests (Roberts and Norman, 1979). They are generally of most use in detecting small intestinal dysfunction because they are specifically absorbed in that part of the alimentary tract.

It has been well-recognised that the large intestine plays an essential role in the metabolic processes of horses (Argenzio, 1975). Many chronic enteropathies are either localised to, or involve part of, the posterior bowel and consequently there will often be disturbances of large intestinal metabolism such that a method of assessment of equine large intestinal function would complement the small intestinal function tests.

Chronic enteropathies are relatively common in human medicine and the so-called "inflammatory bowel diseases" (Crohn's disease and ulcerative colitis) or coeliac disease have some similar features to those of chronic enteritides in horses. For example, affected human and equine cases are frequently found to have a decrease in the serum albumin levels which may result from an increase in the permeability of intestinal mucosa to plasma protein molecules. Alterations in the intestinal permeability have also been proposed as important mechanisms in the pathogenesis of these conditions (Chadwick, Phillips and Hofmann, 1977; Bjarnason and Peters, 1984; Jenkins and others, 1988) and may contribute to the increased incidence of neoplasia in patients with coeliac disease (Walker and Isselbacher, 1974), Crohn's disease (Gyde, Prior, MacArtney,

Thompson, Waterhouse and Allan, 1980) and ulcerative colitis (Lennard-Jones, Morson, Ritchie, Shore and Williams, 1977). Thus, compromise of mucosal integrity may contribute to both cause and effect of certain intestinal enteritides.

To investigate alterations in intestinal permeability, several probes have been used in human medicine including polyethylene glycols (Chadwick and others, 1977), lactulose, mannitol and L-rhamnose (Maxton and others, 1986; Elia and others, 1987). Although all appear to fulfill the requirements for probes of passive intestinal permeation the awkward analytical techniques needed for their quantitation to some extent limit their usefulness. Pathophysiological studies using radioisotopes such as ^{3}H-cellobiotol, ^{14}C-mannitol or ^{51}Cr-Ethylenediaminetetraacetate (Sandhu and Fraser, 1982; Behrens and others, 1987; Fotherby, Wraight and Neale, 1988) are increasingly acquiring more importance in gastroenterology as they are simple, reproducible and they can be easily quantitated without any specimen preparation. Of these, ^{51}Cr-EDTA seems to be the most widely accepted marker both *in vivo* (Bjarnason and others, 1983a; Bjarnason and others, 1983b; O'Morain and others, 1986; Elia and others, 1987; Jenkins and others, 1987; Jenkins and others, 1988; Fotherby and others, 1988) and *in vitro* (Bjarnason and Peter, 1984).

In the preliminary clinical studies undertaken using ^{51}Cr-EDTA in human patients with inflammatory bowel disease (IBD) it was considered that the absorption of the tracer was limited to the small intestine and, consequently, increased ^{51}Cr-EDTA urinary excretion would specifically indicate disease in this portion of the gastrointestinal tract (Bjarnason and others, 1983a, b). However, recent studies confirmed that significant amounts of ^{51}Cr-EDTA were in fact absorbed from the large intestine in patients with IBD (Elia and others, 1987; Jenkins and others, 1987 and 1988). The latter observation may be of relevance in developing a

sensitive test to demonstrate alterations in large intestinal permeability in horses with enteropathy when small intestinal function tests detect no abnormalities.

These experiments were designed to validate ^{51}Cr-EDTA as a test of intestinal permeability in ponies: first, under control conditions; then following both increased and decreased intestinal motility which it was thought might influence the values obtained; and the test was also carried out in ponies with caecal fistulation in order to quantify the absorption of ^{51}Cr-EDTA from the large intestine.

EXPERIMENT 1. INTESTINAL PERMEABILITY TO ^{51}Cr-EDTA IN CONTROL PONIES.

INTRODUCTION.

^{51}Chromium-Ethylenediaminetetraacetate (^{51}Cr-EDTA), administered orally and measured in urine as a reflection of the amount absorbed from the gastrointestinal tract, has been successfully used for the diagnosis and investigation of a variety of conditions affecting the alimentary tract of human patients (*vide supra*). Preliminary studies involving the use of ^{51}Cr-EDTA have also been undertaken in rats (Bjarnason and others, 1985; Ramage and others, 1988), lambs and gerbils (McLean and others, 1989), dogs (Hall and others, 1989; Hall and Batt, 1990) and ponies (Love, 1990) in which the results suggested that ^{51}Cr-EDTA urinary excretion was a suitable method for investigating intestinal permeability in these animals.

The objective of this experiment was to provide further data for ^{51}Cr-EDTA excretion in healthy ponies under control conditions.

MATERIALS AND METHODS.

Four female British native-pony yearlings were administered a solution of ^{51}Cr-EDTA by stomach tube following a period of fasting of 10 hours. The solution was washed through the tube with 300 ml of water. The animals were fasted for a further period of two hours following administration of the test solution.

Total faeces and urine produced in 72 hours were collected at 12-hour intervals. The system used for these collections is illustrated in Figures 1.1 and 1.2.

FIGURE 1.1. Urine collection apparatus.

FIGURE 1.2. Urine and faeces collection apparatus in position.

RESULTS.

The amounts of ^{51}Cr excreted in urine were calculated both as the mean percentage during each collection interval and as the mean cumulative percentage and are illustrated in Figures 1.3 and 1.4, respectively. The same system was used to express radioactivity found in faeces as shown in Figures 1.5 and 1.6. A detailed account of the values obtained at each interval and of the daily subtotals is given in Appendices 1.1 for urine and 2.1 for faeces.

The results obtained for urine in 72 hours ranged from 3.93% to 9.02% of the administered dose with a mean value of 6.64% (SD $\pm$ 2.36). The amount of ^{51}Cr recovered in faeces ranged from 85.51% to 92.99% of the total radioactivity administered, with a mean of 89.85% (SD $\pm$ 3.47).

Approximately 85% of the total ^{51}Cr excreted in urine was detected in urine collected during the first 24 hours following administration of the test dose whereas only 50% of the total amount found in faeces was voided in the same time interval.

DISCUSSION.

In the present study, the mean percentage excretion of ^{51}Cr-EDTA for consecutive 24 hour periods were 5.24 $\pm$ 1.93, 1.11 $\pm$ 0.42 and 0.29 $\pm$ 0.06, respectively (mean $\pm$ SD) which are comparable to the values recorded for ^{51}Cr-EDTA excretion in previous studies in ponies (Love, 1990). Interestingly, in that study only approximately 50% of the total amount of ^{51}Cr excreted in urine was detected in the first 24 hour collection, in contrast to approximately 85% recovered in the present study. This difference might be explained by the protocol used: in the present study experimental animals were fasted for a total of 12 hours

FIGURE 1.3. Experiment 1: Mean percentage of administered ^{51}Cr-EDTA measured in urine in control ponies.

FIGURE 1.4. Experiment 1: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in urine in control ponies.

FIGURE 1.3.

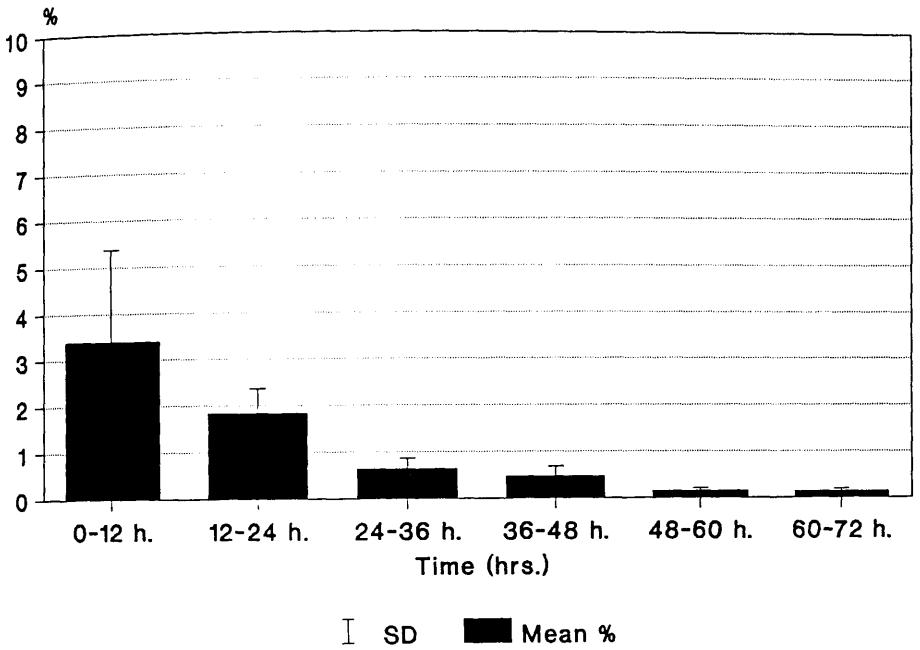


FIGURE 1.4.

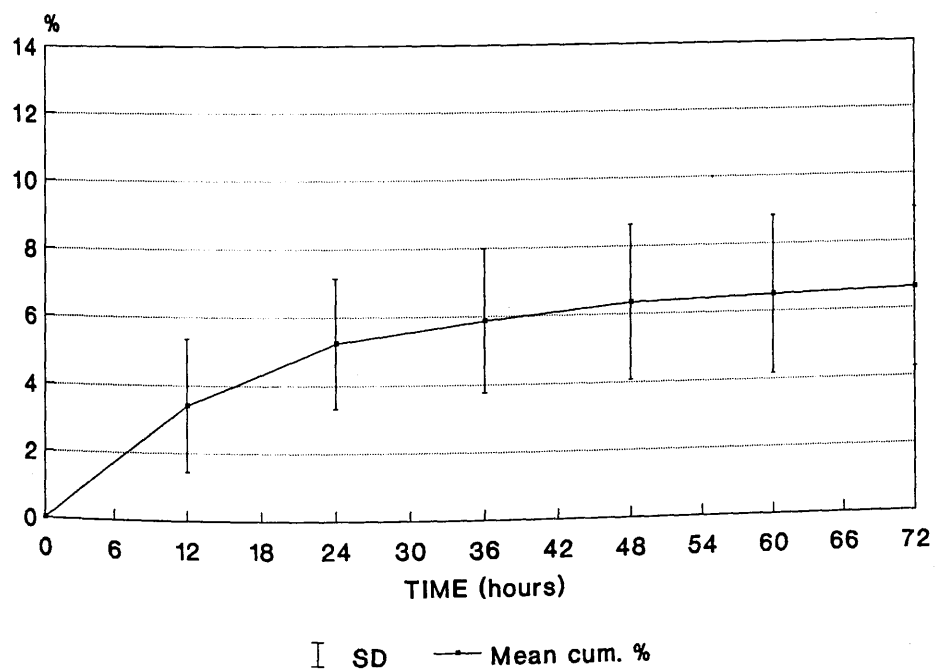


FIGURE 1.5. Experiment 1: Mean percentage of administered ^{51}Cr-EDTA measured in faeces in control ponies.

FIGURE 1.6. Experiment 1: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in faeces in control ponies.

FIGURE 1.5.

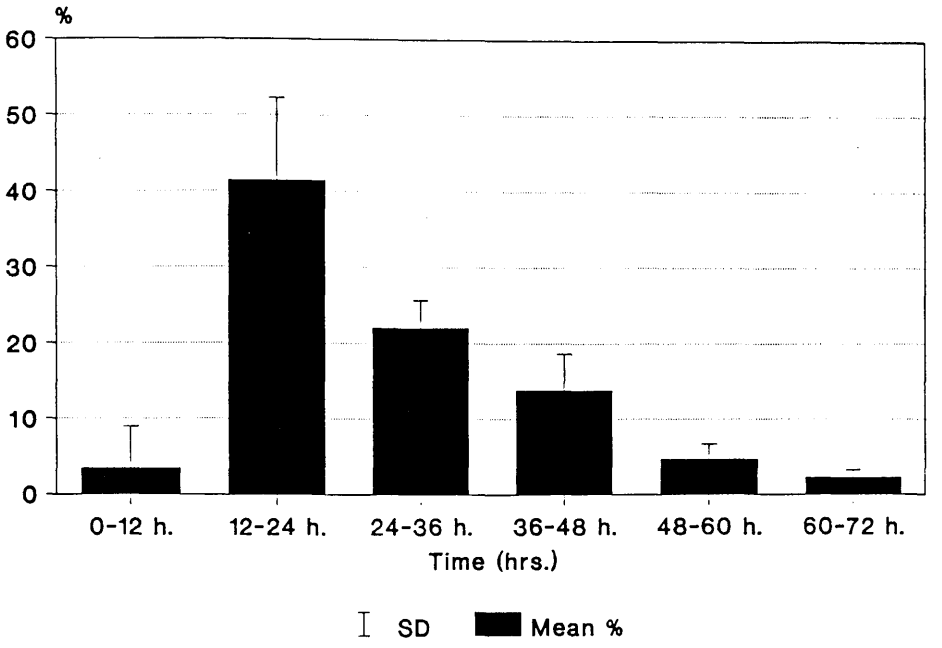
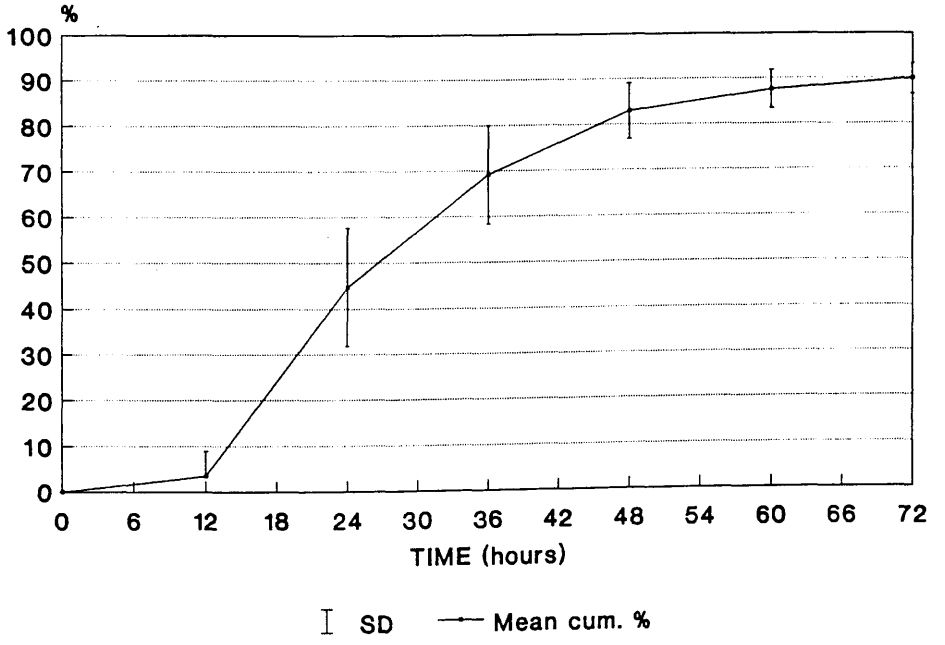


FIGURE 1.6.



around the time of administration of the marker whereas in Love's study, there was no food withdrawal at all. It has been reported (Bjarnason and others, 1985) that the osmolarity and composition of the test dose affects the absorption of ^{51}Cr-EDTA from the gut and it could be hypothesized that the same factors might play a role in the absorption pattern of this marker.

In studies of intestinal permeability, values obtained for 24 hour urinary excretions reported in normal human patients have ranged from 0.61% to 2.82% (Bjarnason and others, 1983a; Bjarnason and others, 1983b; O'Morain and others, 1986; Elia and others, Wraight and Neale, 1987; Fotherby and others, 1988) and values recorded in healthy dogs were between 2.3% and 17.6% with a median of 13% (Hall and others, 1989). The values obtained from ponies in the present work and in previous equine studies were similar to those in man and generally lower than those in dogs. Hall and others (1989) suggested that the high values recovered in urine in dogs might indicate either a greater rate of enterocyte turnover or a major difference in the total surface area of the intestine in this animal species. These differences may reflect the relative importance within each species of the large intestine as a site of absorption of EDTA - which has been presumed to be low in most monogastric animals. However, in horses the large intestine is pivotal to nutrient, electrolyte and water absorption. It was demonstrated by Argenzio, Lowe, Pickard and Stevens (1974) that 68% of a soluble marker was within the caecum two hours after being administered intragastrically and its subsequent retention of about 50 hours in the colon gives support to the idea of a greater absorption of EDTA in this part of the bowel in the equine species.

EXPERIMENT 2. INTESTINAL PERMEABILITY TO ^{51}Cr-EDTA IN PONIES AFTER PREMEDICATION WITH ATROPINE SULPHATE.

INTRODUCTION.

When studying patterns of intestinal permeability, one has to bear in mind that gastric emptying, luminal dilution, intestinal transit time, mucosal surface area, blood and lymphatic flow and renal excretion as well as mucosal permeability determine the absorption of the different markers (Bjarnason and Peters, 1984). When permeability is assessed in normal, 'healthy' animals it is assumed that these factors, with the exceptions of gastric emptying, intestinal transit time and motility patterns, would have no major influence in standardised experimental protocols. However, minor differences in motility may occur in control animals due to the individual endocrine system function and the activity of the autonomic nervous system and reflex neural pathways (Clark, 1990). Further, alterations in intestinal motility have been demonstrated in horses with acute abdominal disease (Gerring, 1989) and strongylosis (Bueno, Ruckebusch and Dorchies, 1979; Berry, Merritt, Burrows, Campbell and Drudge, 1986) and although similar abnormalities have not been reported for the equine chronic enteropathies one must assume that they may be important features of these conditions. In relation to intestinal permeability, motility changes may alter the thorough mixing of the intestinal contents as well as the time the digesta is in contact with the mucosa for optimal absorption (Roger and Ruckebusch, 1987) and thus they might influence the pattern of absorption of intestinal markers.

During the last 15 years much work has been carried out to study the intestinal motility patterns in horses, principally by means of electrodes and strain gauge force transducers, to try to correlate the myoelectrical activity with the mechanical intestinal contractions. These methods have been used to study changes of equine intestinal motility

resulting from feeding (Ruckebusch and Bueno, 1977; Davies and Gerring, 1983; Ross, Cullen and Rutkowsky, 1990) and also following strongyle parasitic infections (Bueno and others, 1979; Berry and others, 1986). There have also been some reported studies on the effect of various drugs on the intestinal motility with special reference to post-prandial motility patterns (Adams, Lamar and Masty, 1984; Roger and Ruckebusch, 1987; Merritt, Campbell-Thompson and Lowrey, 1989; Rutkowsky, Ross and Cullen, 1989).

In the present study healthy, fasted ponies were used and there was only limited data relating to pharmaceutical modulation of intestinal motility in such animals.

Atropine sulphate has been considered to be the prototypical muscarinic blocking agent, preventing acetyl choline (ACh) from affixing to the receptor area in a competitive manner (Adams, 1988). In this way, atropine causes relaxation of gastrointestinal smooth muscle by inhibiting contractile effects of endogenous ACh throughout the length of the bowel. Reduced electrical activity of equine gastrointestinal tract following administration of atropine sulphate at dose rates of 0.05 mg/kg subcutaneously (Roger and Ruckebusch, 1987) and of 0.044 mg/kg intravenously (Adams, 1984) has been recorded in experimental studies in horses. Thus, following administration of this drug it might be expected that transit time of ingesta along the gastrointestinal tract would be prolonged (Roberts and Argenzio, 1986). For the present work it was decided to administer 0.06 mg/kg atropine sulphate subcutaneously 30 minutes before the oral administration of the radioisotope.

MATERIALS AND METHODS.

Four female British native-pony yearlings were administered a solution of ^{51}Cr-EDTA by stomach tube following a period of fasting of 10 hours. The solution was washed through the tube with 300 ml of water. The animals were fasted for a further period of two hours following administration of the test solution.

Total faeces and urine produced in 72 hours were collected at 6-hour intervals on the first day and subsequently at 12-hour intervals.

Thirty minutes before the administration of the ^{51}Cr-EDTA solution, 0.06 mg/kg of atropine sulphate (Bimeda U.K. Limited, Liverpool) was injected subcutaneously. Repeat abdominal and cardiac auscultation was carried out and signs of abdominal pain and evidence of mydriasis were checked for as subjective parameters to quantitate the effect of atropine.

RESULTS.

The amounts of ^{51}Cr excreted in urine were expressed both as the mean percentage for each collection period and as the mean cumulative percentage and they are illustrated in Figures 2.1 and 2.2, respectively. Radioactivity counts found in faeces are shown in Figures 2.3 and 2.4. Details of the values obtained at each interval and of the daily subtotals are given in Appendices 1.2 and 2.2.

Values obtained in urine in the 72 hours collection ranged between 3.60% and 8.80% with a mean of 5.60% (SD $\pm$ 2.39). Approximately 60% of the total amount was excreted during the first 24 hours following

FIGURE 2.1. Experiment 2: Mean percentage of administered ^{51}Cr-EDTA measured in urine in ponies following premedication with 0.06 mg/kg atropine sulphate subcutaneously.

FIGURE 2.2. Experiment 2: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in urine in ponies following premedication with 0.06 mg/kg atropine sulphate subcutaneously.

FIGURE 2.1.

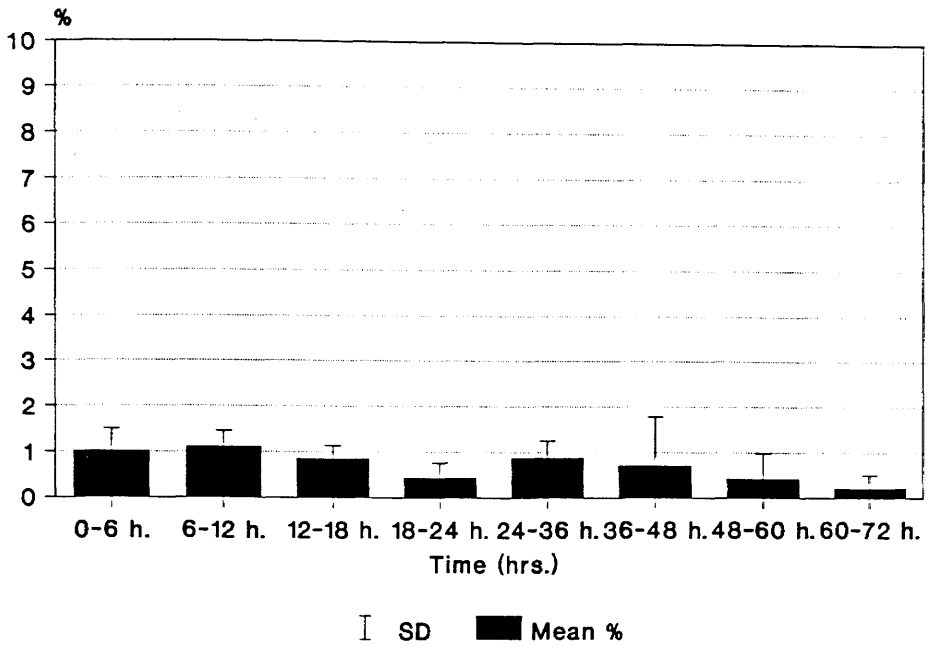


FIGURE 2.2.

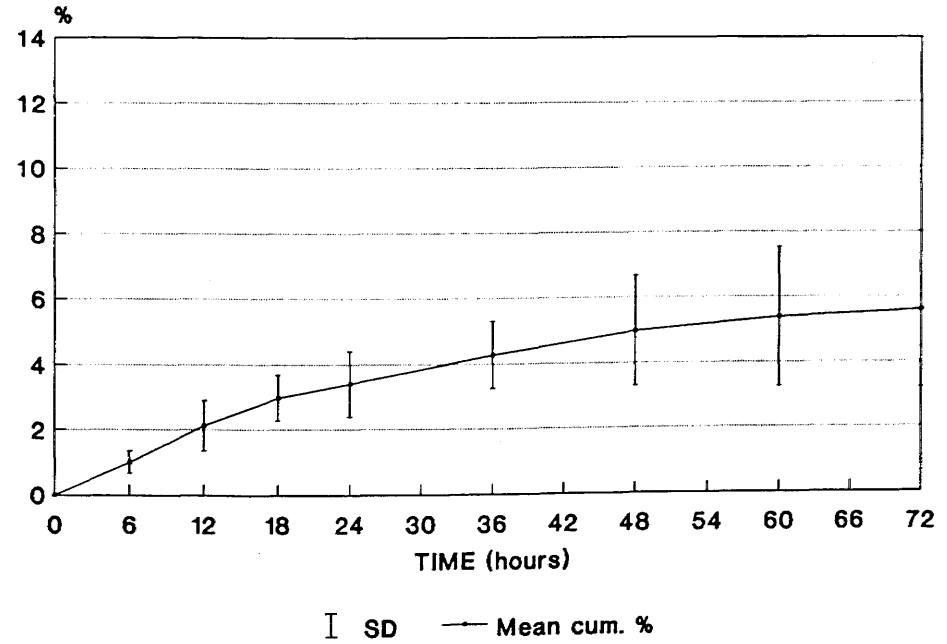


FIGURE 2.3. Experiment 2: Mean percentage of administered ^{51}Cr-EDTA measured in faeces in ponies following premedication with 0.06 mg/kg atropine sulphate subcutaneously.

FIGURE 2.4. Experiment 2: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in faeces in ponies following premedication with 0.06 mg/kg atropine sulphate subcutaneously.

FIGURE 2.3.

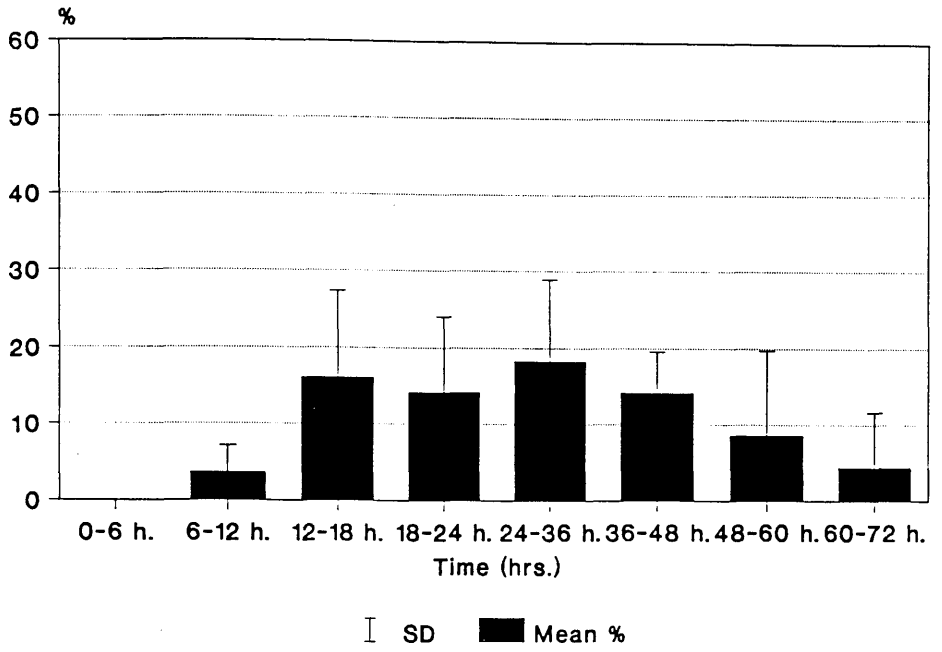
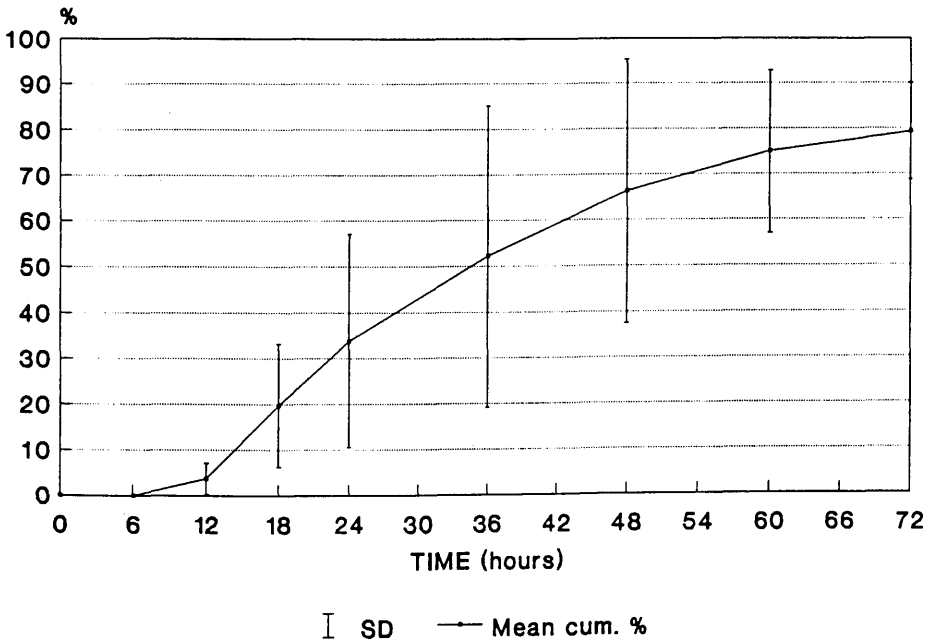


FIGURE 2.4.



^{51}Cr-EDTA administration. The amount of ^{51}Cr measured in faeces ranged from 63.53% to 85.81%, with a mean value of 79.36% (SD ± 10.65) of which approximately 43% was collected on the first day.

DISCUSSION.

None of the ponies showed any of the signs reported to be associated with the administration of atropine to horses (Ducharme, 1983), apart from one of the animals (number 70) which seemed to have a slight, transient pupillary dilation. Borborygmi appeared to be normal and no evidence of increased heart rate or abdominal pain was observed. Interestingly, there was an apparently increased faecal water content (with the exception of Pony number 70). This observation agrees with the results of experimental investigations reported by Alexander (1978). Only 63.53% of the administered dose was recovered in the faeces of pony number 70 in the total time of collection, whereas the mean faecal excretion for the rest of the ponies was 84.64%. The latter figure was slightly lower than a mean of 89.85% observed in control animals in Experiment 1 which might indicate a slower transit time in animals premedicated with atropine sulphate and that this effect was greatest in Pony number 70.

