STUDIES ON CHRONIC GASTROINTESTINAL DISEASE IN THE HORSE

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For the degree of DOCTOR OF PHILOSOPHY

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May 1997

ABSTRACT

Diseases which result in chronic dysfunction of the gastrointestinal tract (GIT) in the horse are an important category of diseases because they are usually severely debilitating and in many cases fatal. However, reaching a definitive diagnosis in such cases is often difficult precluding a rational approach to treatment/management.

A retrospective clinical study of 29 confirmed cases of chronic gastrointestinal disease in the adult horse was undertaken to investigate the relative prevalence of different disorders and their associated historical, clinical and clinicopathological features; chronic inflammatory bowel disease (CIBD) (11 cases), cyathostomosis (9 cases) and alimentary lymphosarcoma (7 cases) were the most common diagnoses. Eight cases of cyathostomosis were diagnosed on the basis of faecal examinations and the clinical features characteristic of this diagnosis included young adult animals presenting in winter/spring because of sudden onset diarrhoea and rapid weight loss with pyrexia, neutrophilia and hypoalbuminaemia. Both CIBD and alimentary lymphosarcoma were diagnosed in older animals and a definitive ante mortem diagnosis was achieved in 2 cases of alimentary lymphosarcoma by histopathological examination of rectal biopsy tissue: An ante mortem diagnosis was not reached in 19 (65.5 %) of the 29 cases. Against this background, a variety of studies were performed to evaluate the ability of different techniques to provide objective information on certain aspects of gastrointestinal function, which may, in turn, improve objective assessment of cases of chronic gastrointestinal disease.

An assay for measurement of fructosamine (glycated serum protein) in equine plasma was validated and a reference range for plasma fructosamine concentration in housed British-native breed ponies was calculated to be 203-310 μmol/L. Plasma fructosamine concentrations decreased in ponies following experimental infection with cyathostome larvae and this was assumed to be due to enteric protein loss and/or altered protein composition and/or increased protein turnover. There was evidence to suggest that fructosamine measurement may be more sensitive than albumin measurement, as an indicator of altered protein metabolism.

In experimental studies, it was shown that both age and diet have a significant effect ($p<0.05$) on plasma glucose concentration measured during an oral glucose tolerance test (OGTT). Retrospective analysis of OGTTs performed on clinical cases demonstrated that the conventional OGTT protocol offers no advantage over a 'modified' test protocol based on a single sample taken at 120 minutes following oral glucose administration. When employing the modified protocol, total malabsorption, defined as failure of plasma glucose concentration to increase above 120 % of the basal glucose concentration, had a sensitivity of 64 % and a specificity of 90 % for the detection/exclusion of small intestinal (SI) pathology.

Several studies were performed in order to investigate the diagnostic/research potential of breath hydrogen (H_2) measurement for assessment of gastrointestinal function in ponies. The initial study indicated that fasting resulted in negligible levels of H_2 excretion and significant concentrations of H_2 were excreted in breath of all ponies following either the ingestion of oats or the administration of wheat flour by stomach tube. However, H_2 was excreted in the breath of three ponies following oral glucose administration which suggests that incomplete glucose absorption may be a normal phenomenon in some healthy ponies. Consequently, this technique is likely to be of limited benefit for the detection of glucose malabsorption in clinical cases. By contrast, H_2 was infrequently excreted in breath following the administration of the non-absorbable carbohydrate, lactulose, the reasons for which are unclear. However, it was confirmed that lactulose is not absorbed from the equine SI and that repeat exposure of intestinal bacteria to this sugar does not enhance hydrogen production/excretion.

The results of another study demonstrated that maintenance diet has a significant effect ($p<0.05$) on breath H_2 excretion following the administration of a test meal, and, by inference, gastrointestinal function. Total H_2 excreted in breath was greater when ponies were maintained on a hay diet compared to when maintained on a high fibre pelleted ration, which presumably reflected an effect of maintenance diet on small intestinal digestive/absorptive capacity and/or conditions in the large intestine which facilitates H_2 production/excretion. In addition, analysis of the patterns of breath H_2 excretion indicated more rapid presentation of the unabsorbed component of the test meal to intestinal bacteria when on the pelleted ration which may reflect an effect of diet on gastrointestinal transit.

In a separate study, measurement of breath H_2 concentration after the ingestion of a high fibre pelleted meal was used to evaluate the effects of cisapride and codeine phosphate on intestinal function in three healthy ponies. Compared to control data, the patterns of hydrogen excretion following premedication with cisapride were interpreted as providing evidence of reduced mouth-to-caecum transit time (MCTT), whereas the results of tests where animals were premedicated with codeine phosphate were indicative of delayed presentation of substrate to large intestinal bacteria (i.e. interpreted as evidence of prolonged gastric/small intestinal transit).

In conclusion, the results presented in this thesis highlight historical, clinical and clinicopathological features which may help differentiate between a variety of chronic GIT diseases in the adult horse. In such cases, identification of total glucose malabsorption, by means of the modified OGTT, is suggestive of severe small intestinal pathology and can be considered an indicator of poor prognosis. In addition, this work provides evidence which indicates that the use of plasma fructosamine measurement, as a means of monitoring protein metabolism, may prove to be a useful parameter for the assessment and/or clinical monitoring of equine chronic enteropathies. Although the findings of these studies indicate that the technique of breath H_2 testing is likely to be of limited benefit for the investigation of individual clinical cases with GIT dysfunction, there is sufficient evidence to suggest that it may be a useful, non-invasive means of assessing MCTT in groups of equine animals.