In this study, one individual animal (Pony no. 70) showed a more protracted excretion of ^{51}Cr in urine over the three days of the experiment - 44% on the first day and 35% on the second day - compared with the excretion of approximately 70% of the administered dose on the first day in the other ponies. This finding implies that there was significant absorption of EDTA from the large intestine of pony no. 70, in which the intestinal transit time was apparently prolonged.

However, no significant differences were found between the values of ^{51}Cr-EDTA excretion obtained in this experiment (mean, 5.60%; SD ± 2.39) and those obtained earlier in the same ponies with no treatment (mean, 6.64%; SD ± 2.36). In conclusion, it would appear that a slight prolongation of the intestinal transit time did not significantly affect the absorption of EDTA from the gastrointestinal tract and this finding is in agreement with the work conducted in rats by Bjarnason and others (1985).

EXPERIMENT 3. INTESTINAL PERMEABILITY TO ^{51}Cr-EDTA IN PONIES AFTER PREMEDICATION WITH BETHANECHOL.

INTRODUCTION.

This experiment was designed to evaluate the influence that the stimulation of the gastrointestinal motility might have on the absorption of ^{51}Cr-EDTA.

There are many parasympathetic compounds which increase the rate of transit of ingesta through the gastrointestinal tract. Adams (1988) classified these agents into two main groups: those acting directly on the muscarinic and/or nicotinic receptors such as methacholine, carbachol and bethanecol, or those which inhibit cholinesterase including physostigmine, neostigmine, edrophonium and organophosphate derivatives.

Many of these compounds also have other parasympathetic activities. For example, carbachol acts on both muscarinic and nicotinic receptors and consequently it effects the cardiovascular system, uterine smooth muscle, skeletal muscle and, particularly, the autonomic ganglia and the chromaffin cells of the adrenal medulla as well as the gastrointestinal tract (Adams, 1988). Similarly, compounds which inhibit cholinesterase do not specifically act upon the gastrointestinal tract and they may also have undesired effects, such as abdominal discomfort associated with neostigmine injection to ponies (Adams, Lamar and Masty, 1984; Gerring, 1989). By contrast, administration of bethanecol has been reported to modify gastrointestinal motility without side effects when given at a dose rate of 0.05 mg/kg (Roger and Ruckebusch, 1987).

Due to this reported specificity of action of bethanecol this compound was considered most suitable to increase gastrointestinal motility in the ponies in this experiment.

MATERIALS AND METHODS.

Four female British native-pony yearlings were administered a solution of ^{51}Cr-EDTA by stomach tube following a period of fasting of 10 hours. The solution was washed through the tube with 300 ml of water. The animals were fasted for a further period of two hours following administration of the test solution.

Total faeces and urine produced in 72 hours were collected at 6-hour intervals on the first day and subsequently at 12-hour intervals.

Thirty minutes prior to the administration of the ^{51}Cr-EDTA solution, each animal was given a subcutaneous injection of 0.05 mg/kg of bethanecol (Carbamyl-b-Methyl-Choline Chloride, SIGMA Chemical Company).

No equipment was available for the study of the myoelectrical activity of the alimentary tract after premedication with bethanecol and abdominal auscultation was the clinical parameter used to monitor this response.

RESULTS.

A slight increase in the audible borborygmi was noticed in the animals as a result of the administration of bethanecol, although no increase in defaecation frequency was observed. No other abnormalities were noticeable on clinical examination of the ponies.

The amounts of ^{51}Cr excreted in urine were expressed both as the mean percentage for each collection period and as the mean cumulative percentage and they are illustrated in Figures 3.1 and 3.2, respectively. Radioactivity counts found in faeces are shown in Figures 3.3 and 3.4. Details of the values obtained at each interval and of the daily subtotals are given in Appendices 1.3 and 2.3.

The total amount of ^{51}Cr measured in urine ranged from 3.05% to 8.01%, with a mean of 4.56% (SD $\pm$ 2.32). Approximately 60% of this amount was excreted during the first 24 hours. The values of ^{51}Cr-EDTA obtained in faeces ranged from 85.87% to 93.55% and the mean excretion was 91.26% (SD $\pm$ 3.65). The mean faecal excretion calculated for the first day accounted for approximately 47% of the total amount in 72 hours.

DISCUSSION.

The mean percentage urinary excretion of administered ^{51}Cr-EDTA for consecutive 24 hour periods obtained in this experiment were 2.72% $\pm$ 0.63, 1.47% $\pm$ 1.31 and 0.38% $\pm$ 0.53, respectively. Although slightly lower, these values were not significantly different to those obtained in Experiments 1 and 2. The values obtained in faeces for all the animals in 72 hours (91.26% $\pm$ 3.65) show an increase in the amount of ^{51}Cr excreted when compared to those obtained in both Experiments 1 and 2 (89.85% $\pm$ 3.47 and 79.36% $\pm$ 10.65, respectively). This finding was taken to indicate an increase in the activity and propulsion of the gastrointestinal tract which was in agreement with previous studies carried out with bethanecol in ponies (Gerring and Hunt, 1986; Roger and Ruckebusch, 1987).

FIGURE 3.1. Experiment 3: Mean percentage of administered ^{51}Cr-EDTA measured in urine in ponies following premedication with 0.05 mg/kg bethanecol subcutaneously.

FIGURE 3.2. Experiment 3: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in urine in ponies following premedication with 0.05 mg/kg bethanecol subcutaneously.

FIGURE 3.1.

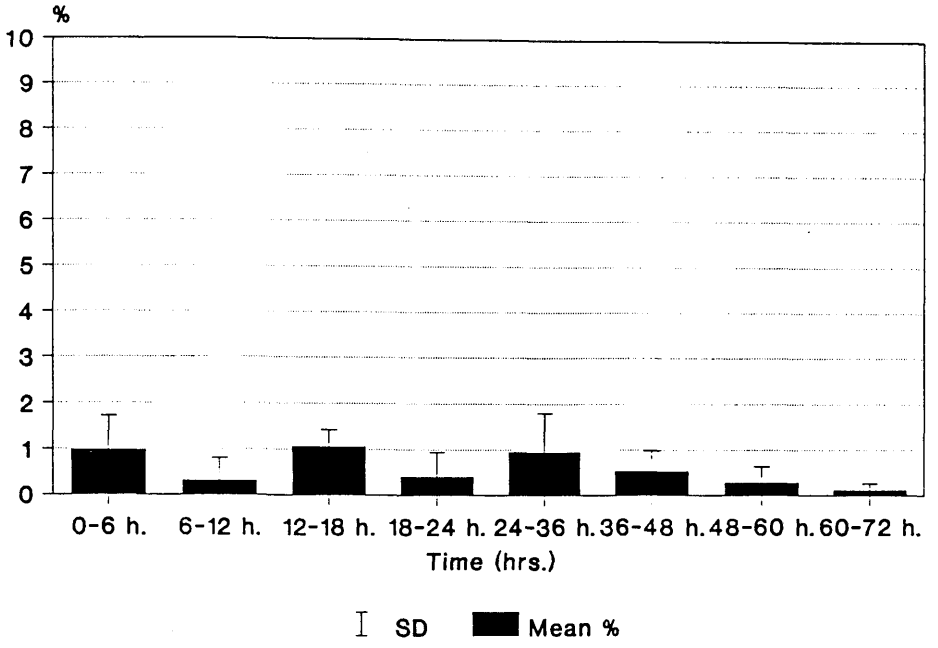


FIGURE 3.2.

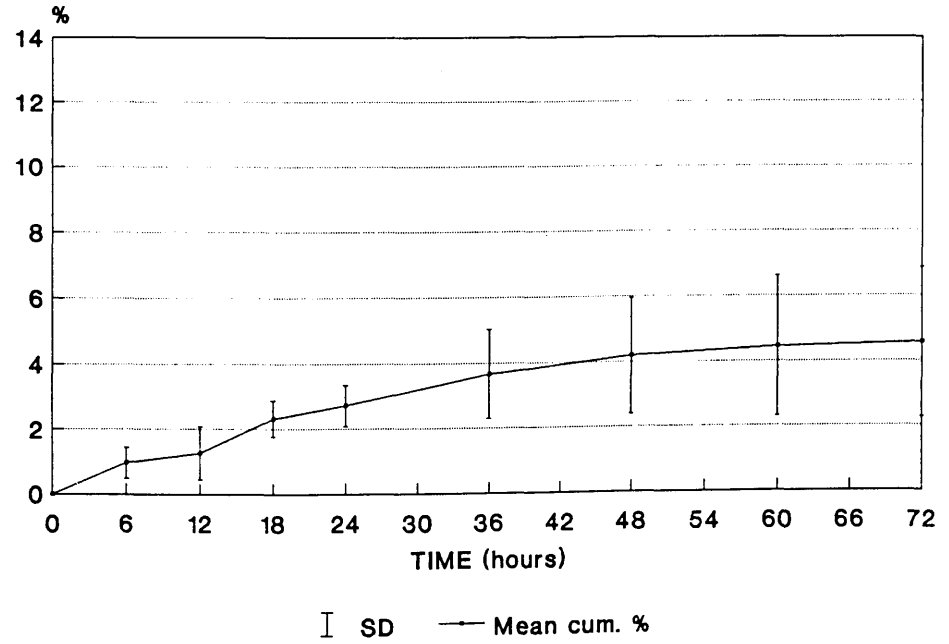


FIGURE 3.3. Experiment 3: Mean percentage of administered ^{51}Cr-EDTA measured in faeces in ponies following premedication with 0.05 mg/kg bethanecol subcutaneously.

FIGURE 3.4. Experiment 3: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in faeces in ponies following premedication with 0.05 mg/kg bethanecol subcutaneously.

FIGURE 3.3.

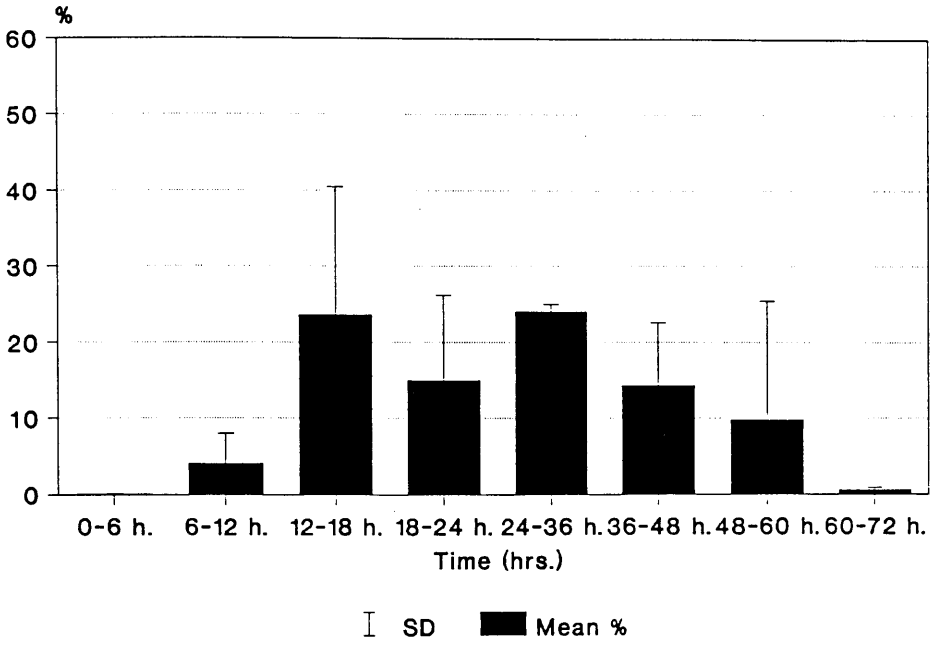
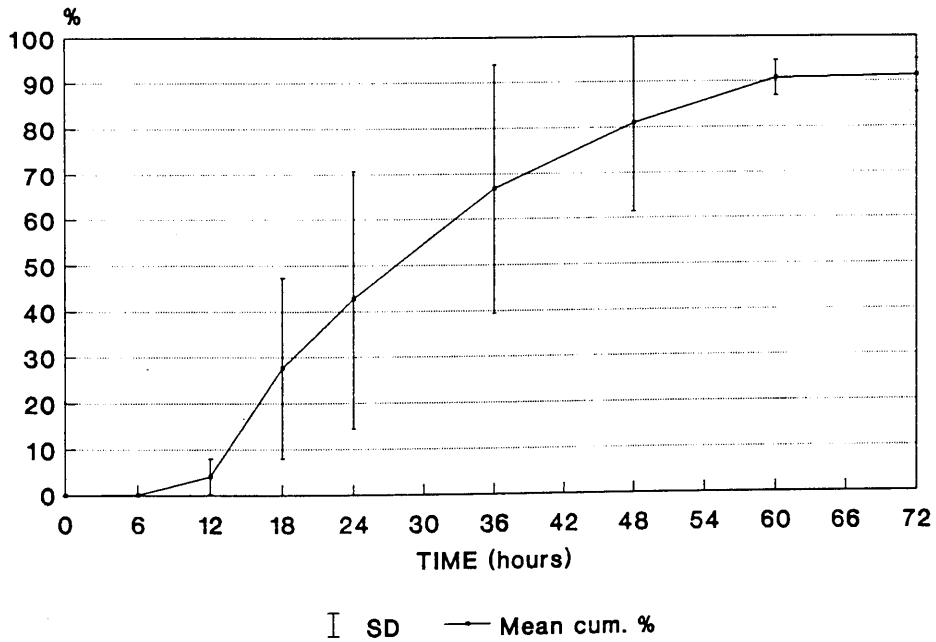


FIGURE 3.4.



In conclusion, the results obtained in the present experiment indicated that a moderate increase in the motility of the gastrointestinal tract of horses did not have a significant influence on the amount of ^{51}Cr-EDTA absorbed.

EXPERIMENT 4: INTESTINAL PERMEABILITY TO ^{51}Cr-EDTA IN PONIES WITH CAECAL FISTULATION.

INTRODUCTION.

The techniques currently in use for the assessment of equine intestinal function have several inherent limitations. Oral monosaccharide tolerance tests have proved of value for the detection of small intestinal dysfunction (Roberts and Pinsent, 1975; Bolton and others, 1976; Merritt and others, 1976; Meuten and others, 1978; Roberts and Kelly, 1980; Hodgson and Allen, 1982; Pass and Bolton, 1982; Mair and others, 1991). However, the absorption of these carbohydrate markers may be influenced by gastric emptying, intestinal transit time, mucosal blood flow (Roberts and Norman, 1979), small intestinal flora and diet (Jacobs and others, 1982) and, further, carbohydrate absorption only reflects function of the anterior portion of the gastrointestinal tract. Radioisotope tracer techniques have been applied to horses with enteropathy to demonstrate loss of proteins into the bowel (Merritt and others, 1976; Merritt and others, 1977; Meuten and others, 1978; Snow and others, 1981; Duncan and Dargie, 1975; Dietz and Nielsen, 1980; Love, 1990). These tests may be of use in assessing intestinal permeability alterations but they cannot be utilised to localise these changes within the gastrointestinal tract. Thus, other methods must be developed which are more sensitive and informative with regard to the site of intestinal dysfunction.

When ^{51}Cr-EDTA was first used by human medical workers (Bjarnason and others, 1983a, b; Bjarnason and others, 1985) it was concluded that ^{51}Cr-EDTA specifically indicated small intestinal disease. By contrast, Elia and others (1987), in studies on intestinal permeability with ^{51}Cr-EDTA in healthy human patients, suggested that differences in the temporal pattern of excretion in urine of orally administered markers might reflect their absorption from different sites and confirmed that significant amounts of ^{51}Cr-EDTA were absorbed from the colon. Jenkins

and others (1987, 1988) further substantiated this finding and they demonstrated that the increased urinary excretion of orally administered ^{51}Cr-EDTA was directly proportional to inflammation of the colon.

The objective of the present study was to evaluate the pattern of absorption of ^{51}Cr-EDTA from the equine large intestine.

MATERIALS AND METHODS.

Two female British native-breed five-year-old ponies were used on this occasion. Both ponies had been kept indoors and dosed at four-monthly intervals with ivermectin (Eqvalan paste, MSD AGVET, Hertfords.) for at least three years. They were kept in individual stalls on woodshavings, and were fed on hay and water *ad libitum*. Five months prior to the experiment a permanent caecal fistula was created in both animals by a modified version of the method described by Simmons and Ford (1988). The design of the acetyl homopolymer plastic cannula is shown in Figure 4.1 and the exact location of the cannula can be appreciated in Figure 4.2. The ponies had been trained to be tethered and to carry the urine collection bags, and during the period of experimentation the floor of the stalls was covered with rubber mats.

Urine was collected by means of Foley catheters (7.33 mm diameter; 40 cm length; 30 cc balloon volume) which were connected to polythene bags contained within canvas bags hanging from the back of the animals by two canvas strips. Faeces were allowed to pass onto the floor from where they were swept up and bagged.

Following a 10-hour fasting period, a solution of ^{51}Cr-EDTA was injected directly into the caecum through the indwelling cannula. A length of plastic tube was connected to the syringe containing the solution in

order to ensure that the radioistope was deposited well away from the cannula and was washed with 300 ml of water. The ponies were allowed to eat two hours following the administration of the solution.

Total urine and faeces produced in 72 hours were collected at 6-hour intervals on the first day and at 12-hour intervals thereafter.

RESULTS.

The amounts of ^{51}Cr-EDTA excreted in urine were expressed both as the percentage for each collection period and as the cumulative percentage and they are illustrated in Figures 4.3 and 4.4, respectively. The same system was used to express ^{51}Cr-EDTA collections in faeces (Figures 4.5 and 4.6). Details of the results obtained at each interval and of the daily subtotals are listed in Appendices 1.4 for urine and 2.4 for faeces.

The total amount of ^{51}Cr-EDTA measured in urine was 1.75% in Pony number 1 and 1.56% in Pony number 2 (mean, 1.66%). The intervals of maximal excretion varied in both animals: approximately 45% of the total urine collection was recovered during the first 24 hours in Pony number 1 whereas approximately 75% was so in the second animal. A similar trend was observed in the faecal excretion of the tracer: although the total excretion was comparable in both animals (73.90% and 76.94%, respectively) only 8.57% of the administered dose was collected during the first 24 hours in Pony number 1, compared to 61.28% in animal number 2.

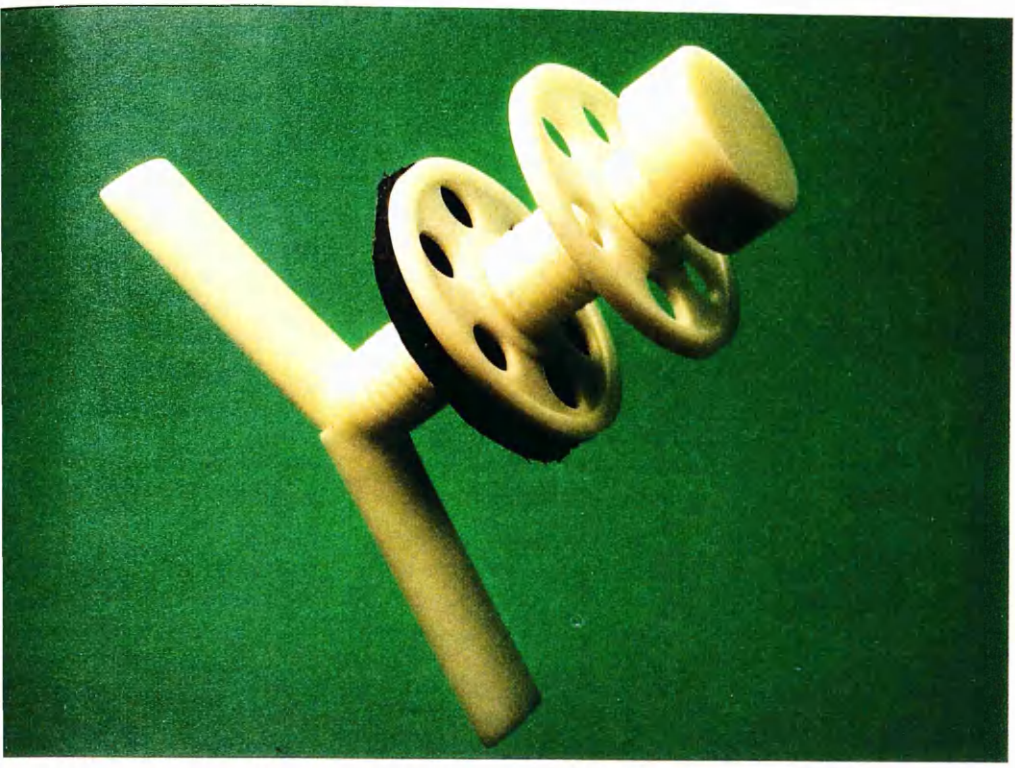


FIGURE 4.1. Experiment 4: Plastic cannula.

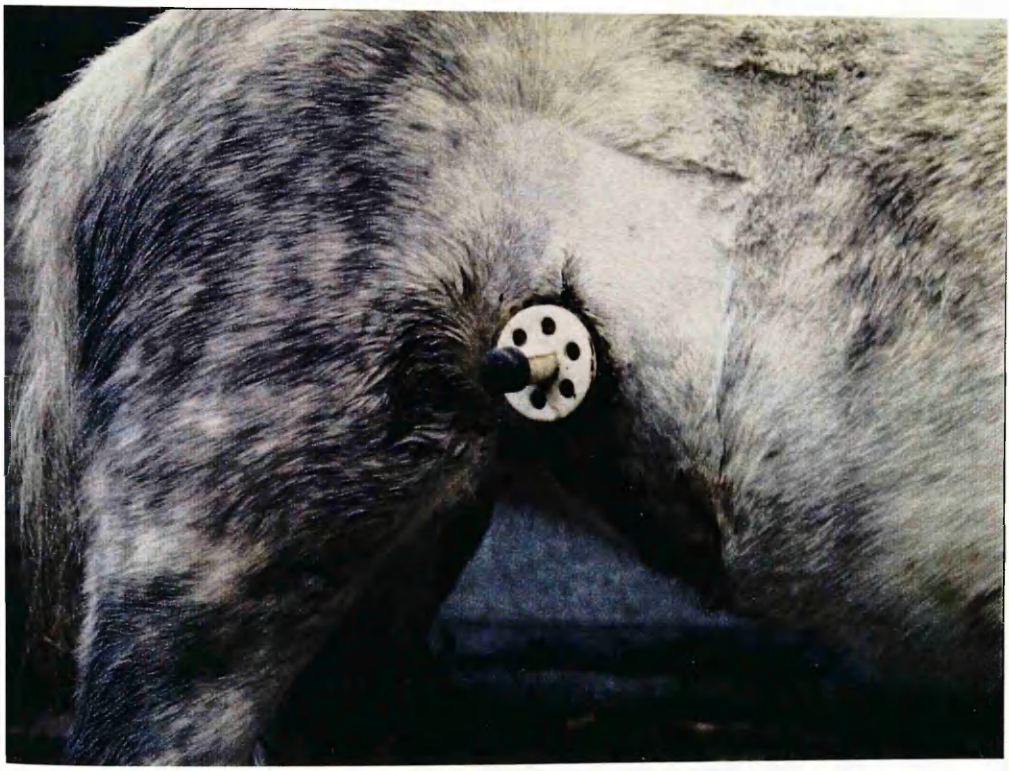


FIGURE 4.2. Experiment 4: Plastic cannula in place.

FIGURE 4.3. Experiment 4: Percentage of administered ^{51}Cr-EDTA measured in urine in ponies with permanent caecal fistulation.

FIGURE 4.4. Experiment 4: Cumulative percentage of administered ^{51}Cr-EDTA measured in urine in ponies with permanent caecal fistulation.

FIGURE 4.3.

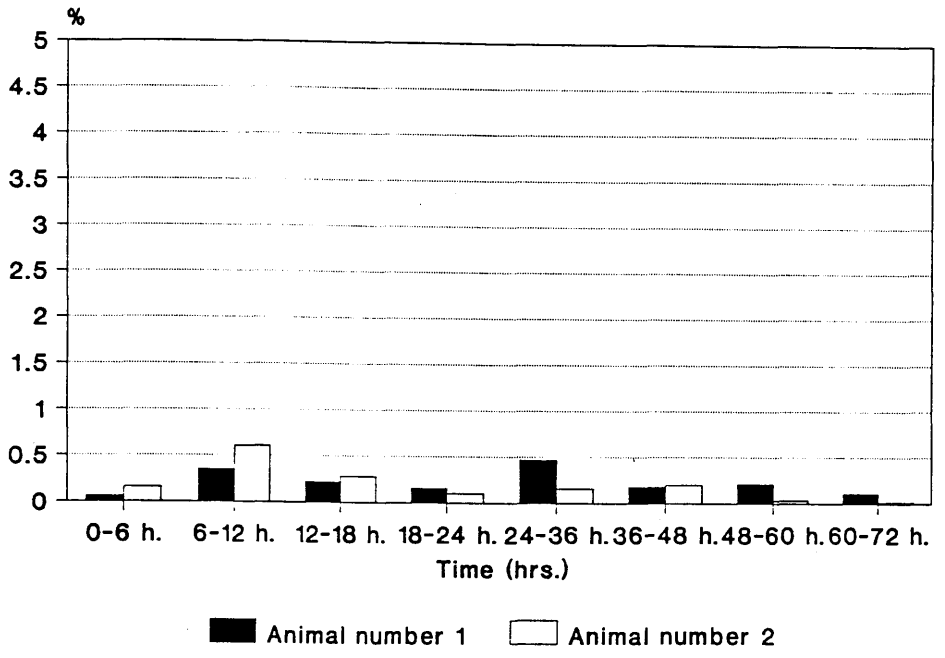


FIGURE 4.4.

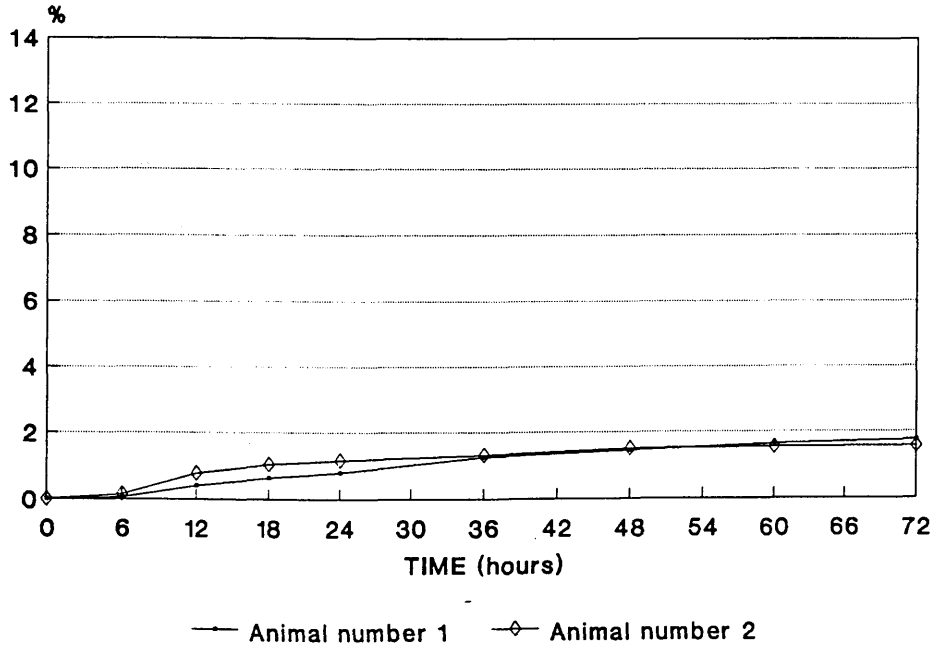


FIGURE 4.5. Experiment 4: Percentage of administered ^{51}Cr-EDTA measured in faeces in ponies with permanent caecal fistulation.

FIGURE 4.6. Experiment 4: Cumulative percentage of administered ^{51}Cr-EDTA measured in faeces in ponies with permanent caecal fistulation.

FIGURE 4.5.

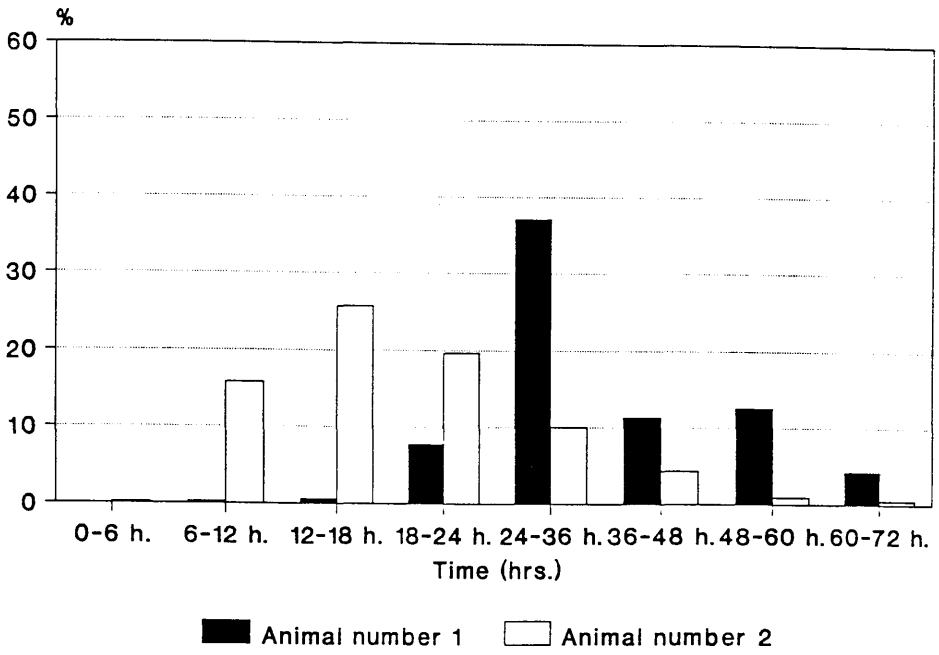
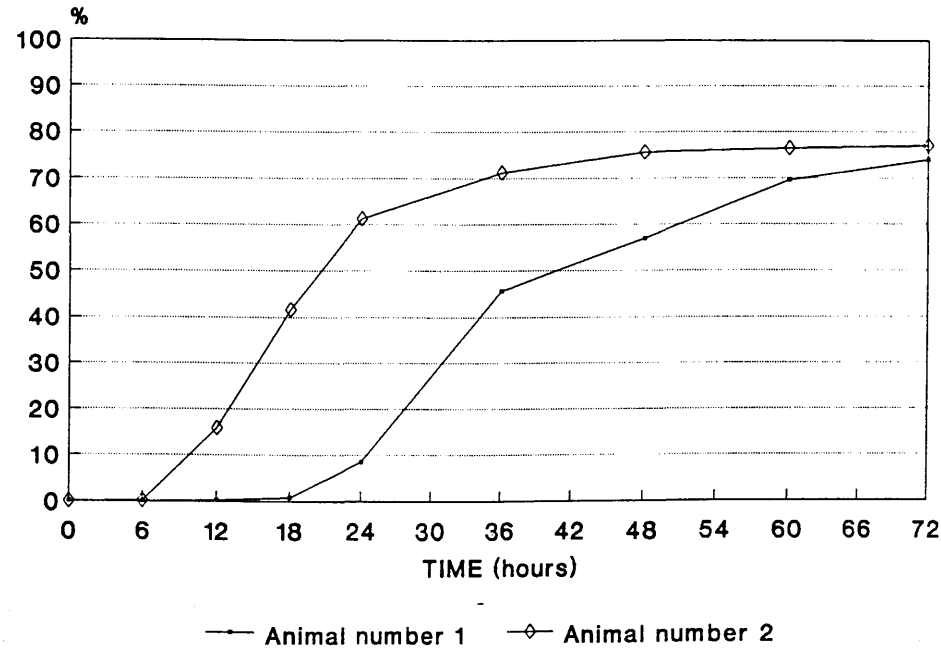


FIGURE 4.6.



DISCUSSION.

The results obtained here confirmed the equine large intestine as a site of absorption of ^{51}Cr-EDTA assuming there was no reflux of marker into the small intestine. In previous studies, when a liquid marker was injected directly into the caecum of ponies with permanent caecal fistulation no marker was found in the ileum (Argenzio and others, 1974). The amounts of ^{51}Cr-EDTA measured in urine were relatively low but one has to consider the possibility that the amount of marker collected in urine in both fistulated ponies (1.66% $\pm$ 0.13) might have been influenced to some extent by partial loss of the administered dose in that there was slight leakage from the caecal cannula in both animals. The relatively low values obtained in faeces would further support this possibility. However, it has been suggested that absorption of ^{51}Cr-EDTA from normal human large intestine is low but it increases markedly when inflammation of this portion of the alimentary tract occurs (Jenkins and others, 1988) and a similar theory may be true for horses.

The marked differences in intestinal transit time observed in the two animals appeared to have no influence in the pattern of absorption of ^{51}Cr-EDTA and this finding further supports the conclusions of previous experiments that alterations of intestinal motility do not affect permeability of the marker from the gastrointestinal tract.

Argenzio and others (1974) reported that approximately 68% of a liquid marker was recovered in the caecum within two hours following intragastric administration. The temporal pattern of ^{51}Cr-EDTA excretion in urine might be of great assistance for the assessment of equine large intestinal permeability in that the urinary excretion values for specific time periods may relate to the permeability of discrete anatomical regions of the gastrointestinal tract.

CHAPTER IV. STUDIES ON ^{51}Cr-EDTA URINARY EXCRETION FOR THE ASSESSMENT OF INTESTINAL PERMEABILITY IN EQUINE CHRONIC ENTEROPATHIES.

CHAPTER IV. STUDIES ON ^{51}Cr-EDTA URINARY EXCRETION FOR THE ASSESSMENT OF INTESTINAL PERMEABILITY IN EQUINE CHRONIC ENTEROPATHIES.

INTRODUCTION.

The suitability of the use of ^{51}Cr-EDTA for the assessment of intestinal permeability has been reported in humans (Bjarnason and others, 1983a, b; Bjarnason and Peters, 1984; O'Morain and others, 1986; Elia and others, 1987; Fotherby and others, 1988), rats (Bjarnason and others, 1985), gerbils and lambs (Sinski and others, 1987; McLean and others, 1989), dogs (Hall and others, 1989; Hall and Batt, 1990) and ponies (Love, 1990).

In Chapter III of this thesis there are details of experiments carried out with the objective of further validating ^{51}Cr-EDTA as a marker of intestinal permeability in ponies. In those experiments there were no significant differences in the amounts of ^{51}Cr-EDTA excreted in urine collected from untreated, control animals and then in the same animals following premedication with either atropine or bethanecol. Thus, it was concluded that ^{51}Cr-EDTA urinary excretion in ponies was not affected by alterations of intestinal motility. It was also confirmed that ^{51}Cr-EDTA is absorbed from the large intestine such that it may be used in the assessment of large intestinal permeability.

In equine medicine, the present knowledge of both the aetiopathogenesis and the approach to the diagnosis of the chronic enteritides is limited (Roberts, 1984). Alterations in the gastrointestinal permeability have been reported to play an important role in the chronic human enteropathies (Chadwick and others, 1977; Bjarnason and Peters, 1984; Jenkins and others, 1988) and it seems likely that this might also be an important factor in the pathogenesis of certain equine enteropathies.

The objective of these studies was to investigate the usefulness of ^{51}Cr-EDTA as a marker for the assessment of intestinal permeability in ponies with experimentally-induced chronic enteropathy. To achieve this, either prolonged oral administration of phenylbutazone or infection with cyathostome larvae were used to induce protein-losing enteropathies in ponies. Additionally, ^{51}Cr-EDTA absorption and urinary excretion were studied in clinical cases with naturally-occurring enteropathy which were referred to the Department of Veterinary Medicine, University of Glasgow Veterinary School.

EXPERIMENT 5. INTESTINAL PERMEABILITY TO ^{51}Cr-EDTA IN PONIES FOLLOWING PROLONGED ORAL ADMINISTRATION OF PHENYLBUTAZONE.

INTRODUCTION.

Phenylbutazone (PBZ) has been widely used in equine medicine for many years for its effects as an anti-inflammatory, anti-pyretic and analgesic agent (Tobin, 1979; Lees and Higgins, 1985). For a long time it was considered to be nontoxic to horses (Jeffcott and Colles, 1977) but more recently there have been several reports which demonstrate the toxicity of PBZ in horses both experimentally (Snow and others, 1979; Snow and others, 1981; Lees and others, 1983; Meschter and others, 1984; Collins and Tyler, 1985; Meschter and others, 1990) and in clinical reports (Gunson and Soma, 1983; Read, 1983; Traub and others, 1983; Behm and Berg, 1987).

Symptoms associated with PBZ toxicosis in horses include anorexia, depression, weight loss, diarrhoea, cyanotic mucous membranes, ventral oedema, and hypoalbuminaemia. At necropsy, marked mucosal atrophy, focal erosions and ulcers can be found in the alimentary tract. Nephrotoxicity, hepatotoxicity, leucopaenia and subsequent leucocytosis, and vasculopathy have also been reported consequent to the administration of PBZ for prolonged periods or at higher dose levels than those recommended by the manufacturers.

In experimental studies in ponies, signs of PBZ toxicity were observed following the oral administration of either 10 to 12 mg/kg/day PBZ in two equal doses for eight to 10 days (Snow and others, 1981) or 8.8 mg/kg/day in two equal doses at 12-hour intervals for 10 days. (Collins and Tyler, 1985). The latter approach resulted in less severe clinical and biochemical effects.

Snow and others (1981) used radioisotope tracer techniques to confirm that the condition constituted a protein-losing enteropathy (PLE).

On the basis that PBZ toxicity has been experimentally reproduced and it results in deranged intestinal mucosal integrity it was considered to be a suitable condition for the study of the usefulness of ^{51}Cr-EDTA in the assessment of altered intestinal permeability in ponies.

MATERIALS AND METHODS.

Four female British native-pony yearlings were given PBZ paste (Phenyzene Oral Paste, C-Vet Limited, Suffolk) at a dose rate of 8.8 mg/kg/day by mouth in 2 equal doses 12 hours apart to effect. When this medication regimen had been maintained for 28 consecutive days the ponies were given a solution of ^{51}Cr-EDTA by stomach tube following a period of fasting of 10 hours. The solution was washed through the tube with 300 ml of water. The animals were allowed to eat two hours following administration of the test solution.

Total faeces and urine produced in 72 hours were collected at 6-hour intervals on the first day and at 12-hour intervals thereafter.

Detailed clinical monitoring of each pony was carried out with particular reference to attitude, appetite, and nature of faeces and urine. A thorough physical examination of each animal was performed daily and the animals were weighed on alternate days.

Haematological and biochemical analyses of blood and plasma were carried out sequentially over the period during which PBZ was administered. The variables which were monitored included: Total and differential white blood cell counts, red blood cell count, packed cell volume, haemoglobin concentration, creatinine, urea, total plasma

proteins, albumin, globulins, alkaline phosphatase, gamma-glutamyl transferase and aspartate aminotransferase. Fractional excretion and renal clearance values of electrolytes were calculated by the method reported by Morris and others (1984) for the two collection periods of the last day of the experiment in order to assess renal function.

RESULTS.

The amounts of ^{51}Cr excreted in urine were calculated both as the mean percentage during each collection interval and as the mean cumulative percentage and are illustrated in Figures 5.1 and 5.2, respectively. The same system was used to express radioactivity found in faeces as shown in Figures 5.3 and 5.4. A detailed account of the values obtained at each interval and of the daily subtotals is given in Appendices 1.5 and 2.5.

The amount of ^{51}Cr-EDTA excreted in urine in 72 hours ranged from 4.20% to 8.25% with a mean total excretion of 6.21% (SD $\pm$ 1.66). The percentages recovered in faeces ranged from 67.53% to 119.71%, with a mean value of 90.20% (SD $\pm$ 21.70). Approximately 61% of the total urinary excretion was collected on the first day whereas about 37% of the total amount recovered in faeces was voided during the same time interval.

None of the signs reported for equine PBZ toxicosis were observed in any of the ponies and no haematological or biochemical abnormalities were detected in the blood samples which were analysed. Details of the calculated fractional excretion (FE) and renal clearance (RC) of electrolytes are shown in Appendices 3.1 and 3.2. Both the FE and RC values of sodium, calcium and phosphate were slightly above the reported reference values.

FIGURE 5.1. Experiment 5: Mean percentage of administered ^{51}Cr-EDTA measured in urine in ponies following oral administration of 8.8 mg/kg/day phenylbutazone for 28 days.

FIGURE 5.2. Experiment 5: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in urine in ponies following oral administration of 8.8 mg/kg/day phenylbutazone for 28 days.

FIGURE 5.1.

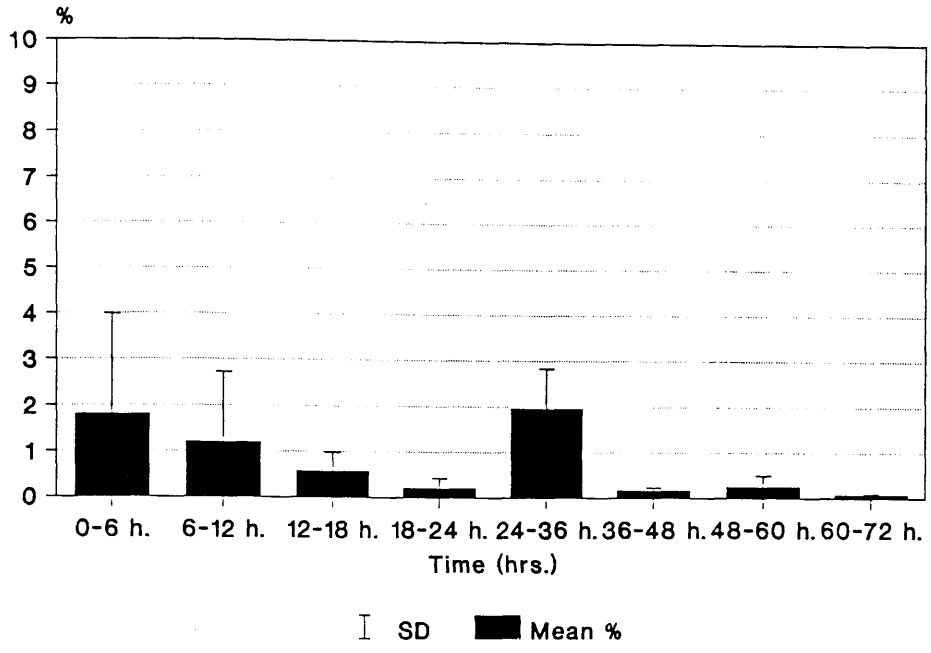


FIGURE 5.2.

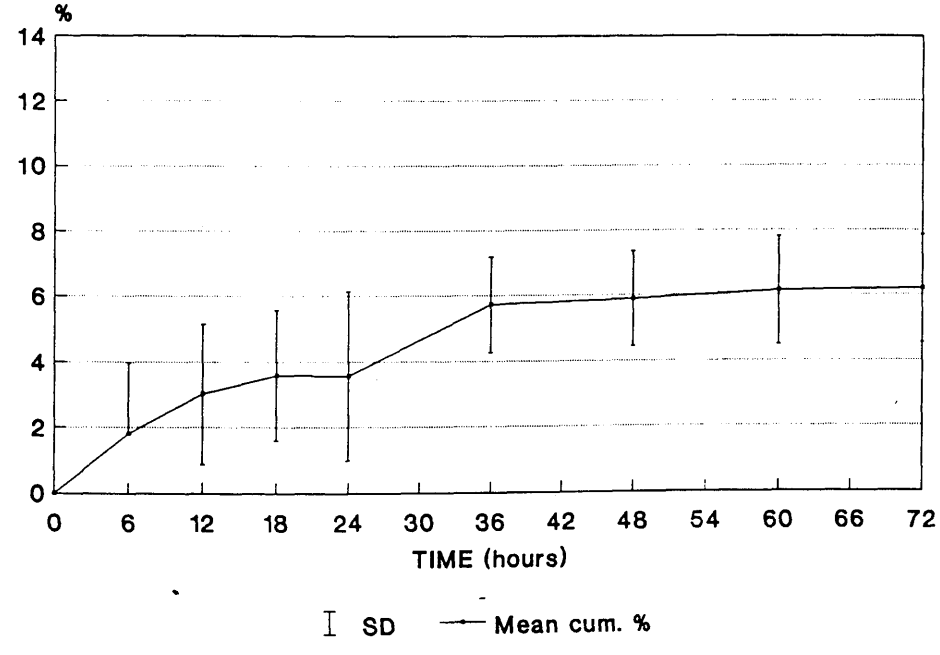


FIGURE 5.3. Experiment 5: Mean percentage of administered ^{51}Cr-EDTA measured in faeces in ponies following oral administration of 8.8 mg/kg/day phenylbutazone for 28 days.

FIGURE 5.4. Experiment 5: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in faeces in ponies following oral administration of 8.8 mg/kg/day phenylbutazone for 28 days.

FIGURE 5.3.

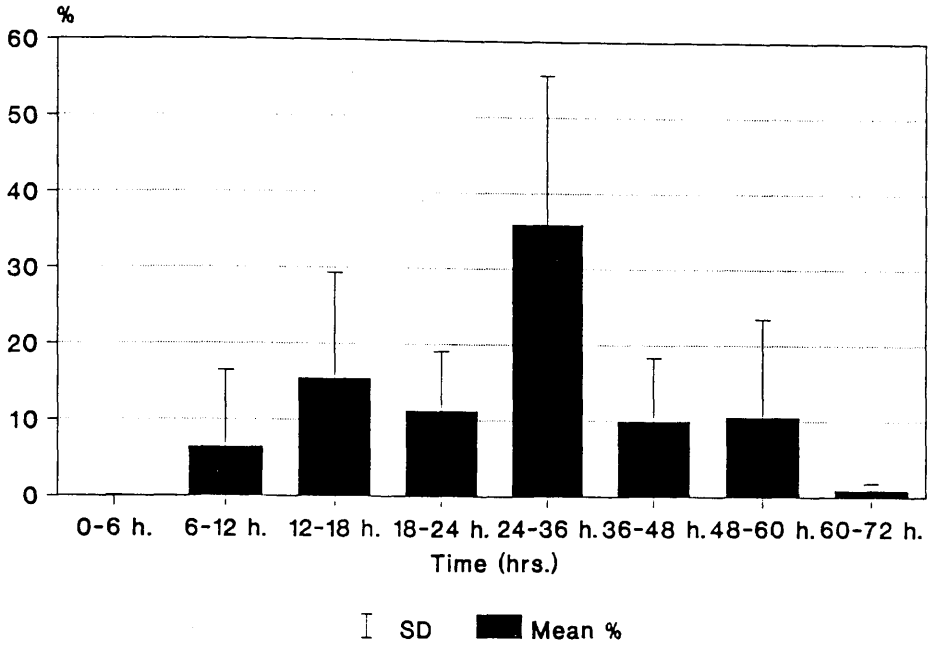
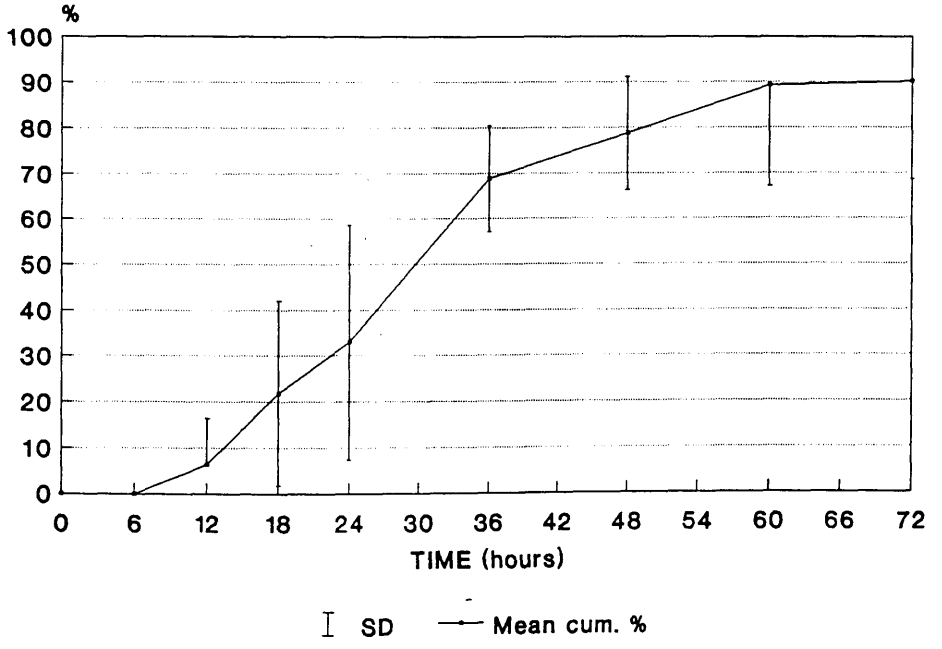


FIGURE 5.4.



DISCUSSION.

In the present experiment, the mean percentage excretions of ^{51}Cr-EDTA for consecutive 24 hour periods were 3.78% ± 2.19, 2.12% ± 0.84 and 0.31% ± 1.65, respectively which were similar to the results obtained Experiments 1, 2 and 3.

Ponies with PBZ toxicosis would be expected to have increased intestinal permeability (Collins and Tyler, 1985) such that increased urinary excretion of ^{51}Cr-EDTA was anticipated in the animals but none was detected. Interestingly, clinical signs of toxicosis were not observed in any of the experimental animals despite the prolonged period (28 days) of administration of PBZ at high dose levels (8.8 mg/kg/day) which contrast with the observations of Collins and Tyler (1985) who recorded clinical disease of PBZ toxicity following a similar experimental protocol. Several reports on PBZ toxicosis following oral administration in ponies and horses recorded hypoproteinaemia (Snow and others, 1981; Collins and Tyler, 1985), increased blood urea nitrogen and creatinine levels (Read, 1983) and initial leucopenia followed by leucocytosis (Lees and others, 1983) but comparable abnormalities were not found in the present study. The only abnormalities found in the laboratory data were increased renal clearance and fractional excretion of sodium, calcium and phosphate. However, the reference values presently available were calculated for the horse, and it it is possible that the values of these tests for ponies might be different.

The explanation for the lack of evidence of toxic effects of PBZ in this study was unclear, but the diet of the experimental ponies might have been a contributing factor. Certainly, different absorption patterns of PBZ have been reported in relation to the type and nature of the feed given to horses or ponies during pharmacokinetic studies of oral medication with

this compound (Lees and Higgins, 1985; Tobin and others, 1986). In those studies, in animals which received hay in their diets, the absorption of PBZ was delayed and reduced compared with that in animals given concentrated feeds. In the present study the ponies were given a proprietary complete, cubed diet such that these animals might have been expected to be predisposed to PBZ toxicity. However, signs of PBZ toxicity in these ponies were absent, which might reflect reduced absorption of the compound but this could not be confirmed because plasma levels of PBZ were not measured. Another possibility to consider is that the time of contact of PBZ with the intestinal mucosa might have been reduced as a result also of the type of feed and, consequently, the direct toxicity that PBZ might have on the mucosa might have been minimised.

Although pathological investigations were not performed, from the clinical and laboratory findings it was concluded that toxicity was not induced in this experiment by the administration of PBZ. The fact that the values obtained for the urinary excretion of ^{51}Cr-EDTA, were not significantly different from those of the previous experiments, was further evidence that the gastrointestinal permeability of these ponies was not altered. The apparent correlation of clinical and experimental results supports the view that ^{51}Cr-EDTA is a reliable marker for the assessment of intestinal permeability in ponies.

EXPERIMENT 6. INTESTINAL PERMEABILITY TO ^{51}Cr-EDTA IN PONIES WITH EXPERIMENTAL CYATHOSTOME INFECTION.

INTRODUCTION.

Until recently it was considered that *Strongylus* spp. including *S. vulgaris, S. edentatus* and *S. equinus* were the most pathogenic parasites in horses (Ogbourne and Duncan, 1985). However, the advent of the modern anthelmintic agents together with their widespread usage in modern parasite prophylactic programmes have caused a marked reduction in the significance of infections by these worms. On the other hand, the clinical importance of cyathostomes has become more evident with an increasing prevalence of clinical cyathostomiasis in the horse reported in recent years (Chiejina and Mason, 1977; Jasko and Roth, 1984; Giles and others, 1985; Church and others, 1986; Love, 1990). The development of resistance of cyathostome species to benzimidazole anthelmintic agents has been widely recognized (Drudge and others, 1979; Herd, 1990) and this phenomenon has to some extent increased the importance of these parasites in equine medicine.

Equine larval cyathostomiasis is distinguished clinically by the sudden onset of chronic diarrhoea in late winter or early spring, resulting in progressive weight loss and eventually death if radical therapy is not instituted promptly (Giles and others, 1985; Love, 1990). Haematological and blood biochemical findings in cases of cyathostomiasis include a moderate leucocytosis due primarily to neutrophilia, increased levels of beta-globulins, raised alkaline phosphatase activity, and hypoalbuminaemia but these are not specific features (Round, 1968; Round, 1970; Blackwell, 1973; Giles and others, 1985; Church and others, 1986; Love, 1990). Larval cyathostomiasis appears to be induced by mass

emergence of inhibited fourth-stage larvae into the gut lumen (Ogbourne, 1978; Giles and others, 1985) which is accompanied by a severe inflammatory response consisting of an infiltration of the intestinal mucosa and submucosa by eosinophils, lymphocytes and some plasma cells, and haemorrhagic foci, submucosal oedema and erosions are generally apparent (Ogbourne, 1978; Reinemeyer, 1986).

Diagnosis of equine chronic enteropathies may present a serious challenge and this is particularly true in cases in which the pathological alterations are limited to the large intestine, as occurs in larval cyathostomiasis, because routine laboratory analyses and small intestinal function tests may show no abnormality. Prompt diagnosis of treatable conditions such as larval cyathostomiasis is of crucial importance for the outcome of the affected animals and it may be extremely difficult if examination of the histopathology of intestinal biopsy samples taken via laparotomy is not practicable (Church and others, 1986; Love, 1990). Although it would be unlikely that a definitive diagnosis could be made on the basis of knowledge of large intestinal dysfunction, a test which quantified the pathophysiology of large bowel enteropathy would be useful in case assessment and prognosis.

Radioisotope tracer techniques used in ruminants for the investigation of the pathogenesis of experimental parasitic infections characterized by marked hypoalbuminaemia have confirmed that the major reason for the reduced plasma levels of albumin is increased losses of proteins as a consequence of altered intestinal mucosal permeability (Nielsen, 1968; Holmes and Mclean, 1971; Mulligan, 1973; Dargie, 1975; Abbott, Parkins and Holmes, 1986). It has been suggested that the pathogenesis of larval cyathostomiasis is similar to some of the parasitic enteropathies of ruminants (Ogbourne, 1978) such that assessment of intestinal permeability might aid in the investigation of this condition.

^{131}Iodine- or ^{125}Iodine-labelled albumin appear to be reliable tracers to assess protein catabolism in animals with gastrointestinal parasitism (Nielsen, 1968; Holmes and Mclean, 1971; Mulligan, 1973; Dargie, 1975; Duncan and Dargie, 1975; Dietz and Nielsen, 1980; Abbott and others, 1986) but it cannot be employed for the quantitation of protein loss into the bowel because of partial reabsorption of the radioisotope following enzymatic degradation of the protein in the intestine (Nielsen, 1968; Mulligan, 1973). This problem may be overcome by the use of radioisotope labels which are not absorbed from the intestine such as ^{51}Chromium, or by administering other compounds which are not broken down in the alimentary tract e.g. polyvinylpyrrolidone (Nielsen, 1968; Mulligan, 1973). Detection of radioactivity in faeces following intravenous administration of either ^{51}Chromium (^{51}Cr)-labelled albumin or ^{51}Cr-chloride has been used in horses to confirm protein-losing enteropathies (Merritt and others, 1976; Merritt and others, 1977; Meuten and others, 1978; Snow and others, 1981; Love, 1990).

^{51}Chromium-ethylenediaminetetraacetate (^{51}Cr-EDTA) is routinely employed in human medicine for the measurement of glomerular filtration rate on the basis that ^{51}Cr-EDTA is not metabolised and it is excreted immediately in urine (Chantler and others, 1969; Granerus and Aurell, 1981). A more recent application of ^{51}Cr-EDTA has been in the assessment of unmediated intestinal permeability by measuring ^{51}Cr in urine following its administration *per os*. ^{51}Cr-EDTA has proved valuable in the assessment of cases of coeliac disease and inflammatory bowel disease in man (Bjarnason and others, 1983a; Bjarnason and others, 1983b; O'Morain and others, 1986; Elia and others, 1987; Jenkins and others, 1987; Jenkins and others, 1988; Fotherby and others, 1988), in dogs with wheat-sensitive enteropathy, small intestinal bacterial overgrowth and giardiasis (Hall and Batt, 1990), in gerbils and lambs experimentally infected with *Trichostrongylus* spp. (Sinski and others, 1987; Maclean and others, 1989) and in experimental nippostrongylosis in

rats (Ramage and others, 1988). Preliminary investigations on the use of ^{51}Cr-EDTA to investigate intestinal permeability of ponies were carried out by Love (1990).

The objective of these studies was to test the suitability of ^{51}Cr-EDTA for the assessment of intestinal permeability alterations in chronic enteropathies by experimental cyathostome infection of ponies.

MATERIALS AND METHODS.

Four female British native-pony yearlings which had been reared indoors and were presumed to be helminth-naive were administered a solution of ^{51}Cr-EDTA following a period of fasting of 10 hours which was continued for two further hours. The solution was given per stomach tube and the tube was washed through with 300 ml of water. Initially all four animals were studied under control conditions. Subsequently three ponies were chosen randomly for 'trickle' experimental cyathostome infection by administration per stomach tube of 250,000 cyathostome third-stage larvae per day for 46 days. ^{51}Cr-EDTA was administered on three occasions: First, when 5.5 million larvae had been dosed (Day 22); secondly, after the administration of 11.5 million larvae (Day 47) and finally, on Day 88 following first infection with 11.5 million larvae when patency was expected to have occurred.

Total faeces and urine produced in 72 hours were collected at 6-hour intervals on the first day and subsequently at 12-hour intervals.

All animals were weighed regularly throughout the period of the studies. Sequential blood samples were collected into vacutainers containing either heparin or EDTA in order to monitor alterations in total and differential white blood cell counts, packed cell volume, total

plasma proteins, albumin and globulins. Faeces were submitted three times weekly for strongyle faecal egg counts to determine the onset of patency of the infections.

RESULTS.

The amounts of ^{51}Cr excreted in urine and faeces were calculated both as the mean cumulative percentage and as the mean percentage during each collection interval and are illustrated in Figures 6.1 to 6.14. Cumulative percentages of ^{51}Cr-EDTA obtained in 72 hours in urine and faeces are listed in Tables 6.1 and 6.2, respectively. A detailed account of the values obtained at each interval and of the daily subtotals is given in Appendices 1.6 to 1.9 for urine and 2.6 to 2.9 for faeces.

All infected animals lost weight during the experimental infection (Appendix 3.9). Two ponies developed watery diarrhoea which contained large numbers of cyathostome parasites and both also became hyperlipaemic. One of these ponies (number 64) died on Day 53 and the other (number 63) was killed on Day 94. At post-mortem examination both animals were found to have enlarged, pale, friable livers. In both ponies, large intestinal contents were scant and markedly fluid, whilst the caecal and colonic mucosae were oedematous, containing large numbers of 2-3 millimetre cyathostome nodules. Microscopic examination revealed fatty change in the liver in both cases. On microscopy, the large intestinal mucosa was found to contain a very high number of parasitic larvae within cysts which were surrounded by an infiltrate of mononuclear and polymorphonuclear cells in the mucosa and lamina propia. Interestingly, few eosinophils could be observed in the large intestinal mucosa of both animals. Diagnoses of larval cyathostomiasis and hepatic lipidosis were made. The worm burdens counted post-mortem are listed in Table 6.3. Figures 6.15 and 6.16 show the gross appearance of the large intestinal mucosa of Pony numbers 64 and 63, respectively.

FIGURE 6.1. Experiment 6: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in urine in control ponies.

FIGURE 6.2. Experiment 6: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in urine in ponies following trickle infection with 5.5 million cyathostome third-stage larvae (Day 22).

FIGURE 6.3. Experiment 6: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in urine in ponies following trickle infection with 11.5 million cyathostome third-stage larvae (Day 47).

FIGURE 6.4. Experiment 6: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in urine in ponies following trickle infection with 11.5 million cyathostome third-stage larvae (Day 88).

FIGURE 6.1.

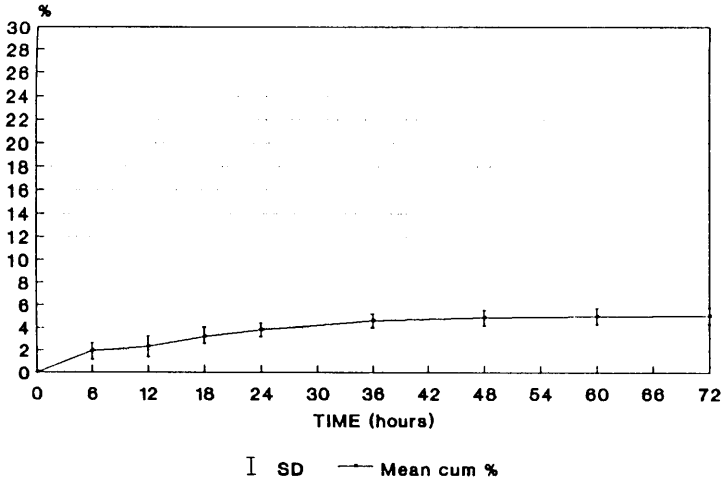


FIGURE 6.2.

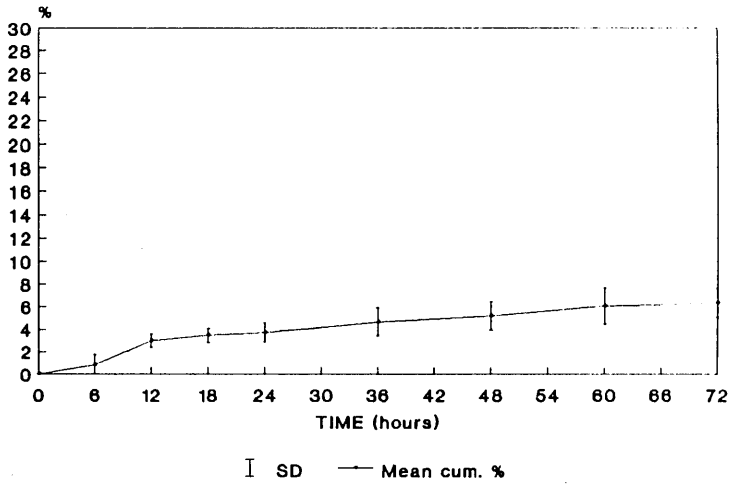


FIGURE 6.3.

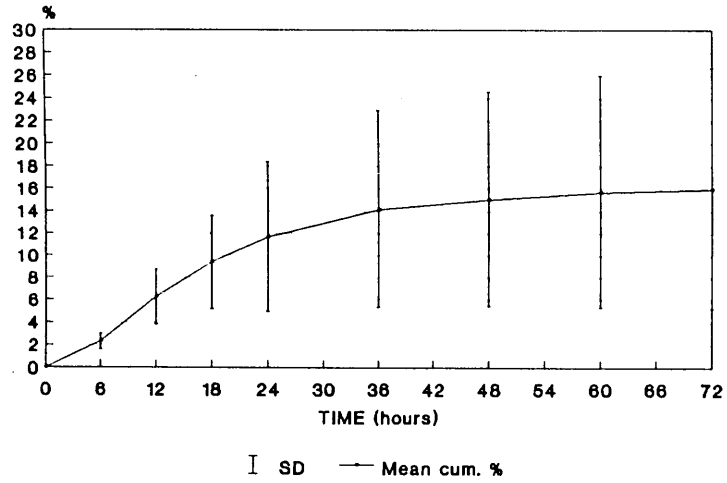


FIGURE 6.4.

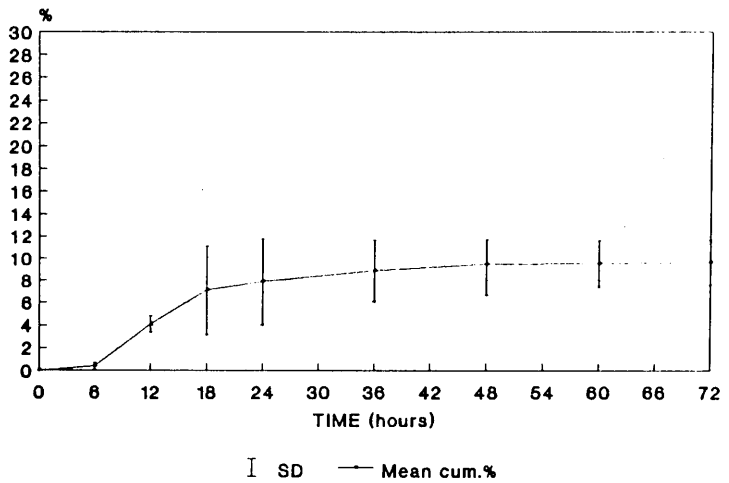


FIGURE 6.5. Experiment 6: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in faeces in control ponies.

FIGURE 6.6. Experiment 6: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in faeces in ponies following trickle infection with 5.5 million cyathostome third-stage larvae (Day 22).

FIGURE 6.7. Experiment 6: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in faeces in ponies following trickle infection with 11.5 million cyathostome third-stage larvae (Day 47).

FIGURE 6.8. Experiment 6: Mean cumulative percentage of administered ^{51}Cr-EDTA measured in faeces in ponies following trickle infection with 11.5 million cyathostome third-stage larvae (Day 88).

FIGURE 6.5.

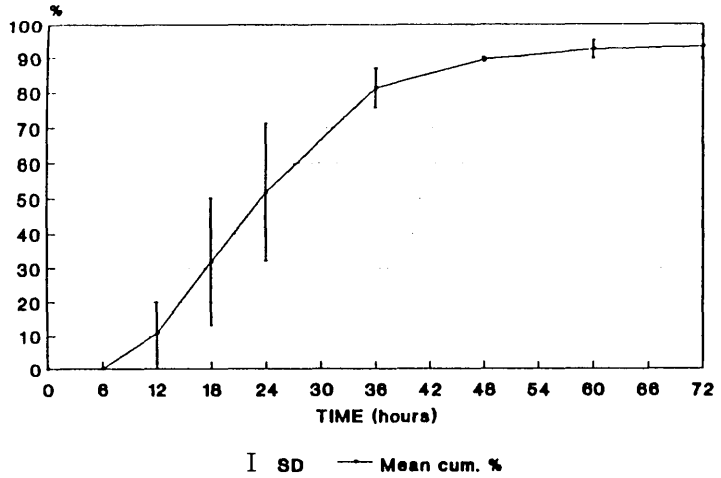


FIGURE 6.6.

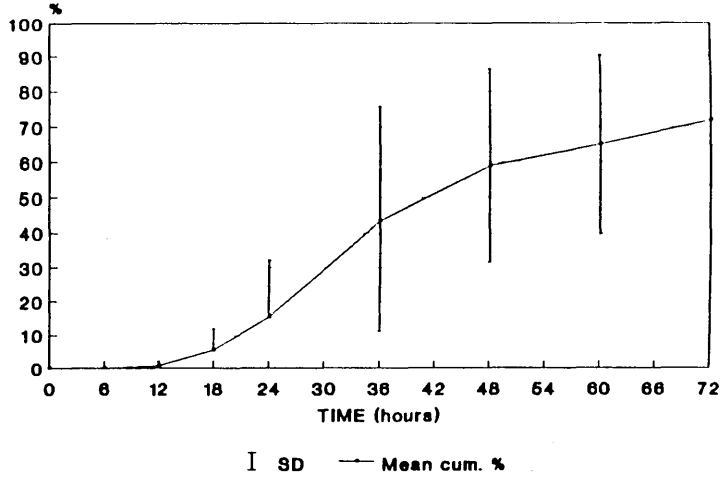


FIGURE 6.7.

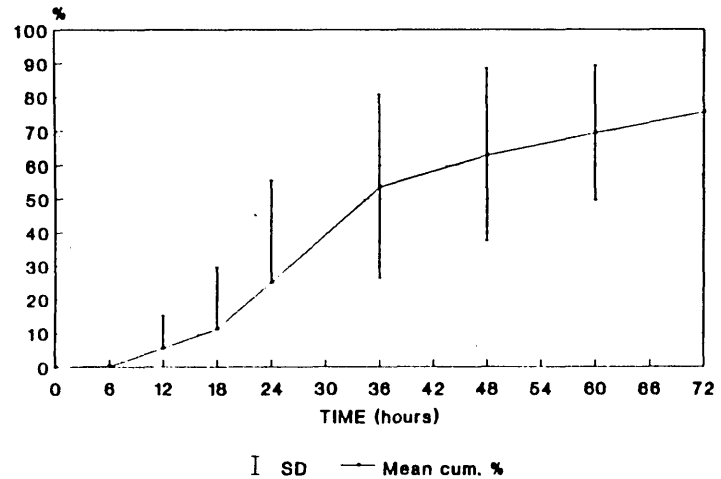


FIGURE 6.8.

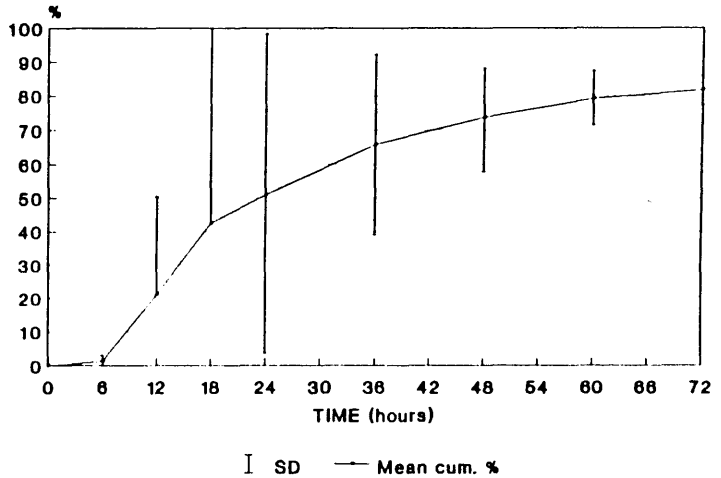


FIGURE 6.9. Experiment 6: Mean percentage of administered ^{51}Cr-EDTA measured in urine in control ponies and in the same ponies following trickle infection with 5.5 million cyathostome third-stage larvae (Day 22).

FIGURE 6.10. Experiment 6: Mean percentage of administered ^{51}Cr-EDTA measured in urine in control ponies and in the same ponies following trickle infection with 11.5 million cyathostome third-stage larvae (Day 47).

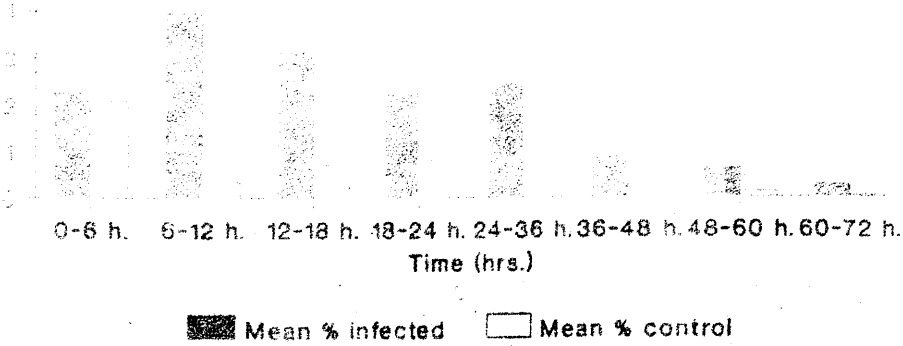


FIGURE 6.9.

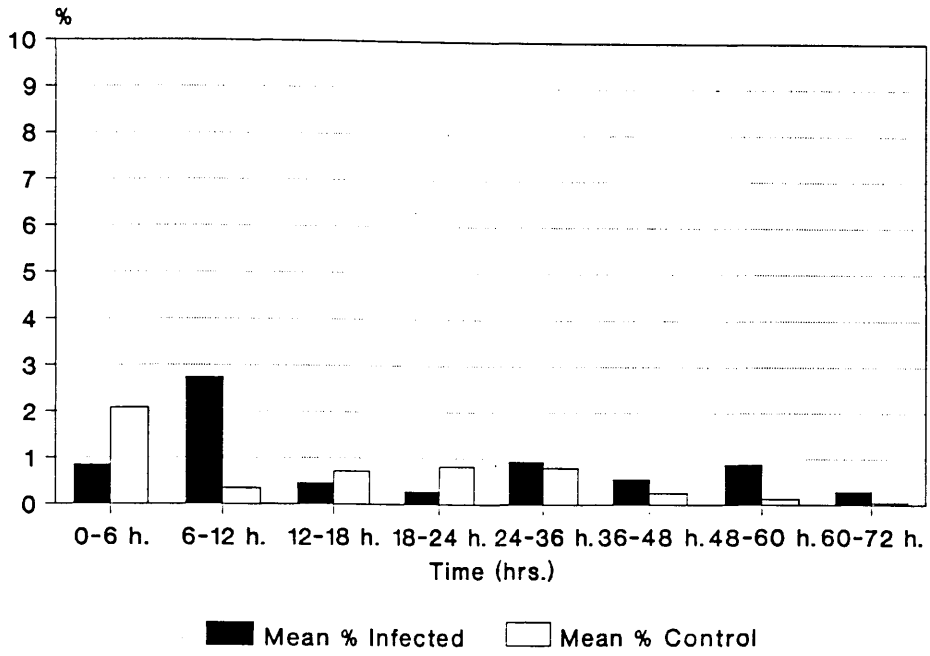


FIGURE 6.10.

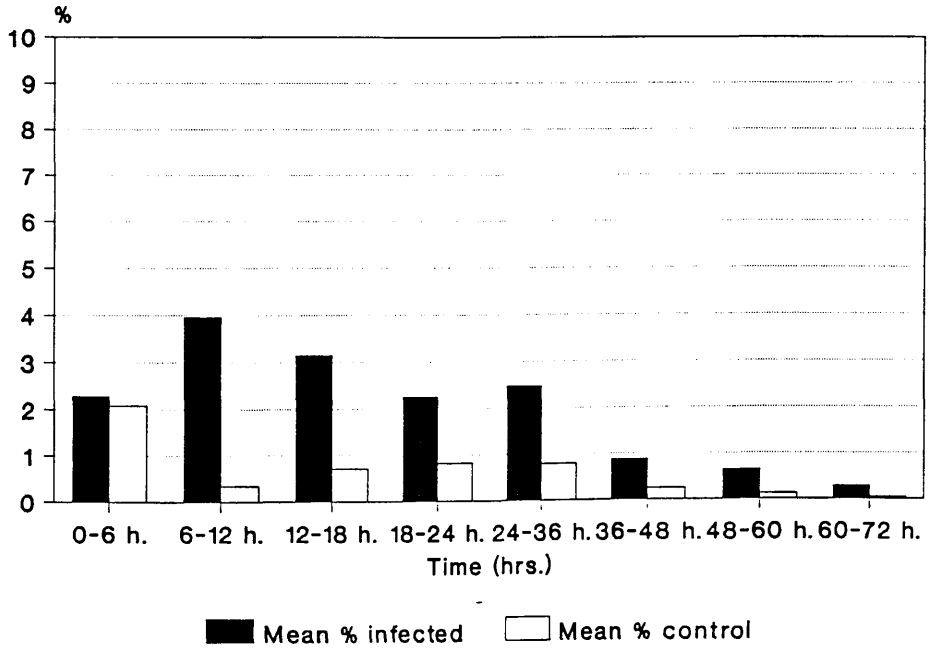


FIGURE 6.11. Experiment 6: Mean percentage of administered ^{51}Cr-EDTA measured in urine in control ponies and in the same ponies following trickle infection with 11.5 million cyathostome third-stage larvae (Day 88).

FIGURE 6.12. Experiment 6: Mean percentage of administered ^{51}Cr-EDTA measured in faeces in control ponies and in the same ponies following trickle infection with 5.5 million cyathostome third-stage larvae (Day 22).

FIGURE 6.11.

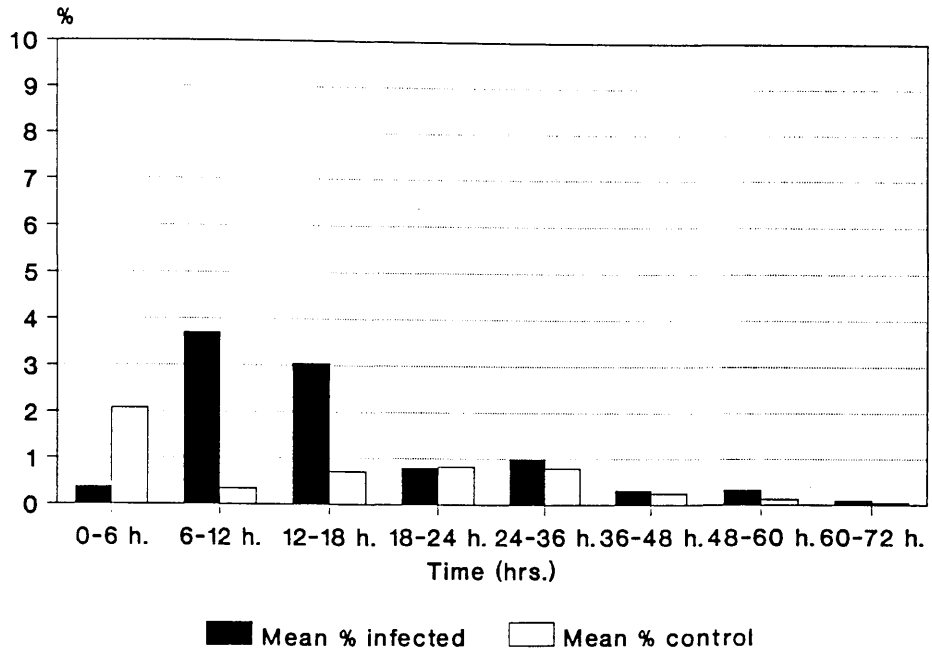


FIGURE 6.12.

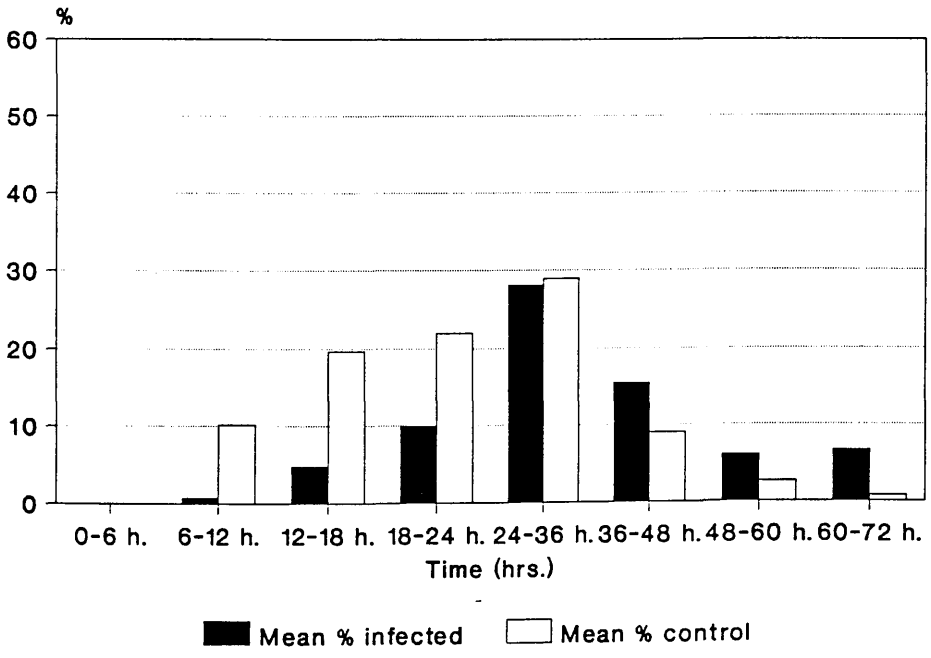


FIGURE 6.13. Experiment 6: Mean percentage of administered ^{51}Cr-EDTA measured in faeces in control ponies and in the same ponies following trickle infection with 11.5 million cyathostome third-stage larvae (Day 47).

FIGURE 6.14. Experiment 6: Mean percentage of administered ^{51}Cr-EDTA measured in faeces in control ponies and in the same ponies following trickle infection with 11.5 million cyathostome third-stage larvae (Day 88).

FIGURE 6.13.

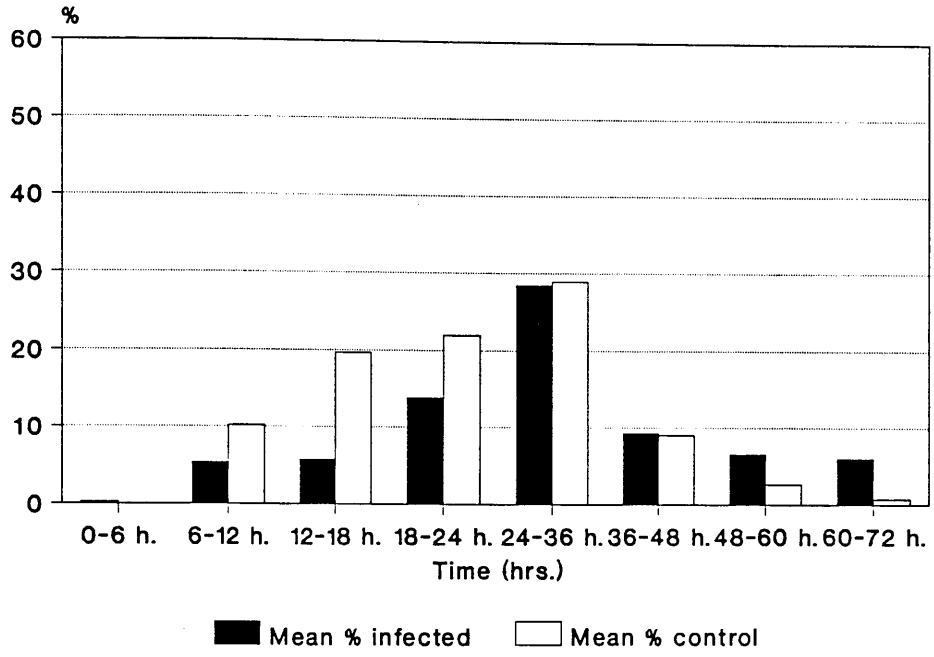


FIGURE 6.14.

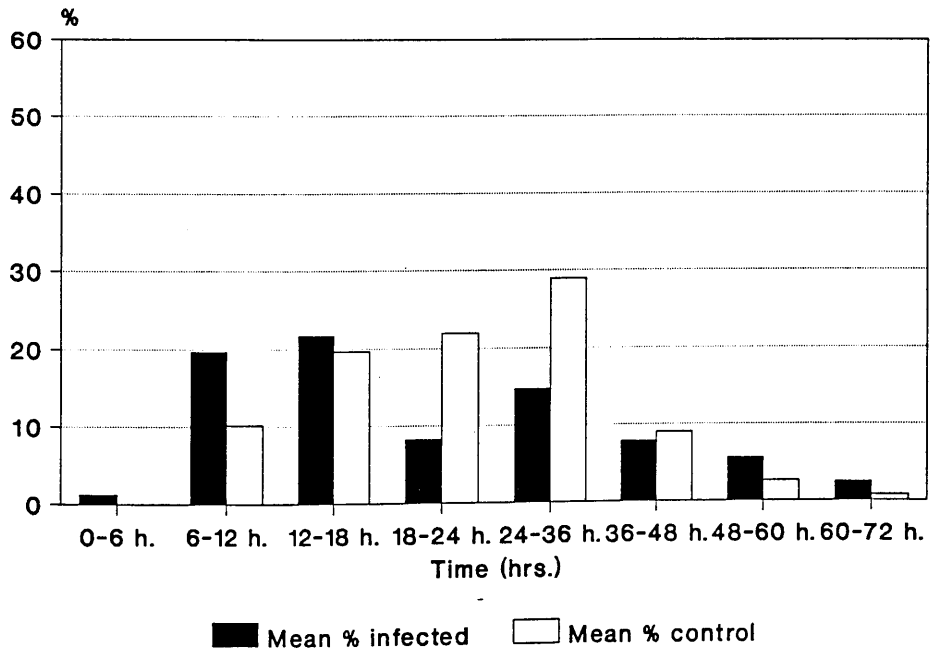


FIGURE 6.15. Macroscopic appearance of the mucosa of the dorsal colon of Pony no. 64. Note autolytic changes - necropsy carried out on Day 54.

FIGURE 6.16. Macroscopic appearance of the mucosa of the ventral colon of Pony no. 63. Necropsy carried out on Day 94.

TABLE 6.1.

Experiment 6: Cumulative percentage of administered ^{51}Cr-EDTA recovered in urine in ponies under control conditions and at three stages of experimental cyathostome infection.

CONDITIONS	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
Control	4.55	5.09	4.35	6.10	5.18	0.88
5.5 million L3 (Day 22)	4.17	6.45	7.58	5.05	6.36	1.27
11.5 million L3 (Day 47)	3.97	9.01	28.34	10.55	15.97	10.74
11.5 million L3 (Day 88)	1.90	11.08	-	8.19	9.64	2.04

Infected animals: 63, 64 and 70.

Mean and standard deviation (SD) refer to infected animals.

L3: Third-stage cyathostome larvae.

TABLE 6.2.

Experiment 6: Cumulative percentage of administered ^{51}Cr-EDTA recovered in faeces in ponies under control conditions and at three stages of experimental cyathostome infection.

CONDITIONS	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
Control	93.74	90.23	91.10	98.01	93.11	4.26
5.5 million L3 (Day 22)	74.78	91.98	67.20	56.16	71.80	18.40
11.5 million L3 (Day 47)	93.91	91.43	55.71	79.42	75.50	18.20
11.5 million L3 (Day 88)	78.06	84.78	-	78.68	81.73	4.31

Infected animals: 63, 64 and 70.

Mean and standard deviation (SD) refer to infected animals.

L3: Third-stage cyathostome larvae.

TABLE 6.3.

Experiment 6: Cyathostome worm burdens.

ANIMAL NUMBER	63	64
Total worm count.	1,208,370	254,870
Percentage of establishment of infected dose.	10.51	2.22
Mucosal larval count.	684,170	210,470
Percentage of total worm count in mucosa.	56.62	82.58

Infective dose: 11.5 million cyathostome third-stage larvae.

Post-mortem examination: Pony number 64, Day 53; Pony number 63, Day 94.

Percentage of establishment of the infective dose refers to the percentage of the infective dose of larvae which was recovered at necropsy.

Strongyle faecal egg counts remained negative in all infected animals with the exception of pony number 70 - the animal which survived the infection - which had a positive count of 700 eggs per gram of faeces on one occasion only (Day 53). This pony (number 70) started to put on weight from approximately Day 110 onwards and seven months after the experimental infection it was clinically normal. On the basis of repeatedly negative faecal worm egg counts it was considered that the entire cyathostome population which established in this animal have undergone inhibited development.

Details of the values obtained for total white blood cell, blood neutrophil and blood lymphocyte counts, packed cell volume, plasma albumin and globulins are given in Appendixes 3.3 to 3.8, respectively. Total white blood cell counts showed a significant increase between Days 20 and 60 of infection which was primarily due to an increase in neutrophil numbers whereas numbers of lymphocytes and eosinophils remained relatively unchanged. Packed cell volume remained unaltered throughout the infection but albumin levels decreased progressively from approximately Day 45 onwards with the exception of the pony that died on Day 53 (number 64) which had lower albumin levels from Day 30 onwards. In the surviving animal the plasma albumin levels remained consistently low throughout the seven month period following infection (25 grams per litre). Plasma globulin levels did not show notable changes in response to cyathostome infection in the animals in this study.

DISCUSSION.

On the first two occasions in the present study, the amounts of ^{51}Cr-EDTA excreted in urine during 72 hours (5.18% $\pm$ 0.88 and 6.36% $\pm$ 1.27, respectively) were similar to those obtained under control conditions in Experiment 1 (6.64% $\pm$ 2.36). On the first occasion, the test was carried out under control conditions and the results further confirm ^{51}Cr-EDTA

as a reliable tracer to assess equine intestinal permeability. Comparable results were obtained when the ^{51}Cr-EDTA solution was administered on Day 22 of the experimental trickle cyathostome infection i.e. after dosing with 5.5 million third-stage larvae. These results indicate either, that no significant changes in the intestinal permeability had occurred at the time the test was carried out or that the technique failed to detect alterations of intestinal permeability. In contrast to previous reports of experimental trickle cyathostome infections (Round, 1970; Love, 1990) in which a decrease in plasma albumin levels was detected during the first weeks following infection, in the present study hypoalbuminaemia was not apparent in any of the animals in the early stages of the infections. Considering together the results of the urinary excretion of the permeability marker and serial plasma albumin levels in the present study, it would appear that intestinal mucosal integrity was not compromised during the first three weeks of cyathostome infection. The recovery of ^{51}Cr-EDTA in faeces of the infected animals (71.80% $\pm$ 18.40) was lower than in the same animals under control conditions (93.11% $\pm$ 4.26) which might suggest a reduced intestinal motility. This finding would be in agreement with the data recorded by other authors (Bueno and others, 1979; Love, 1990) but a similar reduction was observed in the uninfected, control animal which prevents more definitive conclusions.

On the next occasion on which the test was carried out, 11.5 million third-stage larvae had been given. During the 72 hours following the administration of ^{51}Cr-EDTA between Days 47 and 50, the urinary excretion of the marker in pony number 64 was 28.34% of the administered dose. This animal had been diarrhoeic and anorexic for the previous 3 days and died six days later with signs of hyperlipaemia The amounts of ^{51}Cr-EDTA collected in urine in the other infected animals was also significantly increased (9.01% and 10.55%) ($p < 0.05$) when compared to the values obtained in the same animals under control conditions (5.18 $\pm$ 0.88). Thus, increased ^{51}Cr-EDTA urinary excretion appeared to reflect alterations of the intestinal permeability induced by

the experimental cyathostome infection and emergence of larvae was suspected to be the cause of the unusually high excretion recorded in the animal which died (number 64).

Finally, the test was repeated on Day 88 on the two remaining infected animals, of which one (Pony number 63) exhibited signs of diarrhoea and anorexia at the time of study. This animal was killed three days after the end of the 72-hour collection. The values of ^{51}Cr-EDTA urinary excretion in the two infected ponies (11.08% and 8.19%) were increased when compared to those obtained in the same animals under control conditions (5.09% and 6.10%) and also to that of the uninfected animal (1.90%). The value for the control was in fact unusually low possibly due to incomplete urine collection. The amounts of tracer detected in faeces were once more moderately lower than in the control situation.

A noticeable decrease in the plasma albumin levels was observed in all the cyathostome infected animals. This finding is in agreement with the observations reported in naturally occurring cases of larval cyathostomiasis (Giles and others, 1985; Church and others, 1986; Love, 1990). In this study the changes in intestinal permeability detected by measurement of ^{51}Cr-EDTA excretion in urine were coincident with clinical signs and plasma protein abnormalities which suggest that the pathogenic effects of the experimental cyathostome infections were a result of protein-losing enteropathy.

The most marked increase in the urinary ^{51}Cr-EDTA excretion values in cyathostome infected ponies in this study occurred during the first 24 hours and, particularly, from six to 24 hours. This finding was to some extent predictable since the pathological changes of larval cyathostomiasis are limited to the large intestine and in experimental studies on intestinal transit times in ponies it was demonstrated that 75% of a liquid marker had left the stomach within 0.5 hours after intragastric

administration, and approximately 68% was recovered in the caecum after two hours (Argenzio and others, 1974). This finding should be of relevance in the future development of a standard 24-hour test using ^{51}Cr-EDTA to detect alterations in intestinal permeability in horses with suspected chronic enteropathies. Similar techniques have been described for the assessment of intestinal permeability as a screening test for the detection of intestinal mucosal damage and also responses to treatments in clinical cases of enteropathy in man (Bjarnason and others, 1983a and b; O'Morain and others, 1986; Elia and others, 1987; Jenkins and others, 1987 and 1988; Fotherby and others, 1988) and dogs (Hall and Batt, 1990).

EXPERIMENT 7: INTESTINAL PERMEABILITY TO ^{51}Cr-EDTA IN HORSES WITH NATURALLY-OCCURRING CHRONIC ENTEROPATHY.

INTRODUCTION.

In the previous experiments reported in the present thesis, ^{51}Cr-EDTA was validated as a marker for the investigation of equine intestinal permeability and its suitability was further substantiated in ponies with experimental cyathostome infections.

Three clinical cases of suspected chronic enteropathy referred to the Department of Veterinary Medicine, University of Glasgow Veterinary School, were used to test the usefulness of ^{51}Cr-EDTA as a marker for intestinal permeability in the clinical situation.

Case 1: 30 year-old Welsh Mountain pony gelding.

History.

The animal had been losing weight for several weeks and it had been noticed to have very poor teeth.

Clinical findings.

On presentation, the pony was bright and alert but it was in an emaciated condition with generalized muscle atrophy. A full clinical examination was carried out of which the most relevant findings were: appetite was good but mastication was prolonged; faeces were of relatively normal consistency, but contained a large amount of undigested

fibre; some faecal staining was noticed on the hindlimbs; rectal temperature was 36.6°C and heart and respiratory rate were within the normal ranges; oral mucous membranes were normal in colour and the capillary refill time was approximately two seconds; borborygmi were of normal intensity and frequency in all four abdominal quadrants; molar teeth were in very poor condition; no abnormality was noticed in other body systems and on examination *per rectum*.

Laboratory findings.

The only abnormality observed in blood biochemical and haematological analyses of this animal (Appendix 3.10) was a slight lymphopaenia (0.92 x 10^9/l). Faecal strongyle egg count was 550 eggs/gram on presentation. An oral glucose tolerance test (Roberts and Hill, 1973) revealed evidence of small intestinal malabsorption (Appendix 3.11).

Clinical diagnosis and management.

A malabsorption syndrome and possibly a protein-losing enteropathy, of undefined origin were considered. Oral ivermectin (Eqvalan Paste for Horses, MSD AGVET, Hertfords.) was administered at a dose of 0.2 mg/kg.bwt.

^{51}Cr-EDTA test.

During the period in which the test was carried out, the pony was kept in a stall and the floor was covered with rubber mats. Hay and water were available *ad libitum*.

A solution of ^{51}Cr-EDTA was administered per stomach tube following a period of 10-hour fasting. The solution was washed through the tube with 300 ml of water and the animal was allowed to eat two hours after the experiment was started.

Total urine and faeces produced in 72 hours were collected in 12-hour intervals. Urine was collected directly into a polythene bag contained in a plastic bag fixed to the body of the pony by two canvas tapes. Faeces were allowed to pass onto the floor and were swept up and bagged.

The amount of ^{51}Cr-EDTA recovered in urine was expressed as both the percentage during each interval and as the cumulative percentage of the administered dose (Figures 7.1 and 7.2). The same system was used to express data obtained in faeces (Figures 7.3 and 7.4). Details of the values of ^{51}Cr-EDTA measured in urine and faeces are given in Appendixes 1.10 and 2.10, respectively.

The pattern of urinary ^{51}Cr-EDTA excretion for case number 1 was abnormal when compared to the previous experiments, although almost 50% of the total amount excreted in urine (4.73%) was recovered in the first 24 hours. Approximately 54% of the administered dose was recovered in faeces, which is a low value compared with previous experiments and most likely was a result of a marked increase in transit time.

Clinical progression.

Although no definitive diagnosis was reached, on the basis of malabsorption and considering the age and state of the animal, the prognosis was considered to be extremely poor and the animal was destroyed.

FIGURE 7.1. Experiment 7: Percentage of administered ^{51}Cr-EDTA measured in urine in Case no. 1.

FIGURE 7.2. Experiment 7: Cumulative percentage of administered ^{51}Cr-EDTA measured in urine in Case no. 1.

FIGURE 7.1.

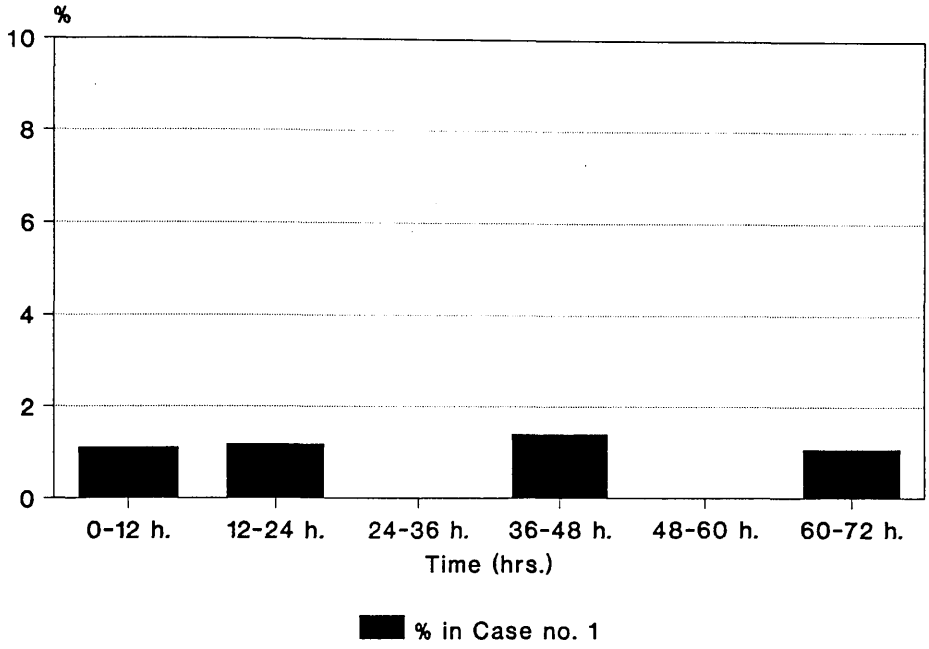


FIGURE 7.2.

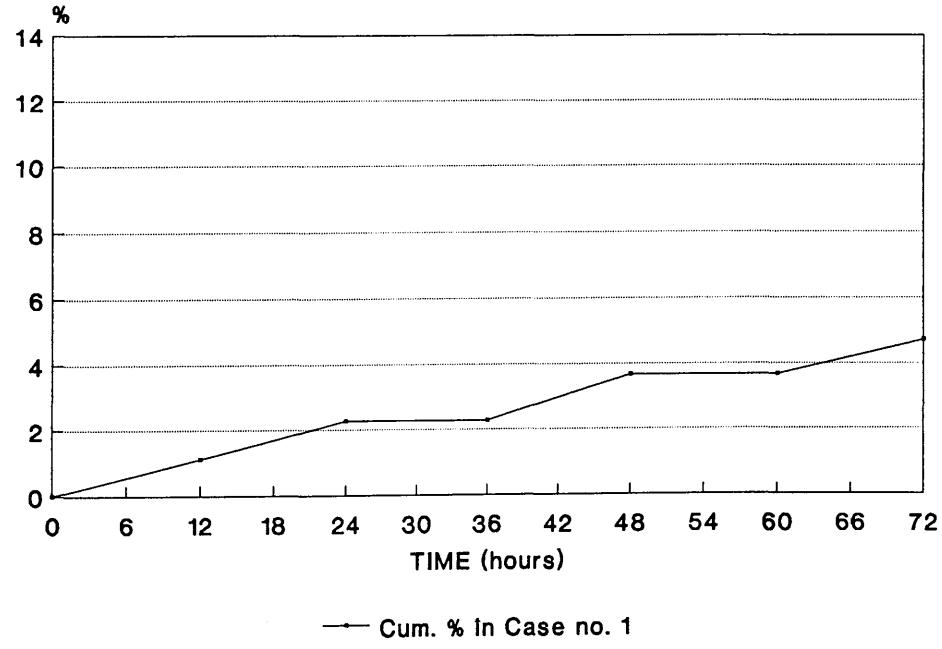


FIGURE 7.3. Experiment 7: Percentage of administered ^{51}Cr-EDTA measured in faeces in Case no. 1.

FIGURE 7.4. Experiment 7: Cumulative percentage of administered ^{51}Cr-EDTA measured in faeces in Case no. 1.

FIGURE 7.3.

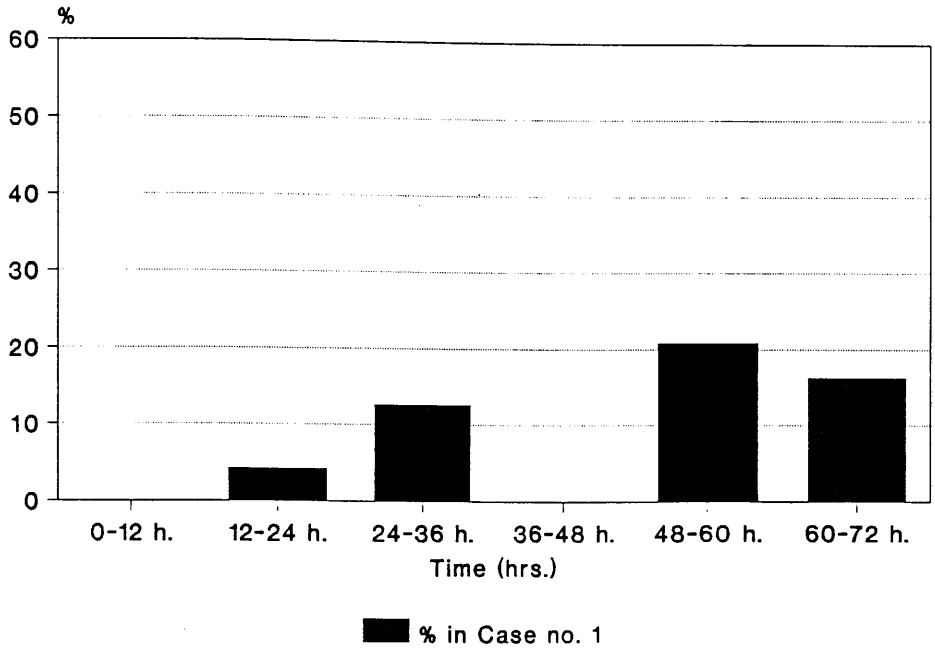
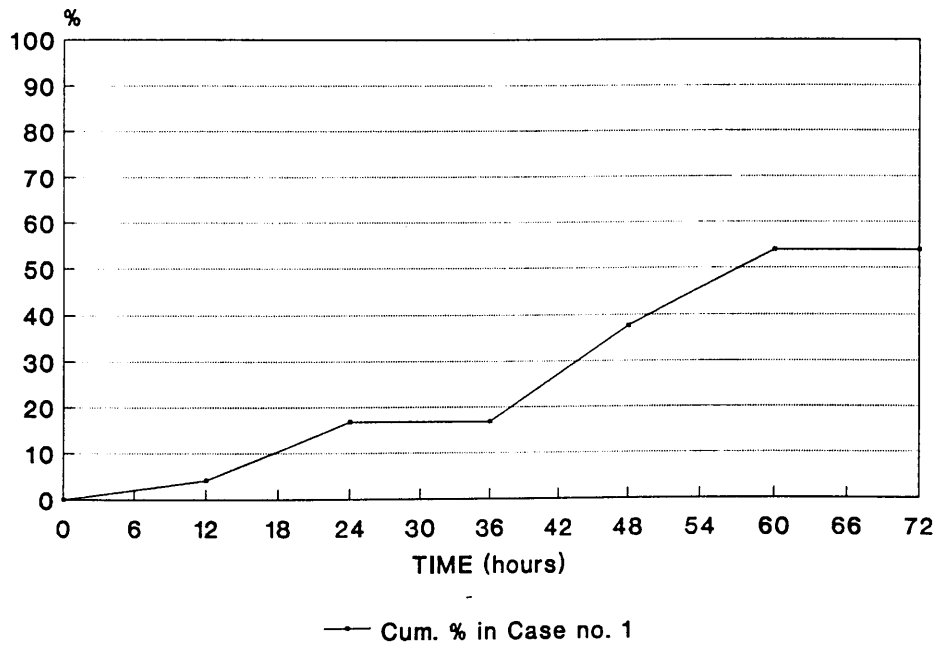


FIGURE 7.4.



Pathological findings.

Post-mortem examination was carried out in the Department of Veterinary Pathology and gross pathology and histopathological examination revealed no signs of any specific enteropathy and only oedema and a moderate infiltrate with mononuclear cells were found in the large intestine. The only abnormality observed was partial gastric impaction. No explanation for the malabsorption was found and the diagnosis remained inconclusive.

Case 2: 13 year-old Arab-cross mare.

History.

This animal had a confusing five-month history of low grade abdominal pain and/or stiffness frequently associated with exercise. During the last two months before presentation the mare had started to lose weight and the attacks of mild abdominal discomfort appeared to occur more frequently.

Clinical findings.

The animal was bright, alert and appetant. The faeces appeared fairly dry. Thorough clinical examination revealed no obvious abnormalities to explain recurrent colic and weight loss but an excess of dry faeces were noticed on examination *per rectum*. On paracentesis, the peritoneal fluid appeared slightly flocular, with some increase in viscosity.

Laboratory findings.

Blood biochemical analysis (Appendix 3.10) revealed no abnormality with the exception of slightly low plasma albumin levels (27 g/l). On haematological examination (Appendix 3.10), moderate leucocytosis (9.1 x 10^9/l) due primarily to neutrophilia (6.96 x 10^9/l) was detected. Examination of faeces for strongyle eggs and bacteriological culture gave negative results. Peritoneal fluid obtained on paracentesis on three occasions showed an increasing level of total proteins (27 g/l on Day 3; 38 g/l on Day 11; normal value: 7-20 g/l) and moderate leucocytosis (13.9 x 10^9/l; normal value: < 10 x 10^9/l) due to neutrophilia (12.16 x 10^9/l). Bacteriological culture of the peritoneal fluid rendered no isolation on Day 7 but significant numbers of non-haemolytic *Escherichia coli* and *Streptococcus* spp. were cultured on Day 12 of hospitalization. An oral glucose tolerance test (Roberts and Hill, 1973) showed a partial malabsorption (Appendix 3.11).

Clinical diagnosis and management.

A clinical diagnosis of chronic enteropathy and mild peritonitis was made and no treatment was attempted.

^{51}Cr-EDTA test.

The mare was kept in an individual stall covered with rubber mats. Hay and water were available *ad libitum*.

A solution of ^{51}Cr-EDTA was administered per stomach tube following a period of 10-hour fasting. The solution was washed through the tube with 300 ml of water and the animal was allowed to eat two hours after the experiment was started.

Total urine and faeces produced in 72 hours were collected at 6-hour intervals on the first day and subsequently at 12-hour intervals. Urine was collected by means of a Foley catheter (7.33 mm diameter; 40 cm length; 30 cc balloon volume) into polythene bags and faeces were swept up from the floor and bagged.

The amount of ^{51}Cr-EDTA recovered in urine was expressed as both the percentage during each interval and as the cumulative percentage of the administered dose (Figures 7.5 and 7.6). The same system was used to express data obtained in faeces (Figures 7.7 and 7.8). Details of the values of ^{51}Cr-EDTA measured in urine and faeces are given in Appendixes 1.10 and 2.10, respectively.

The total urinary excretion of ^{51}Cr-EDTA in 72 hours in case number 2 was abnormally low (1.67%) and approximately 66% of this amount was recovered in the first 24 hours. Most of the amount of ^{51}Cr-EDTA recovered in faeces in 72 hours (85.34%) was detected on the second day.

Clinical progression.

The mare showed continuous signs of mild colic and laparotomy under general anaesthesia was undertaken in the Department of Veterinary Surgery which revealed several nodules in the wall of the small intestine and some adhesions had been formed between these nodules and the mesentery. A diagnosis of intestinal neoplasia was made and euthanasia on human grounds was decided during the operation.

FIGURE 7.5. Experiment 7: Percentage of administered ^{51}Cr-EDTA measured in urine in Case no. 2.

FIGURE 7.6. Experiment 7: Cumulative percentage of administered ^{51}Cr-EDTA measured in urine in Case no. 2.

FIGURE 7.5.

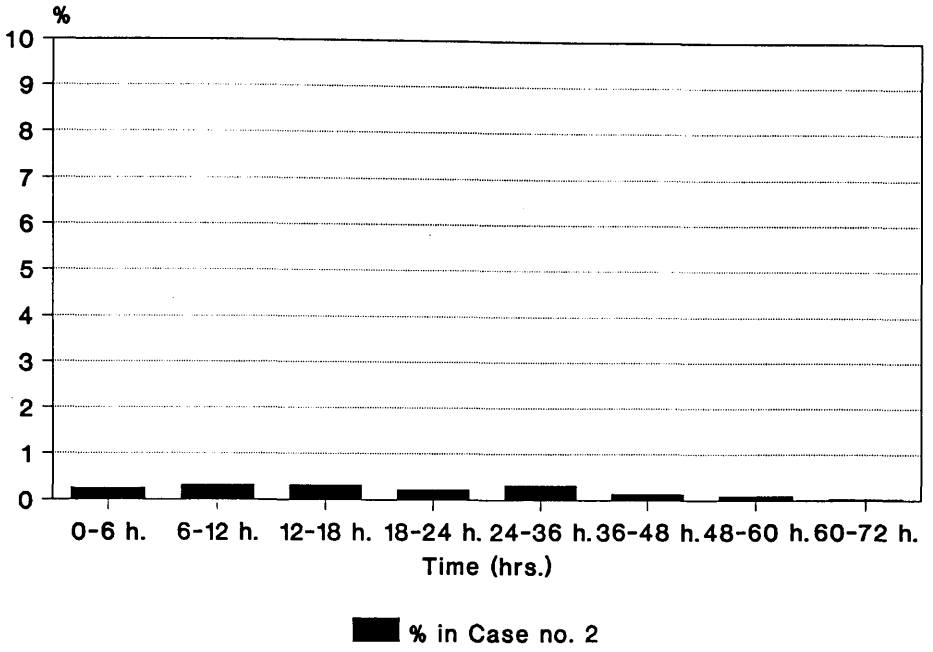


FIGURE 7.6.

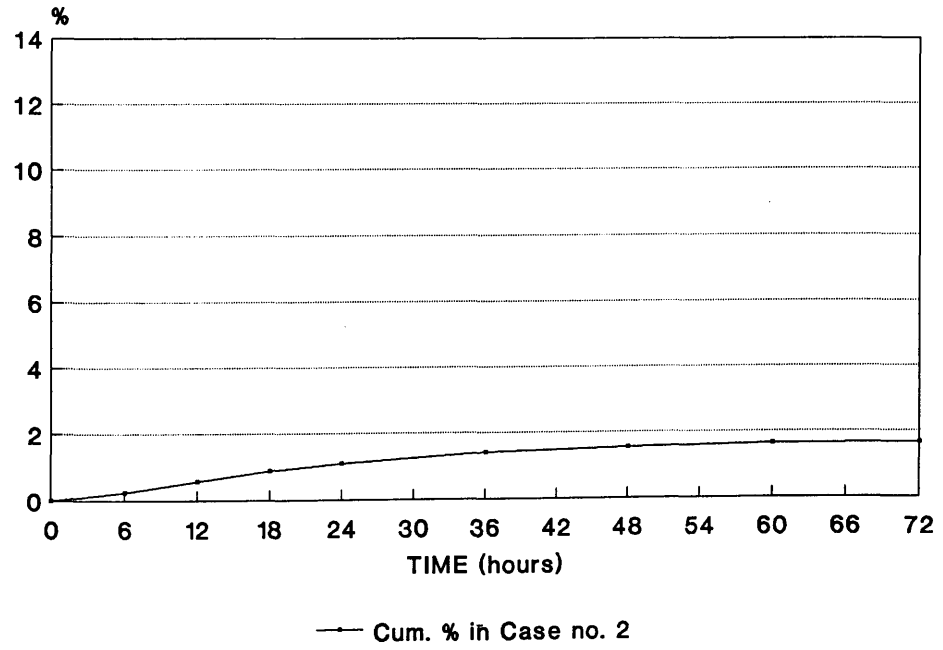


FIGURE 7.7. Experiment 7: Percentage of administered ^{51}Cr-EDTA measured in faeces in Case no. 2.

FIGURE 7.8. Experiment 7: Cumulative percentage of administered ^{51}Cr-EDTA measured in faeces in Case no. 2.

FIGURE 7.7.

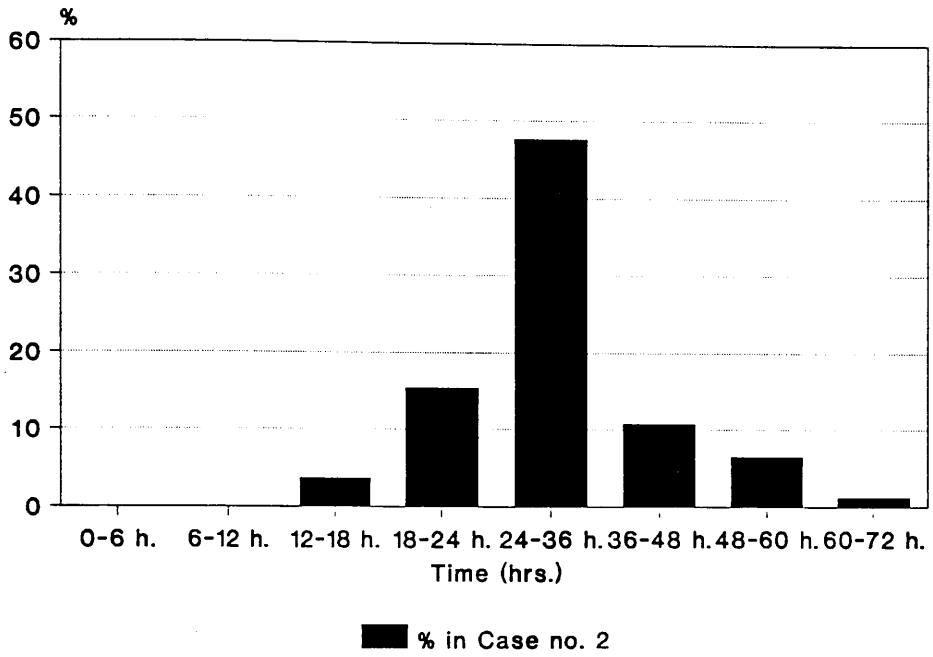
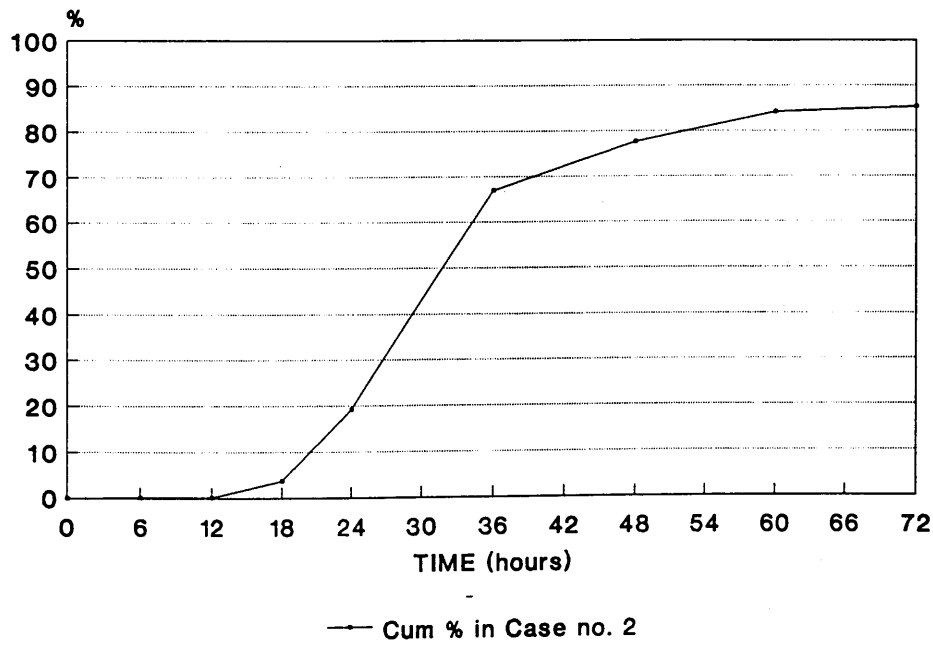


FIGURE 7.8.



Pathological findings.

On post-mortem examination in the Department of Veterinary Pathology several fibrous thichenings were observed in the small intestinal wall which caused stenosis and dilatation proximally and adhesions had been formed between these points and the omentum. The proximal small intestinal wall was diffusely thickened and when it was opened two further nodules with deeply ulcerated necrotic centres were found. A number of ulcers surrounded by scar tissue were present along the mucosa of the small bowel. The stomach, large intestine and abdominal lymph nodes appeared uninvolved.

Histopathology of nodules and ulcers revealed granulation tissue and extensive fibrosis with adjacent epithelioid macrophages. A diffuse infiltrate of lymphoid and plasma cells was present in the mucosa, lamina propia and muscular layers of the small intestine and the mucosa appeared oedematous with either thickened and stunted, or long, frond-like villi. A diagnosis of granulomatous enteropathy was made.

Case 3: 16 year-old Hunter mare (Figure 3.9).

History.

The mare had been moderately diarrhoeic and losing weight for several weeks. On the last days prior to referral the diarrhoea became watery and did not respond to symptomatic treatment.

Clinical findings.

The mare was in very poor condition and weak, but reasonably bright. It picked at hay but was not interested in concentrates when offered. There was some ventral oedema and the tail and hind quarters

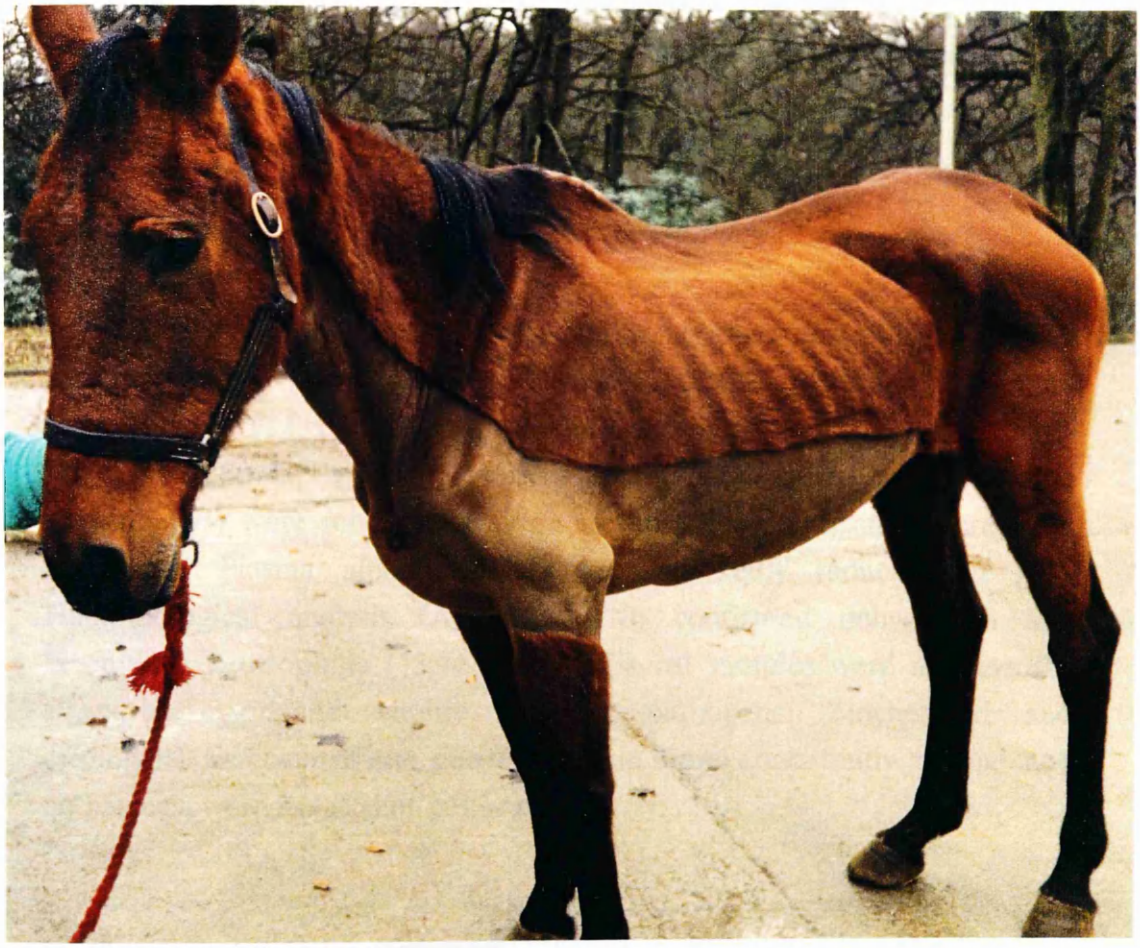


FIGURE 7.9. Experiment 7: Case no. 3: 16 year-old Hunter mare, on Day 19 of hospitalization. Muscle atrophy was evident. Ventral oedema had regressed at that stage.

were stained by faeces, which were of a watery nature. The eyes were sunken and there was a decrease in skin turgor, indicative of dehydration. Thorough clinical examination revealed: rectal temperature was 38.0°C; oral mucous membranes appeared congested; the pulse was slightly elevated in frequency (48/minute); fluid borborygmi could be auscultated in all four quadrants; *per rectum*, the intestinal wall appeared oedematous and the contents were very fluid.

Laboratory findings.

Blood biochemical investigation (Appendix 3.10) showed hyponatraemia (121 mmol/l), hypochloraemia (87 mmol/l) and hypokalaemia (1.8 mmol/l) and alkaline phosphatase activity was slightly increased (480 U/l). Blood levels of urea (15.3 mmol/l) and creatinine (168 umol/l) were moderately elevated, most likely a result of pre-renal azotaemia. Plasma albumin levels were markedly reduced (12 g/l). Haematological analysis (Appendix 3.10) confirmed dehydration and revealed a neutrophilia (13.94 x 10^9/l). Faecal samples were negative for strongyle eggs and enteric bacterial pathogens. Biochemical and cytological analyses of the peritoneal fluid were consistently normal and no bacteria were isolated on culture.

Clinical diagnosis and management.

A diagnosis of protein-losing enteropathy was made and symptomatic therapy was started immediately: initially Hartmann's solution, to which a mixture of sodium and potassium chloride had been added, was administered intravenously. Fluid therapy, including plasma substitutes (Haemaccel Infusion Solution, Hoechst Animal Health, Buckinghams.), was adapted according to the changes detected in the animal's status. Oral administration of codeine phosphate was started at

one mg/kg.bwt b.i.d.. Flunixin meglumine (Finadyne Solution, Schering-Plough Animal Health, Suffolk) was injected at a dose of 0.25 mg/kg.bwt every eight hours for two days for its antiendotoxic properties. Prednisolone (Prednisolone Tablets, Animalcare Ltd., York) tablets were administered from Day 3 of hospitalization onwards at one mg/kg.bwt/day. The animal was stabilized and fluid therapy was discontinued on Day 4.

An oral glucose tolerance test was carried out on Day 5 which showed a poor absorption (Appendix 3.11). The blood biochemical and haematological parameters improved notably by Day 22 (Appendix 3.10) with the exception of plasma albumin levels which were increasing but they were still unusually low. Alkaline phosphatase activity remained elevated and there was a moderate neutrophilia and lymphopaenia, most likely a result of corticosteroid administration. A rectal biopsy revealed no abnormality at that site of the gastrointestinal tract.

^{51}Cr-EDTA test.

During the time the procedure was being carried out, the mare was kept in an individual stall covered with rubber mats. Hay and water were available *ad libitum* and concentrates were fed twice daily.

A solution of ^{51}Cr-EDTA was administered per stomach tube following a period of 10-hour fasting. The solution was washed through the tube with 300 ml of water and the animal was allowed to eat two hours after the experiment was started.

Total urine and faeces produced in 48 hours were collected at 6-hour intervals for the first 24 hours and at 12-hour intervals on the second day. Urine was collected by means of a Foley catheter (7.33 mm diameter; 40 cm length; 30 cc balloon volume) into polythene bags and faeces were swept up from the floor and bagged.

The amount of ^{51}Cr-EDTA recovered in urine was expressed as both the percentage during each interval and as the cumulative percentage of the administered dose (Figures 7.10 and 7.11). The same system was used to report the values obtained in faeces (Figures 7.12 and 7.13). Details of the values of ^{51}Cr-EDTA measured in urine and faeces are given in Appendixes 1.10 and 2.10, respectively.

The total urinary excretion of ^{51}Cr-EDTA in 48 hours in Case number 3 was 9.19% and approximately 68% of this amount was recovered during the first 24 hours. The amount of ^{51}Cr-EDTA measured in faeces in 48 hours was 67.95%.

Clinical progression.

By Day 7, the mare was bright and appetant and was passing normal faeces. It started to put on some weight while in the hospital. The ventral oedema was regressing progressively. Codeine phosphate administration was continued in a reducing dose; a similar protocol was followed for prednisolone administration. The differential diagnosis considered at this stage was one of malabsorption and protein-losing enteropathy, including alimentary lymphosarcoma and chronic infiltrative enteropathy. The mare was discharged on Day 33 and the owner was requested to bring it two weeks later for re-examination.

Examination was repeated on Day 48 and although no abnormality was detected the mare continued to lose weight. An oral glucose tolerance test at this stage (Appendix 3.11) showed that plasma glucose levels doubled by 120 minutes following oral administration. The mare was discharged but it started to deteriorate thereafter and it was decided to euthanase it on Day 56 on the basis of continued weight loss.

FIGURE 7.10. Experiment 7: Percentage of administered ^{51}Cr-EDTA measured in urine in Case no. 3.

FIGURE 7.11. Experiment 7: Cumulative percentage of administered ^{51}Cr-EDTA measured in urine in Case no. 3.

FIGURE 7.10.

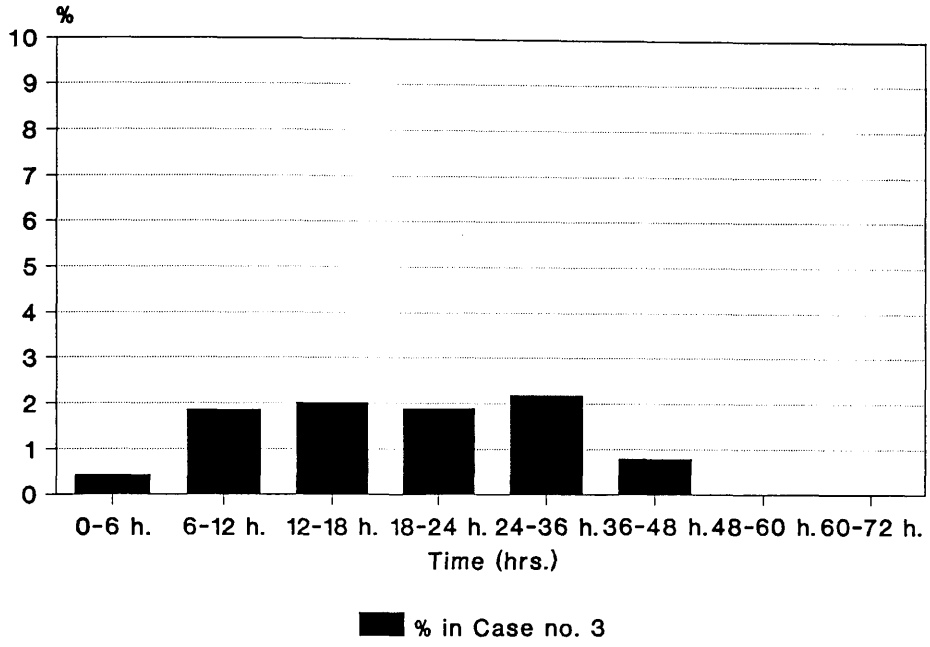


FIGURE 7.11.

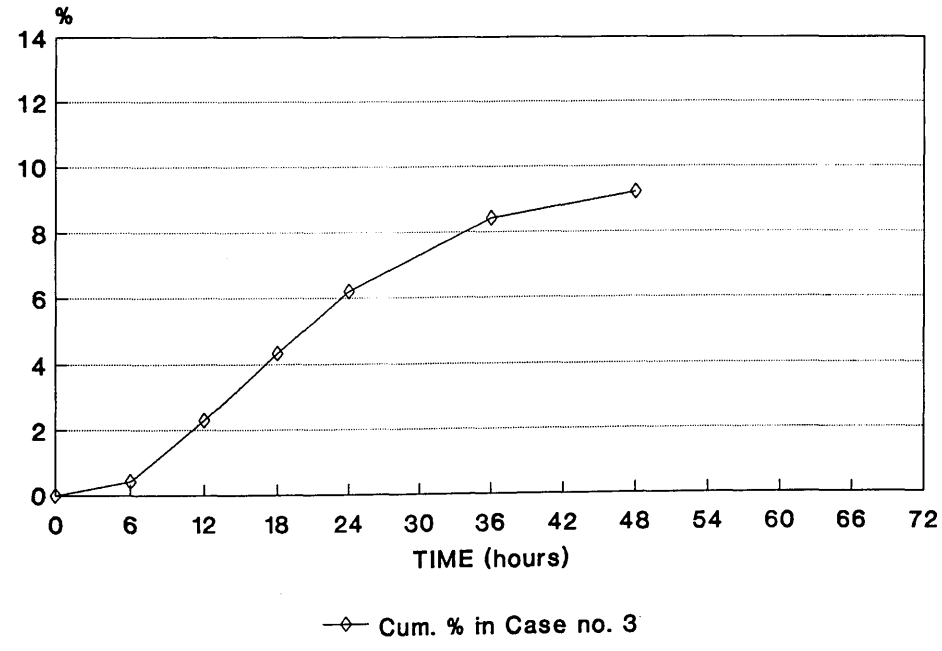


FIGURE 7.12. Experiment 7: Percentage of administered ^{51}Cr-EDTA measured in faeces in Case no. 3.

FIGURE 7.13. Experiment 7: Cumulative percentage of administered ^{51}Cr-EDTA measured in faeces in Case no. 3.

FIGURE 7.12.

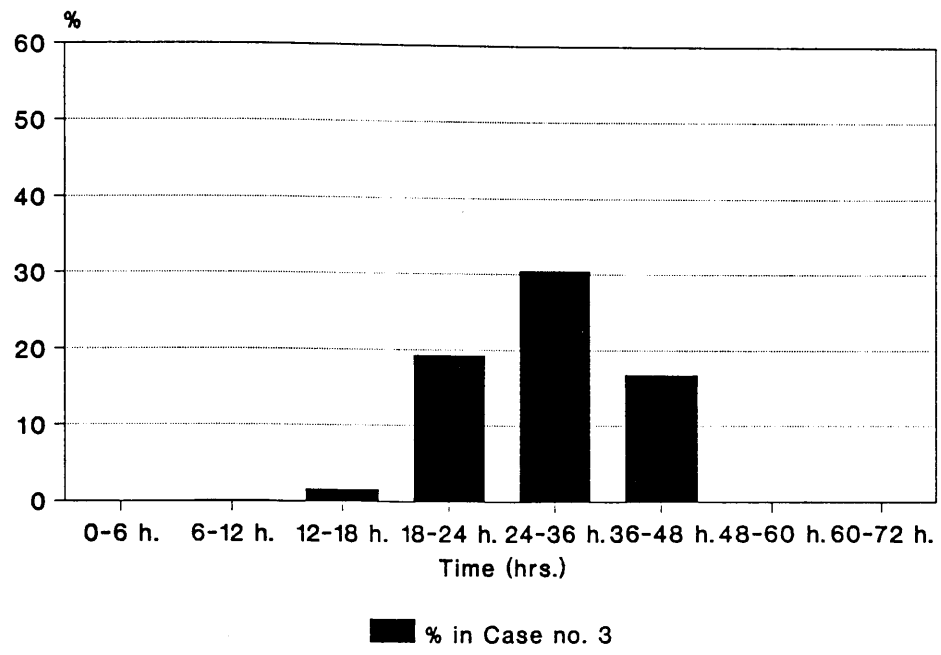
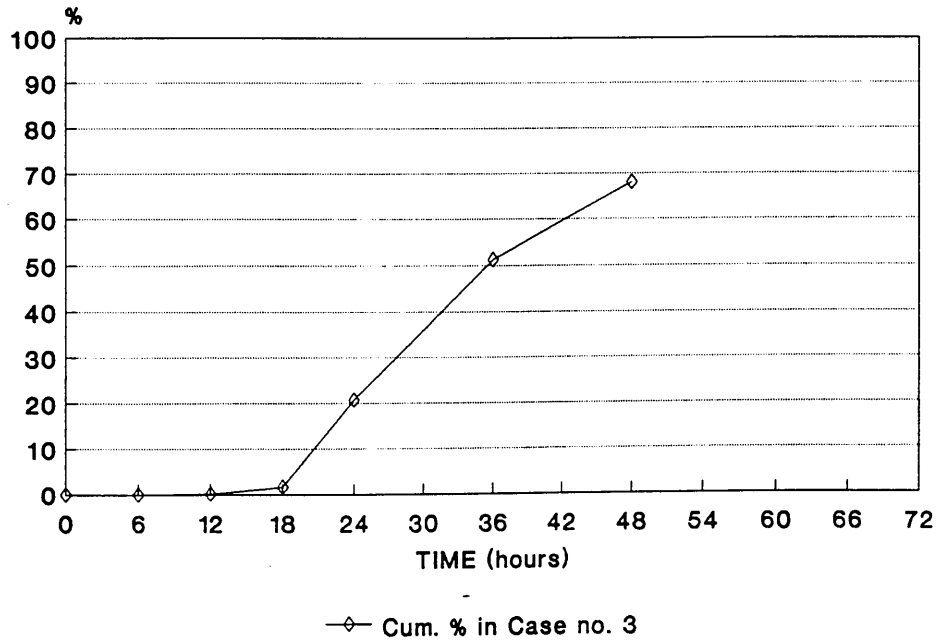


FIGURE 7.13.



Pathological findings.

At necropsy, the large intestinal wall was thickened and histopathology showed that this was due to diffuse infiltration by primarily eosinophils although significant numbers of mononuclear cells were present. The eosinophilic infiltrate was particularly severe around the ileocaecocolic valve. Multiple discrete, 1 cm diameter ulcers were found in the mucosa of the large bowel in which many fungal colonies were observed. Interestingly, the small intestine appeared unaffected. Eosinophilic granulomas were observed in most mesenteric lymph nodes and chronic eosinophilic pancreatitis was evident. A diagnosis of chronic eosinophilic enteritis was made.

DISCUSSION.

The three cases reported in the present study corroborate the view that equine chronic enteropathies may present a serious diagnostic challenge to the clinician.

Case number 1 presented clinically as a 'wasting horse' but clinical examination and laboratory investigations threw no light to the diagnosis. The oral glucose tolerance test (Roberts and Hill, 1973) proved of value in detecting small intestinal malabsorption and on the basis of this finding it was considered that the prognosis for this animal was poor. Although the cumulative percentage of administered ^{51}Cr-EDTA measured in urine during 72 hours (4.73%) was comparable to that obtained in ponies in Experiment 1 (6.64% $\pm$ 2.36), the pattern of intestinal absorption of the marker was abnormal. However, its interpretation was very difficult, even more so when no signs of enteropathy were found when the animal was examined post-mortem. The pattern of ^{51}Cr-EDTA excretion in faeces indicated a dramatic increase in intestinal transit time and the observation

of a full stomach at necropsy appears to confirm this finding. Intestinal dysfunction not accompanied by structural changes was suggested in this case.

Case number 2 presented with a confusing history of mild abdominal discomfort and/or stiffness initially associated with exercise. Moderate weight loss had been evident for several weeks prior to referral. A full clinical examination and the laboratory findings failed to highlight significant alterations, although slight hypoalbuminaemia was detected. An oral glucose tolerance test showed a degree of small intestinal malabsorption but the result was not definitive. The interpretation of the low recovery of ^{51}Cr-EDTA in urine remains unresolved although it might be explained to some extent by the localised pathological lesions observed at necropsy.

The third horse in the present study exhibited more specific and severe signs of enteropathy. The clinical and laboratory findings suggested intestinal mucosal malfunction and this was corroborated by the ^{51}Cr-EDTA test. The increased urinary excretion of ^{51}Cr-EDTA was most likely a result of increased intestinal permeability. Equine chronic eosinophilic gastroenteritis was first reported in 1982 (Pass and Bolton) and was characterised clinically by chronic weight loss and occasionally diarrhoea and, in addition, skin lesions may be observed; it appeared that cellular infiltration of the gastrointestinal tract might result in malabsorption and protein-losing enteropathy. In Case number 3 alterations found at necropsy were limited to the large intestine. Although small intestinal dysfunction was confirmed by the oral glucose tolerance test performed on Day 5, when this was repeated on Day 48 the pattern of absorption of glucose was normal which might indicate that corticosteroid therapy was effective in restoring normal function of the small intestine.

In conclusion, the application of the ^{51}Cr-EDTA test may be useful in future for the assessment of clinical cases of equine enteropathy. However, any judgement must await until the test is performed on more animals. Electron microscopic studies, not available in this study, might have been of great use in determining the pathophysiology of the conditions observed in this animals and its correlation with the results obtained.

CHAPTER V: GENERAL DISCUSSION AND CONCLUSIONS.

CHAPTER V: GENERAL DISCUSSION AND CONCLUSIONS.

In comparison with other domestic animals, progress in the understanding of diseases involving the complex equine gastrointestinal tract is exceptionally limited. This situation does not reflect the importance that the alimentary system holds, which was reported to be involved as the cause of death in one third of cases in a survey of post-mortem findings in 480 horses (Baker and Ellis, 1981). In recent years much thought and study has been focused at the acute abdominal conditions i.e. 'colic' (Gordon and Allen, 1988; White, 1990) and acute enterocolitis (Roberts, 1990; White, 1990). However the limited knowledge of the aetiopathogenesis of equine chronic enteropathies, as discussed in the first chapter of this thesis, may be best illustrated by the results reported in a survey of clinical cases of diarrhoea in adult horses in the UK (Mair and others, 1990a) in which 65% of the cases remained undiagnosed. Thus the diagnosis and management of equine chronic enteropathies present many difficulties for the clinician. This is mainly due to the similar pattern of presenting clinical signs of chronic weight loss and/or diarrhoea and the lack of specificity of the diagnostic techniques currently available.

Human chronic enteropathies exhibit certain similarities to some of the equine enteropathies reviewed in the present study. Hypoalbuminaemia is a common finding in Crohn's disease, ulcerative colitis and coeliac disease and, in the equine species, it has been recorded in cases of alimentary lymphosarcoma (Wiseman and others, 1974; Roberts and Pinsent, 1975; Van den Hoven and Franken 1983; Humphrey and others, 1984; Crawley, 1985; Sheeran, 1987), gastric squamous cell carcinoma (Wrigley and others, 1981; Tennant and others, 1982), granulomatous enteritis (Merritt and others, 1976; Meuten and others,

1978; Roberts and Kelly, 1980; Hodgson and Allen, 1982; Sweeney and others, 1986), chronic eosinophilic gastroenteritis (Pass and Bolton, 1982; Breider and others, 1985; Gibson and Alders, 1987), tuberculosis and histoplasmosis (Roberts, 1984), strongylosis (Round, 1970; Patton and others, 1978; Giles and others, 1985; Church and others, 1986; Love, 1990), PBZ toxicity (Snow and others, 1981; Lees and others, 1983; Collins and Tyler, 1985), and pancreatic adenocarcinoma (Church and others, 1987). In equine medicine, reduced plasma albumin levels generally result from increased loss of proteins into the gastrointestinal tract due to increased intestinal permeability (Merritt and others, 1977; Coffman, 1979 and 1984; Dietz and Nielsen, 1980; Pearson, 1990).

Alterations in intestinal permeability have been suggested to be involved in the pathogenesis of chronic enteropathies both as a consequence of a compromised intestinal mucosa, and as a primary defect permitting the access through the intestinal epithelium of substances which are not absorbed under normal circumstances (Chadwick and others, 1977; Bjarnason and others, 1983a; Bjarnason and Peters, 1984; Jenkins and others, 1988; Hall and Batt, 1990). Further, abnormalities in the intestinal permeability have been shown to be associated with an increase in the incidence of neoplasia in patients with coeliac disease (Walker and Isselbacher, 1974) and inflammatory bowel disease (Lennard-Jones and others, 1977; Gyde and others, 1980). Interestingly, alterations in intestinal permeability may not always result in detectable changes in plasma variables such as albumin levels when an increase in the intake of proteins may compensate for the increased loss from plasma, which has been demonstrated in studies on small ruminants experimentally infected with helminth parasites (Abbott and others, 1986). Recent studies in human and veterinary medicine have tried to delineate the mechanisms by which alterations in intestinal permeability develop and the effect that they have on the gastrointestinal tract.

The first objective of the present study was to assess the suitability of ^{51}Cr-EDTA for the assessment of intestinal permeability in ponies. The first four experiments were designed for that purpose. The results obtained in ponies under control conditions were between 3.93% and 9.02% (mean ± SD; 6.64% ± 2.36) and in the same animals following modification of the intestinal motility with either atropine or bethanecol there were no significant differences between the percentages of ^{51}Cr-EDTA recovered in urine.

Alterations in intestinal motility have been confirmed in horses with strongylosis (Bueno and others, 1979; Berry and others, 1986). It would seem likely that similar alterations of motility occur in other equine chronic enteropathies which might modify the pattern of absorption of intestinal permeability markers, rendering such markers unsuitable. On the basis of the results obtained in Experiments 1 to 3 of the present study, it was concluded that ^{51}Cr-EDTA was a reliable tracer to assess intestinal permeability in ponies and that minor alterations in intestinal motility did not affect the absorption and subsequent urinary excretion pattern of the probe. This conclusion is in agreement with the observations reported in previous studies using ^{51}Cr-EDTA in man and rats (Bjarnason and others, 1985; Maxton and others, 1986).

Some debate has arisen between human medical research workers on the anatomical site of the alimentary tract from which ^{51}Cr-EDTA is absorbed. Initial studies concluded that most, if not all, of the ^{51}Cr-EDTA absorption occurred in the small intestine (Bjarnason and others, 1983a, b; Bjarnason and others, 1985). This is in contrast with more recent studies on intestinal permeability in man in which significant absorption of the marker was reported to occur in the large intestine (Elia and others, 1987; Jenkins and others, 1987 and 1988). The implications of this debate in equine medicine are important in that, in cases in which histopathological examination of intestinal specimens obtained by laparotomy is frequently not practicable, no diagnostic technique has been

developed to assess large intestinal function. Therefore demonstration of ^{51}Cr-EDTA absorption from this portion of the gastrointestinal tract might be of great interest in the development of a test which could demonstrate large intestinal dysfunction in horses. Experiment 4 of this series was designed to analyse the pattern of absorption of ^{51}Cr-EDTA from the equine large intestine. Two ponies with permanent caecal fistulation were used on this occasion and 1.75% and 1.56% of the intracaecally administered ^{51}Cr-EDTA dose was recovered in urine. Thus absorption of the probe from the large intestine was confirmed although caecal/colonic permeability to ^{51}Cr-EDTA appeared to be relatively low in normal ponies, which is in agreement with previous reported studies in human patients and dogs (Bjarnason and others, 1985; Hall and others, 1989). However, a marked increase in the urinary excretion of ^{51}Cr-EDTA was noticed in the cyathostome infected ponies in Experiment 6 of the present series which was presumably due to increased absorption of the marker through a diseased large intestine with markedly altered permeability. Thus, there is evidence from the present studies that inflammatory changes of the equine large intestine affect ^{51}Cr-EDTA absorption in a similar way to human enteropathy (Jenkins and others, 1988).

The objective of Experiment 5 was to study intestinal permeability alterations in ponies with phenylbutazone toxicity. Failure to demonstrate changes in the permeability of the bowel in these animals might suggest that ^{51}Cr-EDTA is not a suitable marker to assess this aspect of gastrointestinal function in ponies. However three main observations oppose this view: first, the consistency of the ^{51}Cr-EDTA recoveries in urine in control animals and in the same animals following pharmacological modulation of intestinal motility (Experiments 1 to 3) appeared to confirm ^{51}Cr-EDTA as a reliable marker for the assessment of intestinal permeability; secondly, urinary excretion of ^{51}Cr-EDTA was successfully applied in Experiment 6 of the present thesis to detect alterations in intestinal permeability in ponies with experimental

cyathostome infections; thirdly, no evidence of PBZ toxicity could be ascertained in any of the animals after continuous administration of this agent for 28 days. Therefore the comparable values of ^{51}Cr-EDTA urinary excretions obtained in Experiment 5 and on the previous occasions in which the tracer was tested in the present work further substantiate that PBZ toxicity was not achieved and may encourage the use of ^{51}Cr-EDTA to assess equine intestinal permeability.

In Experiment 6 of the present study ^{51}Cr-EDTA was administered to ponies at three stages of experimental cyathostome infection. When the test was performed under control conditions and shortly (Day 22) after the initiation of the administration of cyathostome third-stage larvae, the amounts of ^{51}Cr-EDTA recovered in urine were comparable to control values. In contrast, the urinary excretion of the radioisotope increased markedly when the test was repeated on Days 47 and 88 of the trickle infection and the ponies had received 11.5 million cyathostome larvae. The amounts of ^{51}Cr-EDTA measured in urine appeared to correlate with alterations in intestinal permeability and these alterations appeared to correspond with the clinical signs observed in the infected animals as well as changes in serum albumin and haematological values.

A major application of a test to assess intestinal permeability in horses could be to expand the present knowledge on equine enteropathies, both to clarify their aetiopathogenic mechanisms and to allow the clinician to diagnose these conditions in the live animal. Three horses with chronic enteropathy were tested with ^{51}Cr-EDTA in the present study. One animal (Case number 1) showed an abnormal temporal pattern of urinary excretion of the marker which presumably reflected the functional derangements of the alimentary tract suggested by the post-mortem findings of partial gastric impaction but the exact pathogenic mechanisms involved in this condition could not be fully ascertained. An equally abnormal result was obtained in Case number 2 which was found to have a decrease in intestinal permeability as judged by

the amounts of ^{51}Cr-EDTA detected in urine. The localised pathological lesions observed in this animal at necropsy may partially explained this finding but a straight explanation was not available.

An increased intestinal permeability was demonstrated in an animal with chronic diarrhoea (Case number 3), even when the test was performed when the mare was in remission following symptomatic anti-diarrhoeic treatment and administration of corticosteroid agents. It is felt that a more marked increase in the urinary excretion of ^{51}Cr-EDTA would have been detected should the technique have been applied earlier in the course of the disease. In previous radioisotope tracer studies it was reported that most chronically diarrhoeic horses do not have protein-losing enteropathy (Merritt and others, 1977). However the results of increased permeability recorded in this animal were strong evidence that the extremely low plasma albumin levels detected were due to protein-losing enteropathy (PLE). Indeed, the condition diagnosed in this animal - chronic eosinophilic enteritis - characterised by diffuse cellular infiltration of the gastrointestinal tract by primarily eosinophils has been considered to be a PLE (Pass and Bolton, 1982; Roberts, 1984).

In previous studies, ^{51}Cr-EDTA has been used to investigate intestinal transit time in ponies (Argenzio and others, 1974; Clayton and others, 1980). Although the primary aim of the present study was to quantify the urinary excretion of ^{51}Cr-EDTA as a reflection of intestinal permeability, total faeces produced in 72 hours following intragastric administration of the marker were collected and the amounts of ^{51}Cr-EDTA measured in the same way as urine. The results obtained gave an estimate of the intestinal transit time and demonstrated alterations in this parameter following modifications of intestinal motility by pharmacological agents and experimental cyathostome infections. However the results were very variable and the conclusions which may be drawn from the available data have limited application in the study of equine chronic enteropathies.

For clinical purposes, the 72-hour collection period is likely to be impractical. The protocol for the ^{51}Cr-EDTA test used in human patients and dogs involves the collection of urine for 24 hours. The results reported in ponies in the present study clearly indicate that most absorption occurs during the first 24 hours and consequently should a clinical ^{51}Cr-EDTA test be developed for horses, a similar approach to that in man and dogs might be followed. The amount of ^{51}Cr-EDTA recovered in urine in 24 hours in the control ponies was 5.24% $\pm$ 1.93 (mean $\pm$ SD) which may be used as guideline values for future studies but verification of reference values would require to be substantiated with greater numbers of animals.

Although the total amount of ^{51}Cr-EDTA recovered in urine during the 24 or 72 hours following intragastric administration may reliably reflect the state of equine intestinal permeability, the study of the temporal pattern of the urinary excretion of the probe may also be informative. In studies on the intestinal transit time in ponies, Argenzio and others (1974) found that 68% of a soluble marker was within the caecum within two hours following intragastric administration. However, the rate of passage appeared to slow markedly thereafter and the authors estimated that the marker was retained in the colon for approximately 50 hours. If one assumes the same rate of movement for the ^{51}Cr-EDTA solution along the gastrointestinal tract, then the amounts of ^{51}Cr-EDTA excreted in urine between six and 24 hours following the administration per stomach tube of the dose solution would be a reflection of the state of large intestinal permeability.

Repeatability of the ^{51}Cr-EDTA test was not studied in the present series and it would be of interest to assess this in future, with a larger number of experimental animals, to confirm the sensitivity of this technique. Recent studies in human medicine reported an increase in the diagnostic specificity of the ^{51}Cr-EDTA test when it was used in

combination with other probes. For example, ^{51}Cr-EDTA and ^{14}C-mannitol were administered simultaneously *per os* and urine was collected for six hours; the ratio of the percentage urinary excretion of the probes was reported to clearly separate patients with coeliac disease or inflammatory bowel disease from control subjects (Behrens and others, 1987; Fotherby and others, 1988). Alternatively, ^{51}Cr-EDTA has been administered separately *per os* and *per rectum* to human beings with inflammatory bowel disease to differentiate small intestinal from colonic disease (O'Morain, 1986; Jenkins and others, 1988). Similar protocols may be developed for the equine species.

It has been demonstrated in studies in man and rats (Bjarnason and others, 1985; Maxton and others, 1986) that osmolarity and composition of the ^{51}Cr-EDTA solution administered influence the intestinal absorption of the marker; this appears to be associated with water absorption via intercellular pathways or through areas of cell extrusion (Bjarnason and others, 1983a; Bjarnason and others, 1985; Maxton and others, 1986; Ramage and others, 1988). Therefore it must be emphasized that a standard protocol be used and the animals should be fasted to try to minimize this influence.

In conclusion, the characteristics of ^{51}Cr-EDTA as a marker - i.e. it is stable, biologically inert, chemically nontoxic, confined to an extracellular location and it can be easily measured in biologic fluids by means of a gamma-counter - and the consistency of the results obtained in the present study appear to confirm ^{51}Cr-EDTA as a reliable probe to assess intestinal permeability in horses. To some extent the conclusions drawn here must be considered preliminary due to the small number of animals studied and the absence of parallel investigations such as electron microscopic studies or subcellular studies on the distribution of ^{51}Cr-EDTA in the equine intestinal gastrointestinal tract. Nevertheless,

the results of this study suggest that novel approaches to assess large intestinal function should lead to improved understanding of equine chronic enteropathy.

APPENDIX 1.

APPENDIX 1.1.

Experiment 1. Percentages of administered ^{51}Cr-EDTA measured in urine in control ponies.

TIME	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
0-12 h.	3.10	1.01	3.72	5.79	3.41	1.97
12-24 h.	1.23	2.05	2.46	1.61	1.84	0.53
DAY 1	**4.33**	**3.06**	**6.18**	**7.40**	**5.24**	**1.93**
24-36 h.	0.56	0.36	0.89	0.73	0.64	0.23
36-48 h.	0.29	0.32	0.71	0.57	0.47	0.20
DAY 2	**0.85**	**0.68**	**1.60**	**1.30**	**1.11**	**0.42**
48-60 h.	0.16	0.08	0.14	0.22	0.15	0.05
60-72 h.	0.12	0.11	0.21	0.11	0.14	0.05
DAY 3	**0.28**	**0.19**	**0.35**	**0.33**	**0.29**	**0.06**
TOTAL	**5.46**	**3.93**	**8.13**	**9.02**	**6.64**	**2.36**

APPENDIX 1.2.

Experiment 2. Percentages of administered ^{51}Cr-EDTA measured in urine in ponies following premedication with 0.06 mg/kg atropine sulphate subcutaneously.

TIME	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
0-6 h.	1.06	1.40	0.56	1.05	1.02	0.35
6-12 h.	0.00	0.65	2.11	1.69	1.11	0.96
12-18 h.	1.20	0.47	1.02	0.71	0.85	0.32
18-24 h.	0.00	0.42	0.86	0.40	0.42	0.35
DAY 1	**2.26**	**2.94**	**4.55**	**3.85**	**3.40**	**1.00**
24-36 h.	1.30	0.44	1.04	0.73	0.88	0.37
36-48 h.	0.13	0.06	0.32	2.30	0.70	1.07
DAY 2	**1.43**	**0.50**	**1.36**	**3.03**	**1.58**	**0.91**
48-60 h.	0.18	0.11	0.10	1.27	0.42	0.57
60-72 h.	0.09	0.05	0.04	0.65	0.14	0.05
DAY 3	**0.27**	**0.16**	**0.14**	**1.92**	**0.62**	**0.75**
TOTAL	**3.96**	**3.60**	**6.05**	**8.80**	**5.60**	**2.39**

APPENDIX 1.3.

Experiment 3. Percentages of administered ^{51}Cr-EDTA measured in urine in ponies following premedication with 0.05 mg/kg bethanecol subcutaneously.

TIME	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
0-6 h.	0.51	1.23	0.61	1.51	0.97	0.48
6-12 h.	0.00	0.62	0.00	0.60	0.31	0.35
12-18 h.	1.39	0.76	1.21	0.87	1.06	0.29
18-24 h.	0.00	0.33	0.82	0.44	0.40	0.34
DAY 1	**1.90**	**2.94**	**2.64**	**3.42**	**2.72**	**0.63**
24-36 h.	0.66	0.51	0.38	2.22	0.94	0.86
36-48 h.	0.35	0.18	0.34	1.20	0.52	0.46
DAY 2	**1.01**	**0.69**	**0.74**	**3.42**	**1.47**	**1.31**
48-60 h.	0.10	0.06	0.08	0.83	0.27	0.38
60-72 h.	0.05	0.02	0.04	0.34	0.11	0.15
DAY 3	**0.15**	**0.08**	**0.12**	**1.17**	**0.38**	**0.53**
TOTAL	**3.05**	**3.70**	**3.47**	**8.01**	**4.56**	**2.32**

APPENDIX 1.4.

Experiment 4. Percentages of administered ^{51}Cr-EDTA measured in urine in ponies with permanent caecal fistulation.

TIME	ANIMAL NUMBER		MEAN	SD
	1	2		
0-6 h.	0.06	0.16	0.38	0.33
6-12 h.	0.35	0.61	3.71	1.05
12-18 h.	0.22	0.28	3.05	3.26
18-24 h.	0.16	0.10	0.79	0.11
DAY 1	**0.79**	**1.15**	**0.97**	**0.25**
24-36 h.	0.47	0.16	0.98	1.12
36-48 h.	0.18	0.20	0.32	0.25
DAY 2	**0.65**	**0.36**	**0.51**	**0.21**
48-60 h.	0.21	0.03	0.33	0.37
60-72 h.	0.11	0.01	0.10	0.09
DAY 3	**0.32**	**0.04**	**0.18**	**0.20**
TOTAL	**1.75**	**1.56**	**1.66**	**0.13**

APPENDIX 1.5.

Experiment 5. Percentages of administered ^{51}Cr-EDTA measured in urine in ponies following oral administration of 8.8 mg/kg/day phenylbutazone for 28 days.

TIME	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
0-6 h.	0.00	2.94	0.00	4.31	1.81	2.17
6-12 h.	0.00	0.64	3.45	0.70	1.20	1.54
12-18 h.	0.87	0.89	0.00	0.51	0.57	0.42
18-24 h.	0.00	0.36	0.00	0.43	0.20	0.23
DAY 1	**0.87**	**4.83**	**3.45**	**5.95**	**3.78**	**2.19**
24-36 h.	3.05	1.03	2.22	1.55	1.96	0.87
36-48 h.	0.12	0.25	0.19	0.08	0.16	0.08
DAY 2	**3.17**	**1.28**	**2.41**	**1.63**	**2.12**	**0.73**
48-60 h.	0.07	0.13	0.21	0.60	0.25	0.24
60-72 h.	0.09	0.03	0.03	0.08	0.06	0.03
DAY 3	**0.16**	**0.16**	**0.24**	**0.68**	**0.31**	**0.22**
TOTAL	**4.20**	**6.27**	**6.11**	**8.25**	**6.21**	**1.66**

APPENDIX 1.6.

Experiment 6. Percentages of administered ^{51}Cr-EDTA measured in urine in control ponies.

TIME	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
0-6 h.	1.41	2.33	1.15	2.76	2.08	0.83
6-12 h.	0.58	1.03	0.00	0.00	0.34	0.60
12-18 h.	1.36	0.81	0.87	0.46	0.71	0.22
18-24 h.	0.00	0.37	1.22	0.89	0.83	0.43
DAY 1	**3.35**	**4.54**	**3.24**	**4.11**	**3.95**	**0.66**
24-36 h.	0.99	0.38	0.76	1.26	0.80	0.44
36-48 h.	0.23	0.09	0.21	0.46	0.25	0.19
DAY 2	**1.22**	**0.47**	**0.97**	**1.72**	**1.05**	**0.63**
48-60 h.	0.16	0.06	0.10	0.21	0.12	0.08
60-72 h.	0.05	0.02	0.03	0.07	0.04	0.03
DAY 3	**0.21**	**0.08**	**0.13**	**0.28**	**0.16**	**0.10**
TOTAL	**4.77**	**5.09**	**4.35**	**6.10**	**5.18**	**0.88**

APPENDIX 1.7.

Experiment 6. Percentages of administered ^{51}Cr-EDTA measured in urine in ponies following trickle infection with 5.5 million cyathostome third-stage larvae (Day 22).

TIME	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
0-6 h.	0.25	0.77	0.00	1.79	0.85	0.90
6-12 h.	0.00	2.49	3.43	0.51	2.74	0.60
12-18 h.	1.27	0.84	0.00	0.52	0.45	0.42
18-24 h.	0.00	0.60	0.00	0.21	0.27	0.30
DAY 1	**1.52**	**4.70**	**3.43**	**3.03**	**3.72**	**0.87**
24-36 h.	0.92	1.05	1.48	0.27	0.93	0.61
36-48 h.	0.22	0.29	0.93	0.44	0.55	0.33
DAY 2	**1.14**	**1.34**	**2.41**	**0.71**	**1.99**	**0.70**
48-60 h.	0.69	0.28	1.71	0.65	0.80	0.74
60-72 h.	0.83	0.14	0.03	0.68	0.28	0.35
DAY 3	**1.52**	**0.42**	**1.74**	**1.33**	**1.16**	**0.55**
TOTAL	**4.17**	**6.45**	**7.58**	**5.05**	**6.36**	**1.27**

NOTE. Infected animals: 63, 64 and 70. Control animal: 68. Mean and standard deviation (SD) refer to infected animals.

APPENDIX 1.8.

Experiment 6. Percentages of administered ^{51}Cr-EDTA measured in urine in ponies following trickle infection with 11.5 million cyathostome third-stage larvae (Day 47).

TIME	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
0-6 h.	0.00	2.73	2.65	1.50	2.29	0.69
6-12 h.	0.56	2.55	6.40	2.94	3.96	2.12
12-18 h.	1.86	1.21	5.15	3.08	3.15	1.97
18-24 h.	0.34	0.73	5.18	0.87	2.26	2.53
DAY 1	**2.76**	**7.22**	**19.38**	**8.39**	**11.66**	**6.71**
24-36 h.	0.53	1.34	4.87	1.24	2.48	2.07
36-48 h.	0.30	0.16	1.80	0.67	0.88	0.84
DAY 2	**0.83**	**1.50**	**6.67**	**1.91**	**3.36**	**2.35**
48-60 h.	0.24	0.22	1.48	0.25	0.65	0.72
60-72 h.	0.14	0.08	0.80	0.00	0.29	0.44
DAY 3	**0.38**	**0.30**	**2.28**	**0.25**	**0.94**	**0.95**
TOTAL	**3.97**	**9.01**	**28.34**	**10.55**	**15.97**	**10.74**

NOTE. Infected animals: 63, 64 and 70. Control animal: 68. Mean and standard deviation (SD) refer to infected animals.

APPENDIX 1.9.

Experiment 6. Percentages of administered ^{51}Cr-EDTA measured in urine in ponies following trickle infection with 11.5 million cyathostome third-stage larvae (Day 88).

TIME	ANIMAL NUMBER			MEAN	SD
	68	63	70		
0-6 h.	0.03	0.14	0.61	0.38	0.33
6-12 h.	0.00	4.45	2.96	3.71	1.05
12-18 h.	0.38	5.35	0.74	3.05	3.26
18-24 h.	0.00	0.71	0.87	0.79	0.11
DAY 1	**0.41**	**10.65**	**5.18**	**7.91**	**3.87**
24-36 h.	0.64	0.19	1.77	0.98	1.12
36-48 h.	0.28	0.14	0.49	0.32	0.25
DAY 2	**0.92**	**0.33**	**2.26**	**1.30**	**0.97**
48-60 h.	0.50	0.07	0.59	0.33	0.37
60-72 h.	0.07	0.03	0.16	0.10	0.09
DAY 3	**0.57**	**0.10**	**0.75**	**0.43**	**0.33**
TOTAL	**1.90**	**11.08**	**8.19**	**9.64**	**2.04**

NOTE. Infected animals: 63 and 70. Control animal: 68. Mean and standard deviation (SD) refer to infected animals.

APPENDIX 1.10.

Experiment 7. Percentages of administered ^{51}Cr-EDTA measured in urine in horses with chronic enteropathy.

TIME	ANIMAL NUMBER		
	1	2	3
0-6 h.	|	0.25	0.43
6-12 h.	1.11	0.32	1.87
12-18 h.	|	0.31	2.02
18-24 h.	1.17	0.22	1.89
DAY 1	**2.28**	**1.10**	**6.21**
24-36 h.	0.00	0.31	2.19
36-48 h.	1.40	0.14	0.79
DAY 2	**1.40**	**0.45**	**2.98**
48-60 h.	0.00	0.10	-
60-72 h.	1.06	0.02	-
DAY 3	**1.06**	**0.12**	-
TOTAL	**4.73**	**1.67**	**9.19**

NOTE. Collection of urine in Case number 1 during the first day was divided in two 12-hour intervals. Collection of urine in Case number 3 was limited to the first 48 hours.

APPENDIX 2.

APPENDIX 2.1.

Experiment 1. Percentages of administered ^{51}Cr-EDTA measured in faeces in control ponies.

TIME	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
0-12 h.	11.57	1.90	0.01	0.01	3.37	5.54
12-24 h.	41.47	56.70	33.94	33.52	41.41	10.83
DAY 1	**53.04**	**58.60**	**33.95**	**33.53**	**44.78**	**12.95**
24-36 h.	20.61	23.70	25.98	27.16	21.98	3.65
36-48 h.	13.07	7.24	17.26	17.61	13.80	4.83
DAY 2	**33.68**	**30.94**	**43.24**	**44.77**	**38.16**	**5.95**
48-60 h.	3.43	2.61	5.66	7.01	4.68	2.02
60-72 h.	2.13	0.84	2.66	3.29	2.23	1.04
DAY 3	**5.56**	**3.45**	**8.32**	**10.30**	**6.91**	**2.61**
TOTAL	**92.29**	**92.99**	**85.51**	**88.61**	**89.85**	**3.47**

APPENDIX 2.2.

Experiment 2. Percentages of administered ^{51}Cr-EDTA measured in faeces in ponies following premedication with 0.06 mg/kg atropine sulphate subcutaneously.

TIME	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
0-6 h.	0.00	0.00	0.00	0.00	0.00	0.00
6-12 h.	6.89	6.30	1.38	0.00	3.64	3.46
12-18 h.	15.80	25.79	22.41	0.41	16.10	11.25
18-24 h.	14.28	22.53	19.67	0.28	14.19	9.88
DAY 1	**36.97**	**54.62**	**43.46**	**0.69**	**33.90**	**23.30**
24-36 h.	21.34	20.28	28.25	3.23	18.27	10.63
36-48 h.	18.38	8.38	10.88	19.15	14.20	5.38
DAY 2	**39.72**	**28.66**	**39.13**	**22.38**	**32.47**	**7.30**
48-60 h.	4.86	1.76	2.61	25.14	8.59	11.11
60-72 h.	1.15	0.37	0.61	15.33	4.36	7.32
DAY 3	**6.01**	**2.13**	**3.22**	**40.47**	**12.96**	**19.95**
TOTAL	**82.71**	**85.41**	**85.81**	**63.53**	**79.36**	**10.65**

APPENDIX 2.3.

Experiment 3. Percentages of administered ^{51}Cr-EDTA measured in faeces in ponies following premedication with 0.05 mg/kg bethanecol subcutaneously.

TIME	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
0-6 h.	0.01	0.00	0.00	0.14	0.04	0.07
6-12 h.	8.18	0.83	6.62	0.66	4.07	3.90
12-18 h.	28.29	25.67	40.34	0.11	23.60	16.91
18-24 h.	16.21	27.17	16.51	0.01	14.97	11.20
DAY 1	**52.69**	**53.67**	**63.47**	**0.92**	**42.70**	**28.30**
24-36 h.	23.95	23.24	23.39	25.41	24.00	0.99
36-48 h.	12.08	13.92	5.55	25.53	14.27	8.32
DAY 2	**36.03**	**37.16**	**28.94**	**50.94**	**38.27**	**7.97**
48-60 h.	2.84	2.18	0.97	33.14	9.78	15.59
60-72 h.	0.75	0.36	0.17	0.87	0.54	0.33
DAY 3	**3.59**	**2.52**	**1.14**	**34.01**	**10.32**	**13.71**
TOTAL	**92.31**	**93.35**	**93.55**	**85.87**	**91.26**	**3.65**

APPENDIX 2.4.

Experiment 4. Percentages of administered ^{51}Cr-EDTA measured in faeces in ponies with permanent caecal fistulation.

TIME	ANIMAL NUMBER		MEAN	SD
	1	2		
0-6 h.	0.00	0.14	0.38	0.33
6-12 h.	0.20	15.79	3.71	1.05
12-18 h.	0.58	25.70	3.05	3.26
18-24 h.	7.79	19.65	0.79	0.11
DAY 1	**8.57**	**61.28**	**34.93**	**37.27**
24-36 h.	37.09	9.98	0.98	1.12
36-48 h.	11.32	4.34	0.32	0.25
DAY 2	**48.41**	**14.32**	**31.37**	**24.11**
48-60 h.	12.60	0.90	0.33	0.37
60-72 h.	4.33	0.45	0.10	0.09
DAY 3	**16.93**	**1.35**	**9.14**	**11.02**
TOTAL	**73.90**	**76.94**	**75.42**	**2.15**

APPENDIX 2.5.

Experiment 5. Percentages of administered ^{51}Cr-EDTA measured in faeces in ponies following oral administration of 8.8 mg/kg/day phenylbutazone for 28 days.

TIME	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
0-6 h.	0.00	0.02	0.00	0.00	0.01	0.01
6-12 h.	3.03	21.35	1.25	0.00	6.41	10.04
12-18 h.	32.09	21.39	6.11	1.96	15.39	13.92
18-24 h.	18.10	12.28	14.35	0.00	11.18	7.83
DAY 1	**53.22**	**55.04**	**21.71**	**1.96**	**33.00**	**25.70**
24-36 h.	22.28	24.63	31.68	64.71	35.83	19.67
36-48 h.	5.10	4.92	7.33	22.35	9.93	8.36
DAY 2	**27.38**	**29.55**	**39.01**	**87.06**	**45.75**	**24.25**
48-60 h.	3.58	3.91	4.59	30.01	10.52	13.00
60-72 h.	0.58	0.22	2.22	0.68	0.93	0.89
DAY 3	**4.16**	**4.13**	**6.81**	**30.69**	**11.45**	**11.16**
TOTAL	**84.77**	**88.72**	**67.53**	**119.71**	**90.20**	**21.70**

APPENDIX 2.6.

Experiment 6. Percentages of administered ^{51}Cr-EDTA measured in faeces in control ponies.

TIME	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
0-6 h.	0.00	0.00	0.00	0.00	0.00	0.00
6-12 h.	12.49	22.19	0.04	8.35	10.19	11.19
12-18 h.	25.09	32.30	23.63	3.03	19.65	15.03
18-24 h.	13.91	21.34	28.01	16.49	21.95	5.78
DAY 1	**51.49**	**75.83**	**51.68**	**27.87**	**51.80**	**24.00**
24-36 h.	31.59	11.30	29.56	45.85	28.93	17.29
36-48 h.	5.88	2.24	8.02	16.82	9.03	7.34
DAY 2	**37.47**	**13.54**	**37.58**	**62.67**	**37.83**	**24.57**
48-60 h.	3.82	0.67	1.53	5.75	2.65	2.72
60-72 h.	0.96	0.18	0.30	1.72	0.73	0.86
DAY 3	**4.78**	**0.85**	**1.83**	**7.47**	**3.38**	**3.57**
TOTAL	**93.74**	**90.23**	**91.10**	**98.01**	**93.11**	**4.26**

APPENDIX 2.7.

Experiment 6. Percentages of administered ^{51}Cr-EDTA measured in faeces in ponies following trickle infection with 5.5 million cyathostome third-stage larvae (Day 22).

TIME	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
0-6 h.	0.00	0.00	0.00	0.00	0.00	0.00
6-12 h.	0.00	2.15	0.00	0.00	0.72	1.24
12-18 h.	0.13	10.73	2.22	1.41	4.79	5.16
18-24 h.	0.00	21.15	8.60	0.00	9.92	10.64
DAY 1	**0.13**	**34.03**	**10.82**	**1.41**	**15.42**	**16.79**
24-36 h.	25.18	45.10	24.39	14.89	28.13	15.45
36-48 h.	20.82	9.89	17.32	19.20	15.47	4.92
DAY 2	**46.00**	**54.99**	**41.71**	**34.09**	**43.60**	**8.64**
48-60 h.	10.85	2.08	11.23	5.03	6.11	4.67
60-72 h.	17.80	0.88	3.43	15.63	6.65	7.88
DAY 3	**28.65**	**2.96**	**14.66**	**20.66**	**12.76**	**7.35**
TOTAL	**74.78**	**91.98**	**67.20**	**56.16**	**71.80**	**18.40**

NOTE. Infected animals: 63, 64 and 70. Control animal: 68. Mean and standard deviation (SD) refer to infected animals.

APPENDIX 2.8.

Experiment 6. Percentages of administered ^{51}Cr-EDTA measured in faeces in ponies following trickle infection with 11.5 million cyathostome third-stage larvae (Day 47).

TIME	ANIMAL NUMBER				MEAN	SD
	68	63	64	70		
0-6 h.	0.00	0.75	0.00	0.00	0.25	0.43
6-12 h.	0.06	15.95	0.00	0.00	5.32	9.21
12-18 h.	5.96	15.61	1.73	0.00	5.78	8.56
18-24 h.	10.07	27.98	3.55	9.85	13.79	12.68
DAY 1	**16.09**	**60.29**	**5.28**	**9.85**	**25.10**	**30.50**
24-36 h.	36.87	22.30	23.34	39.80	28.48	9.82
36-48 h.	24.87	6.25	9.13	12.59	9.32	3.17
DAY 2	**61.74**	**28.55**	**32.47**	**52.39**	**37.80**	**10.44**
48-60 h.	11.07	1.78	13.58	4.42	6.59	6.19
60-72 h.	5.00	0.82	4.38	12.75	5.98	6.12
DAY 3	**16.07**	**2.60**	**17.96**	**17.17**	**12.58**	**7.06**
TOTAL	**93.91**	**91.43**	**55.71**	**79.42**	**75.50**	**18.20**

NOTE. Infected animals: 63, 64 and 70. Control animal: 68. Mean and standard deviation (SD) refer to infected animals.

APPENDIX 2.9.

Experiment 6. Percentages of administered ^{51}Cr-EDTA measured in faeces in ponies following trickle infection with 11.5 million cyathostome third-stage larvae (Day 88).

TIME	ANIMAL NUMBER			MEAN	SD
	68	63	70		
0-6 h.	0.00	2.52	0.00	1.26	1.78
6-12 h.	0.00	39.35	0.00	19.70	27.80
12-18 h.	4.48	41.85	1.57	21.70	28.50
18-24 h.	15.05	0.80	15.88	8.34	10.66
DAY 1	**19.53**	**84.52**	**17.45**	**51.00**	**47.40**
24-36 h.	20.94	0.01	29.48	14.70	20.80
36-48 h.	22.54	0.21	15.58	7.89	10.87
DAY 2	**43.48**	**0.22**	**45.06**	**22.64**	**22.42**
48-60 h.	10.71	0.03	11.30	5.66	7.97
60-72 h.	4.34	0.00	4.87	2.43	3.44
DAY 3	**15.05**	**0.03**	**16.17**	**8.10**	**8.07**
TOTAL	**78.06**	**84.78**	**78.68**	**81.73**	**4.31**

NOTE. Infected animals: 63 and 70. Control animal: 68. Mean and standard deviation (SD) refer to infected animals.

APPENDIX 2.10.

Experiment 7. Percentages of administered ^{51}Cr-EDTA measured in faeces in horses with chronic enteropathy.

TIME	ANIMAL NUMBER		
	1	2	3
0-6 h.	|	0.01	0.00
6-12 h.	0.00	0.01	0.05
12-18 h.	|	3.73	1.55
18-24 h.	4.18	15.42	19.19
DAY 1	**4.18**	**19.17**	**20.79**
24-36 h.	12.61	47.67	30.42
36-48 h.	0.00	10.81	16.74
DAY 2	**12.61**	**58.48**	**47.16**
48-60 h.	20.83	6.48	-
60-72 h.	16.23	1.21	-
DAY 3	**37.06**	**7.69**	-
TOTAL	**53.84**	**85.34**	**67.95**

NOTE. Collection of faeces in Case number 1 during the first day was divided in two 12-hour intervals. Collection of faeces in Case number 3 was limited to the first 48 hours.

APPENDIX 3.

APPENDIX 3.1.

Experiment 5. Fractional excretion of electrolytes (%) in urine.

PONY	PERIOD	Na	K	Cl	Ca	PO_4
63	3.1	0.62	62.32	1.61	9.49	2.88
	3.2	0.62	39.60	1.07	7.10	6.04
64	3.1	0.81	58.29	1.43	4.53	1.81
	3.2	0.69	67.69	1.80	12.52	6.34
68	3.1	0.54	32.94	1.50	5.05	2.87
	3.2	0.25	35.54	1.17	6.19	2.98
70	3.1	0.53	80.32	1.49	10.23	3.30
	3.2	0.90	51.21	1.16	4.53	5.91
NORMAL RANGES:		.03-.52	35-80	.58-1.86	.15-6.72	0 - 0.2

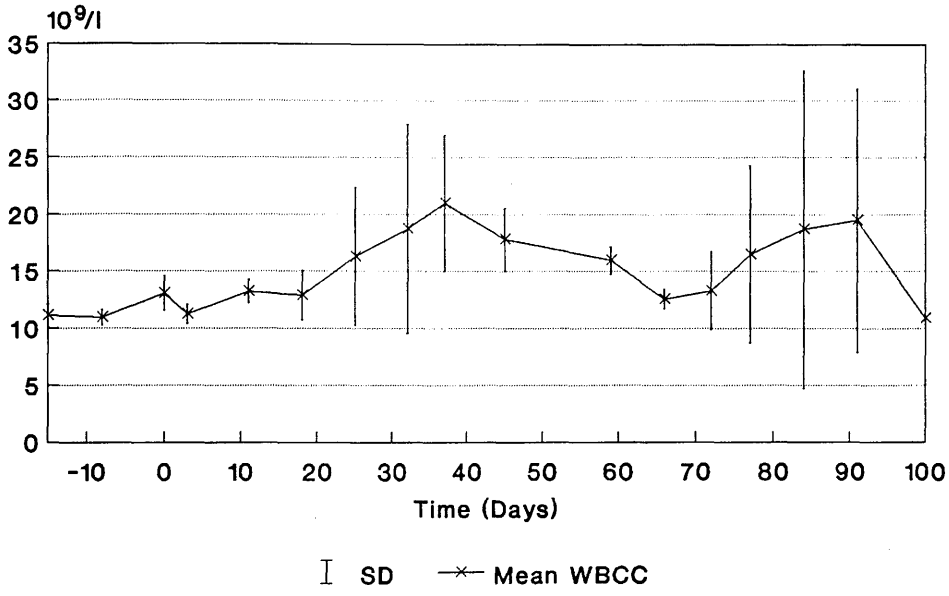
APPENDIX 3.2.

Experiment 5. Renal clearance of electrolytes (ml/min/kg).

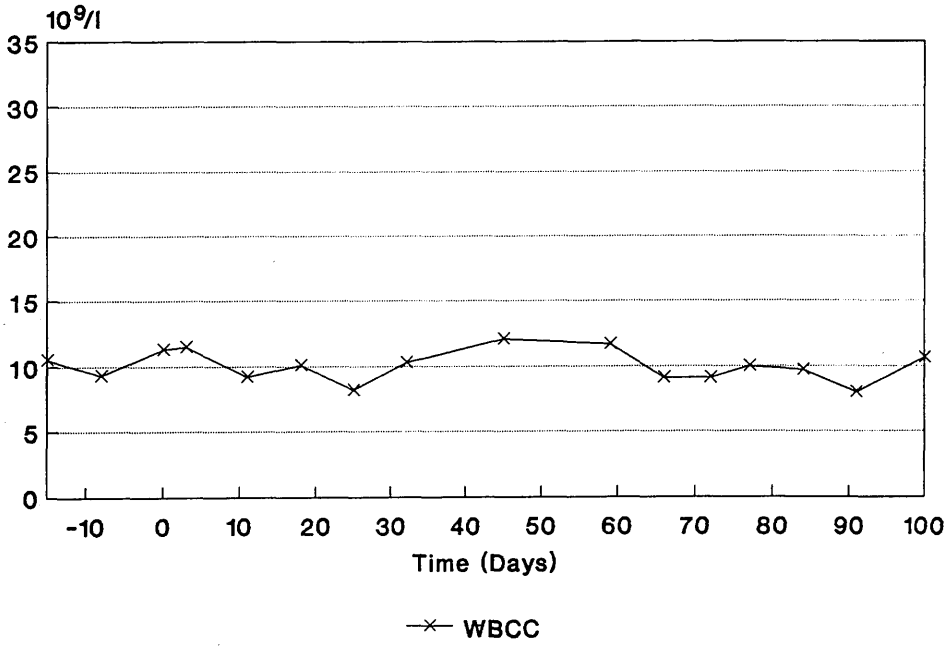
PONY	PERIOD	CREAT	Na	K	Cl	Ca	PO_4
63	3.1	2.66	0.02	1.66	0.04	0.25	0.08
	3.2	1.24	0.01	0.49	0.01	0.09	0.07
64	3.1	4.33	0.04	2.52	0.06	0.20	0.12
	3.2	0.66	0.00	0.45	0.01	0.08	0.04
68	3.1	2.22	0.01	0.73	0.03	0.11	0.06
	3.2	3.46	0.01	1.23	0.04	0.21	0.10
70	3.1	1.41	0.30	1.13	0.02	0.14	0.05
	3.2	1.78	0.02	0.91	0.02	0.08	0.11
NORMAL RANGES:		1.42-2.32	0.002-0.009	0.53-1.05	0.01-0.03	0.02-0.09	N.A.

NOTE. N.A.: Not available.

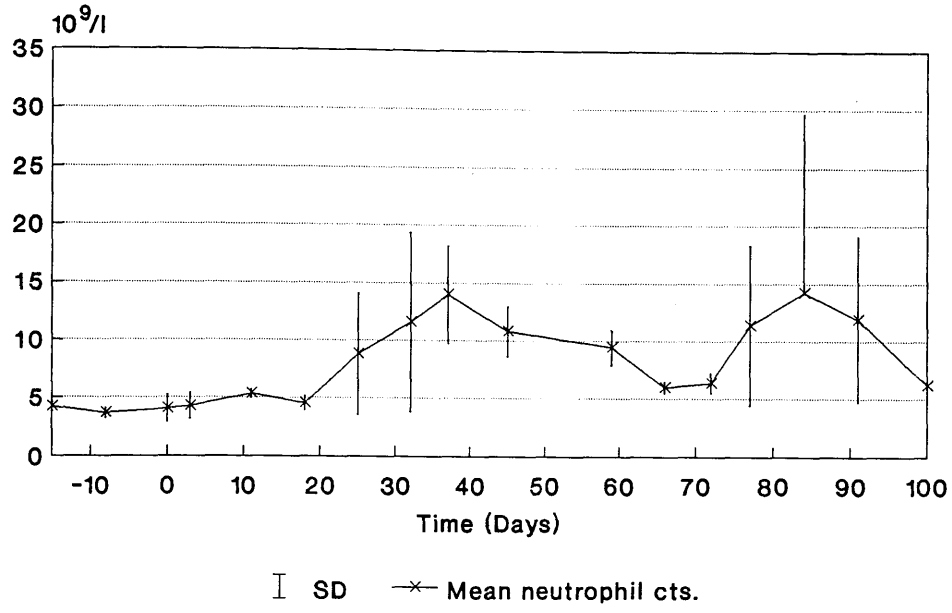
APPENDIX 3.3.
Experiment 6:Mean Total White Cell Count
(A) In cyathostome infected ponies.



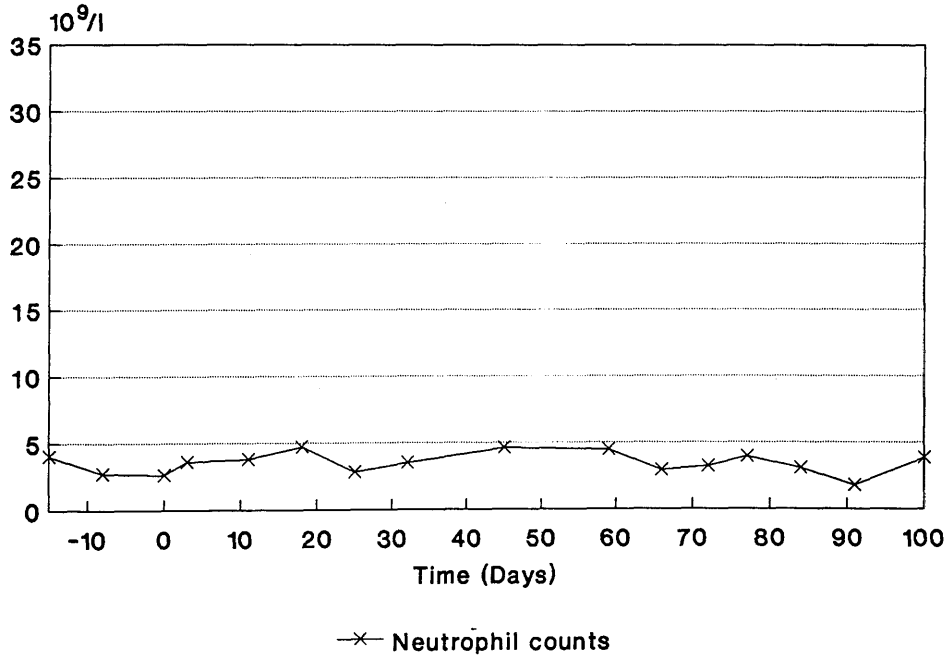
(B) In control, uninfected animal.



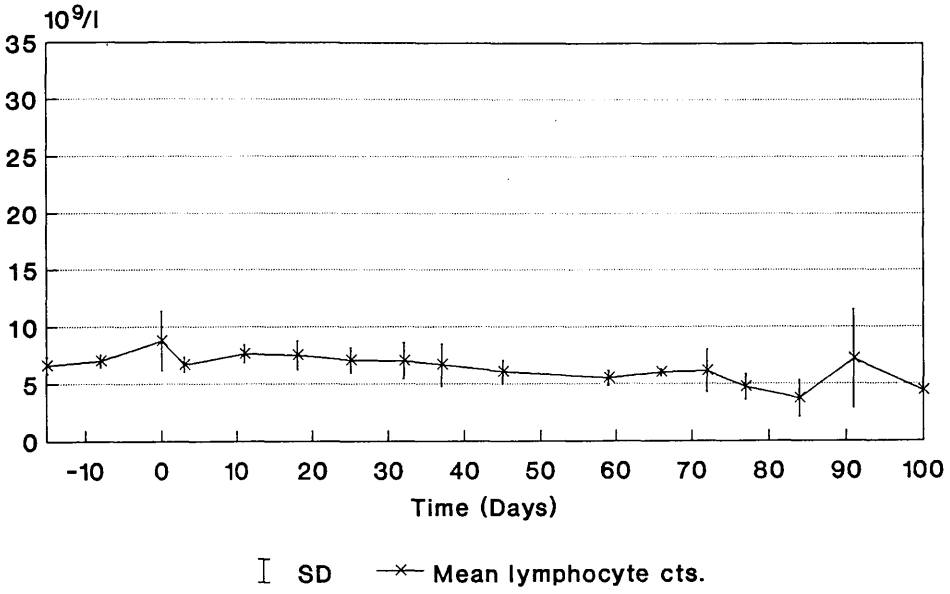
APPENDIX 3.4.
Experiment 6: Mean neutrophil counts.
(A) In cyathostome infected animals.



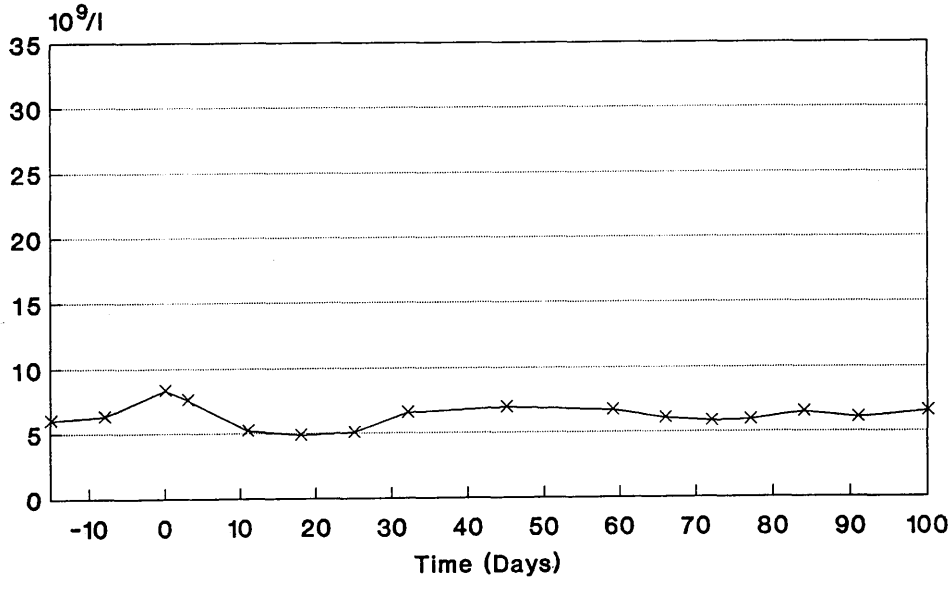
(B) In control, uninfected animal.



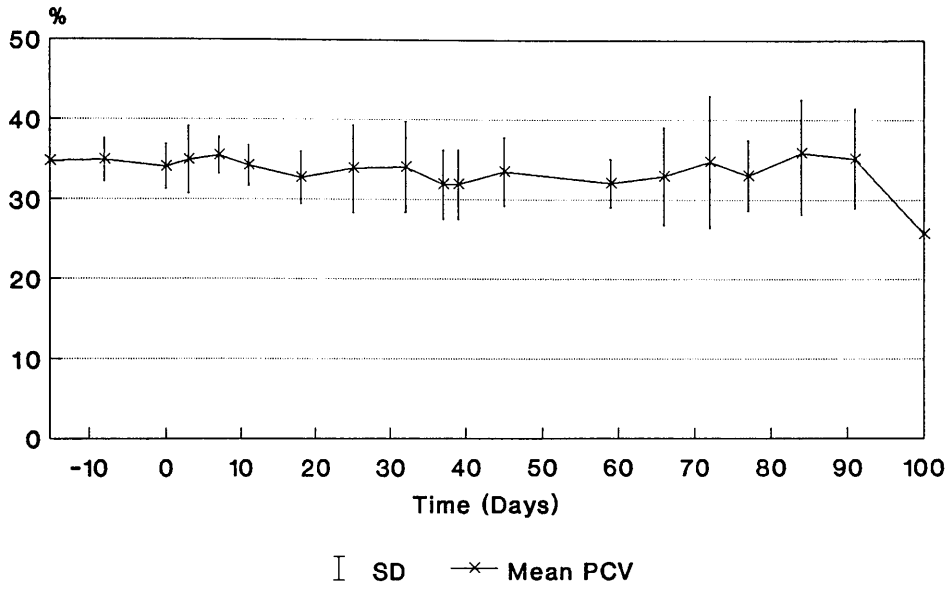
APPENDIX 3.5.
Experiment 6: Mean lymphocyte counts.
(A) In cyathostome infected animals.



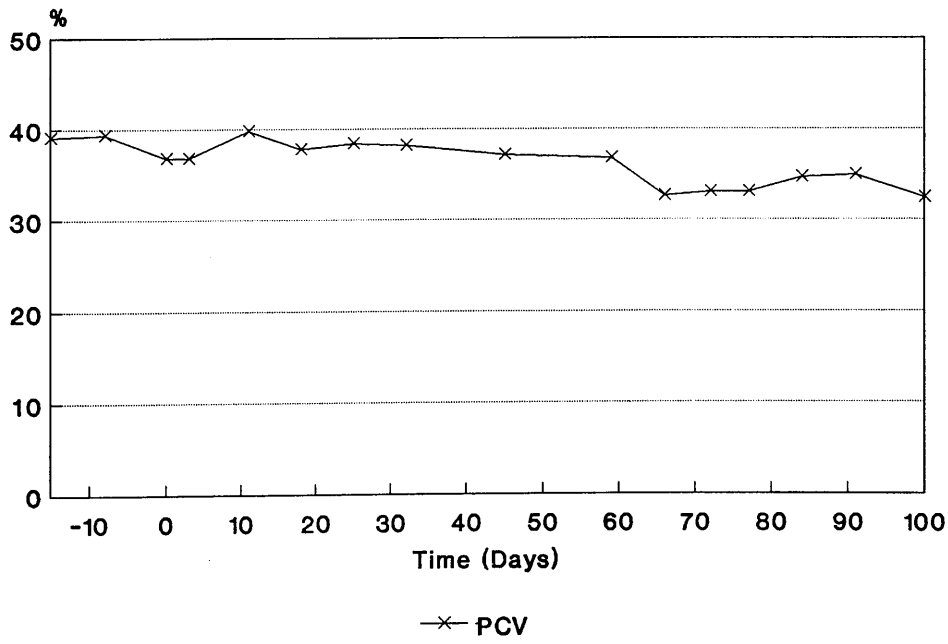
(B) In control, uninfected animal.



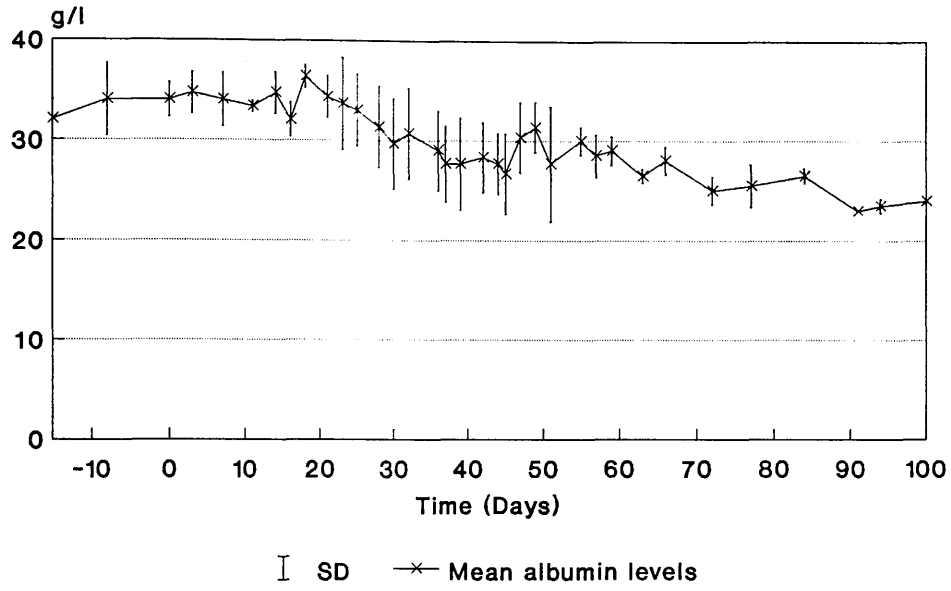
APPENDIX 3.6.
Experiment 6: Mean Packed Cell Volume.
(A) In cyathostome infected animals.



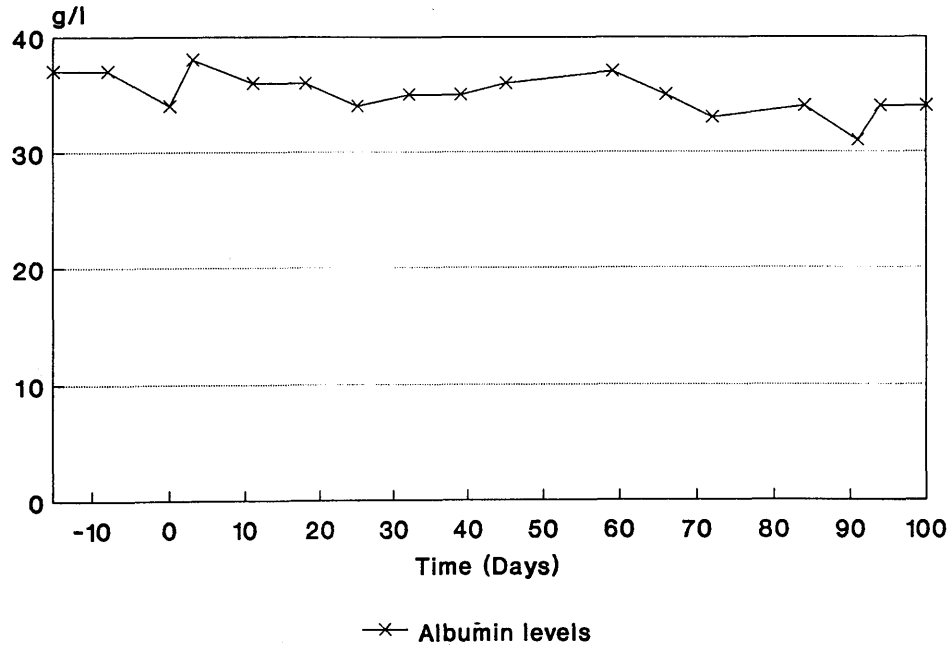
(B) In control, uninfected animal.



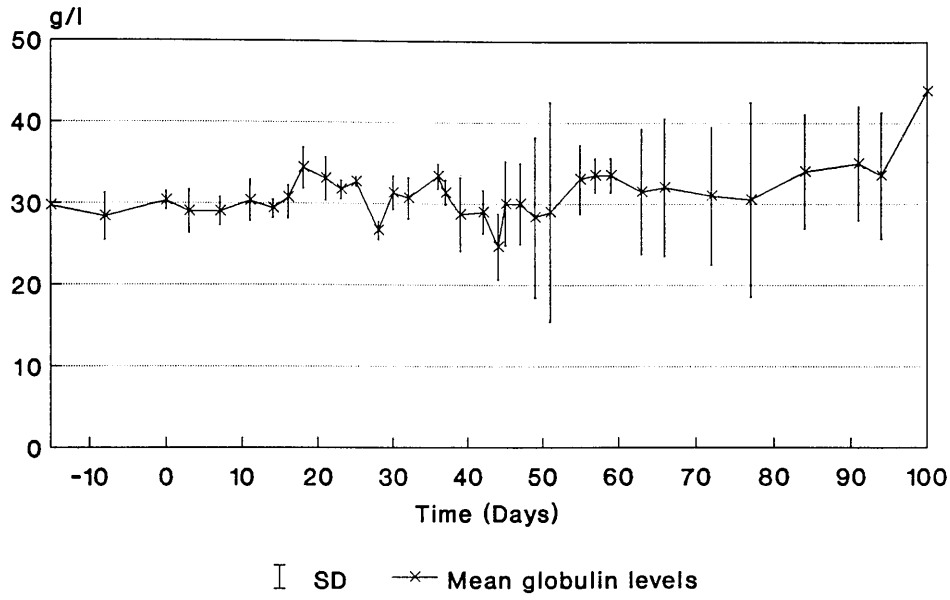
APPENDIX 3.7.
Experiment 6: Mean plasma albumin levels
(A) In cyathostome infected animals.



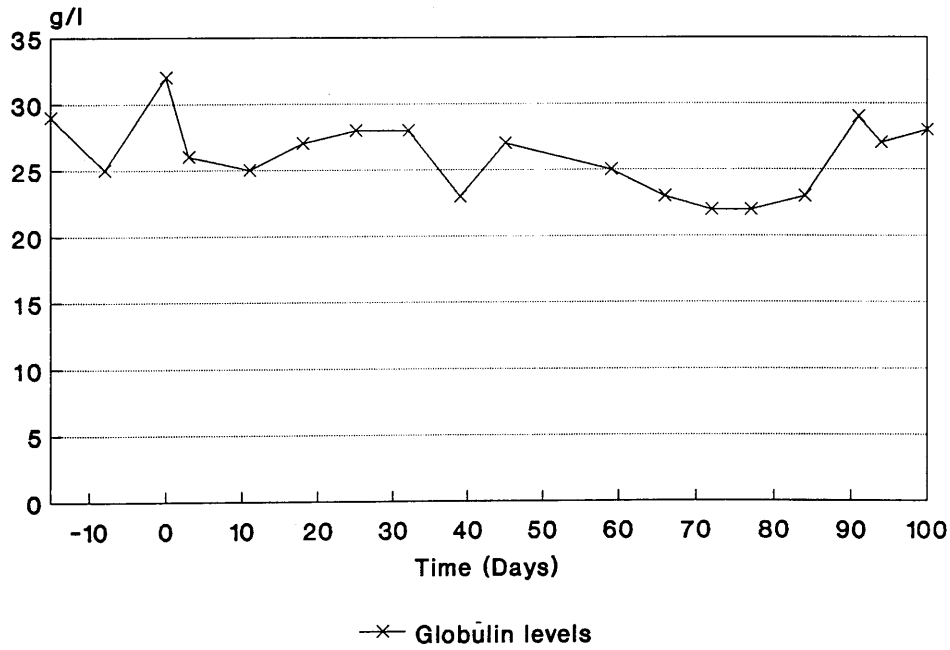
(B) In control, uninfected animal.



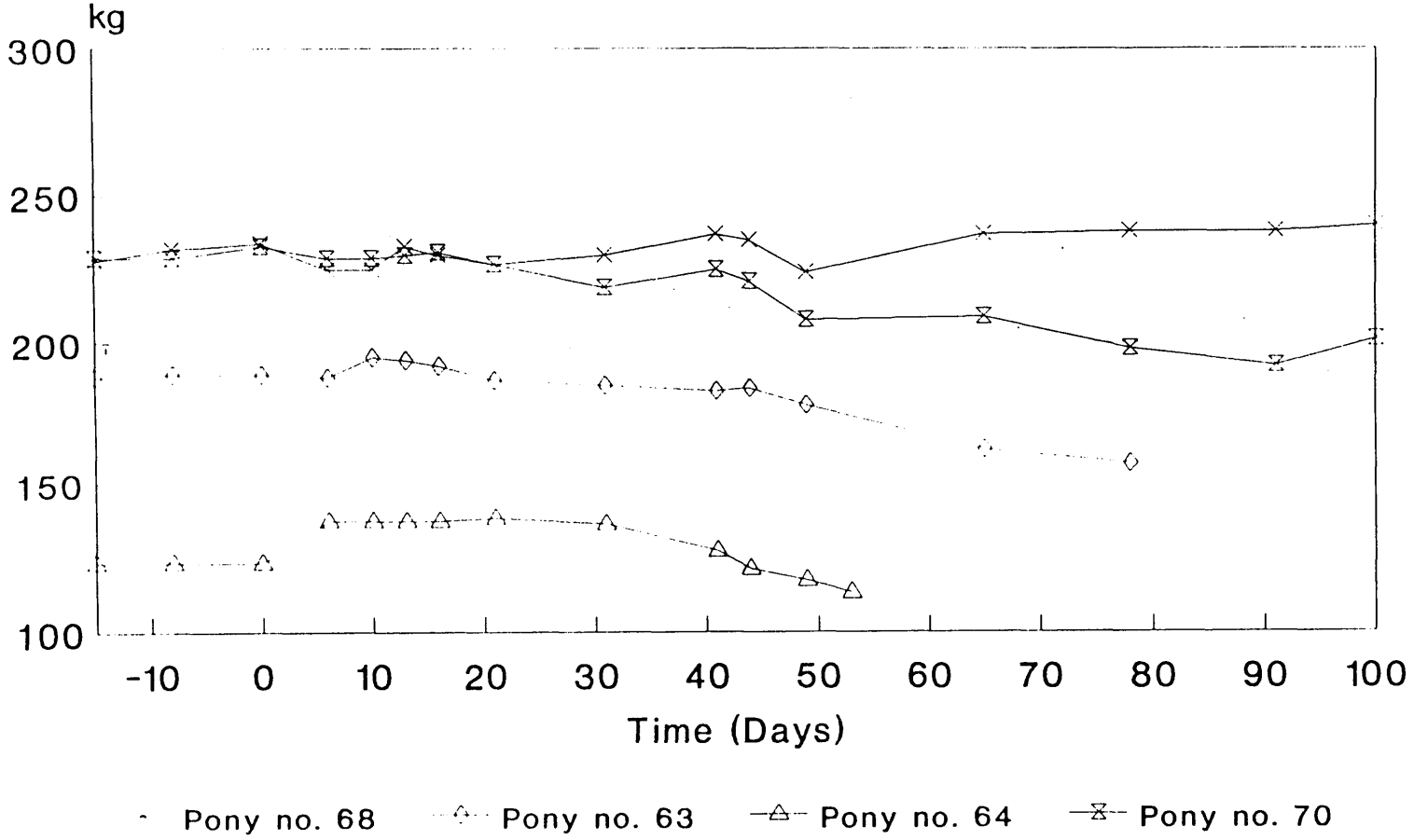
APPENDIX 3.8.
Experiment 6: Mean plasma globulin level
(A) In cyathostome infected animals.



(B) In control, uninfected animal



APPENDIX 3.9.
Experiment 6: Weights of infected and control animals during experimental cyathostome infection.



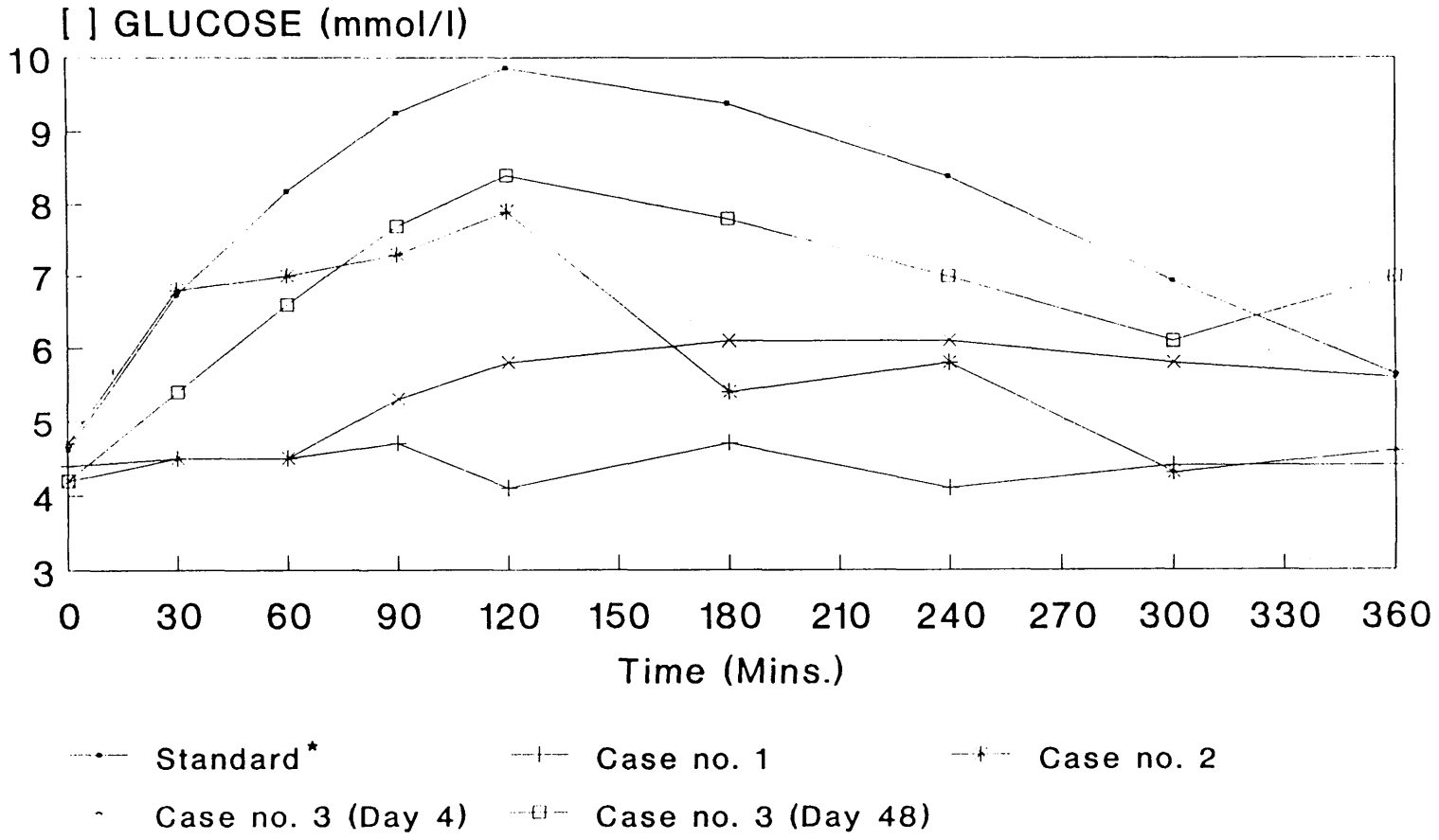
APPENDIX 3.10.

Experiment 7: Plasma biochemistry and haematology results in three horses with chronic enteropathy.

	CASE NUMBER				
	1	2	3		Normal Values (range)
	DAY 1	DAY 1	DAY 1	DAY 22	
Sodium mmol/l	133	136	121	136	132-146
Potassium mmol/l	4.6	3.9	1.8	2.7	2.4-4.7
Chloride mmol/l	99	100	87	98	99-109
Calcium mmol/l	3.08	3.17	2.42	2.36	2.7-3.2
Urea mmol/l	4.7	2.7	15.3	3.6	3.6-8.6
Creatinine umol/l	131	153	168	102	62-159
Alkaline Phosphatase IU/l	249	228	480	687	83-283
Aspartate Transaminase IU/l	367	304	ND	182	153-411
Gamma Glutamyltransferase IU/l	22	26	ND	ND	11-44
Bilirubin umol/l	23	21	26	21	1.7-42.5
Total Protein g/l	71	62	37	63	59-84
Albumin g/l	28	27	12	16	28-32
Globulin g/l	43	35	25	47	31-52
Haemoglobin g/dl	12.8	12.1	20.8	14.3	8-15
Packed Cell Volume %	35.3	33.7	56.1	39.9	24-46
Red Cell Count x10 ^{12}/l	6.6	6.82	12.14	8.46	5-10
White Cell Count x10 ^{9}/l	7.5	9.8	18.1	12.0	5.4-14.3
Neutrophils x10 ^{9}/l (%)	5.18(69)	6.27(64)	13.9(77)	10.2(85)	2.7-6.8 (30-75)
Lymphocytes x10 ^{9}/l (%)	1.88(25)	3.23(33)	3.62(20)	1.26(11)	1.5-5.5 (10-70)
Monocytes x10 ^{9}/l (%)	0.38 (5)	0.29 (3)	0.18 (1)	0.30(2)	0.03-0.84 (1-7)
Eosinophils x10 ^{9}/l (%)	0.00 (0)	0.00 (0)	0.36 (2)	0.12 (1)	0-1.0 (0-11)
Basophils x10 ^{9}/l (%)	0.08 (1)	0.00 (0)	0.00 (0)	0.12 (1)	0-0.2 (0-3)

NOTE. Figures in brackets indicate percentages of Total White Blood Cell Counts.

APPENDIX 3.11.
Experiment 7: Oral glucose tolerance test in horses with chronic enteropathy.



* From Roberts and Hill (1973)

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