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ACKNOWLEDGEMENTS

I would like to acknowlegde the assistance of Professors Max Murray and Jim Parkins of the Department of Veterinary Clinical Studies and Professor Andy Tait of the Department of Veterinary Parasitology for the provision of facilities in which this work was undertaken and for their support in its final completion. I am also grateful to Professor Onions and colleagues in the Department of Veterinary Pathology for readily providing the facilities of the post mortem room and for performing pathological examinations on the clinical cases.

This thesis could not have been completed without the sound technical support of a number of colleagues. I wish to extend my sincere gratitude to: Ronnie Barron, Amanda Davidson, June Downs, James Harvey, Richard Irvine, Arlene Macrae, Kathyrn McBride, James McGoldrick, David Moffet, Jimmy Murphy, Kerry O'Neill, Mark McIlveen, John Ramsey, Kenny Williamson and Brian Wright. Alan May produced the original photographic material included in this thesis and Vicki Dale assisted in its final format. Outwith the Veterinary School, Dr. Simon Fleming of Treliske Hospital, Truro, kindly assessed urinary lactulose concentrations and Dr. Andy Duncan of the Glasgow Royal Infirmary assisted in preliminary breath hydrogen studies.

The clinical scholars Sue, Donal, Tom, Rose, Mick, Gareth, Chans and Gina were largely responsible for the day-to-day management of the clinical cases which they approached with enthusiasm and always good humour. I am also grateful for the dedicated care given to experimental ponies by Tom Kelly and clinical cases by Alison Graham and Manon van Nimwegen. Mary Gatherer who cared for many of the animals, both experimental and clinical cases, deserves special mention for her good humoured, unfailing assistance, which in many instances was extended above and beyond the call of duty.

I am indebted to Dr. Peter Graham of the then Department of Veterinary Medicine who advised on the fructosamine analysis and Professor Stuart Reid of the Department of Veterinary Clinical Studies who was called upon frequently for his expert statistical advise.

The initial ideas for this work would never have resulted in a cohesive study without the foresight and dedication of my supervisor Professor Sandy Love of the Department of Veterinary Clinical Studies. He has given unstintingly of his time and effort and continually encouraged me through the years of work.

Finally, I wish to acknowledge the support of my family and the unfailing encouragement and assistance given to me by Carmel.

AUTHOR'S DECLARATION

The work presented in this thesis was performed solely by the author, except where the assistance of others is acknowledged.

David Murphy

May, 1997

Publications

Murphy, D., Edwards, S.E., Russell, T., Sammin, D. & Love, S. (1996) Chronic diarrhoea in the adult horse: a review of 18 cases. *Irish Veterinary Journal* **46**, 216-210.

Murphy, D., Reid, S.W.J. & Love, S. (1997) A modified glucose tolerance test for use in the horse. *Veterinary Record* **140**, 342-343.

Murphy, D. & Love, S. (1997) The pathogenic effects of experimental cyathostome infection in ponies. *Veterinary Parasitology* (in press).

Murphy, D., Reid, S.W.J. & Love, S. (1997) The effect of age and diet on the oral glucose tolerance test in healthy ponies. *Equine Veterinary Journal* (in press).

Murphy, D., Reid, S.W.J., Graham, P.A. & Love, S. (1997) Fructosamine measurement in ponies: Validation and response following experimental cyathostome infection. *Research Veterinary Science* (in press).

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Murphy, D. & Love, S. Use of breath hydrogen measurement to evaluate intestinal transit in ponies. *The 5th Equine Colic Research Symposium*, University of Georgia 1994.

Murphy, D. & Love, S. A preliminary study on the use of breath hydrogen measurement in ponies. *European Society of Veterinary Internal Medicine*, 5th Annual Congress, Cambridge 1995

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CHAPTER 1

CHRONIC GASTROINTESTINAL DISEASE IN THE ADULT HORSE

1.1 Introduction

Diseases which result in chronic dysfunction of the gastrointestinal tract are an important category of conditions because they are usually severely debilitating and in many cases fatal. Causes of chronic gastrointestinal dysfunction are numerous and diverse in nature: most are associated with pathological/physiological changes in the gastrointestinal tract itself, but diseases of other body systems may result in secondary gastrointestinal disturbances. The principle pathophysiological mechanisms by which gastrointestinal function is influenced include one or a combination of the following: disruption of normal mucosal/submucosal architecture leading to reduced absorptive capacity and/or enteric protein loss; altered gastrointestinal motility and/or physical obstruction of the gastrointestinal lumen; increased intestinal secretion and disruption to the normal balance of intestinal microflora. None of these mechanisms are specific for a single disease process, and consequently, there is a paucity of specific diagnostic tests for the investigation of these disorders. This, coupled with the inaccessible nature of the gastrointestinal tract, makes the investigation of chronic equine gastrointestinal disease a difficult, time-consuming task which is often both expensive and unrewarding. The diagnostic challenge posed by equine chronic gastrointestinal disease was evident in a recent review by Love, Mair & Hillyer (1992); of 51 adult equine animals which presented with chronic diarrhoea, a diagnosis was reached in 37 animals, of which only 15 were diagnosed *ante mortem*.

The purpose of this chapter is to review the possible causes of chronic gastrointestinal disease in the adult horse and the techniques which may be used in their investigation.

1.2 Aetiology

1.2.1 Intestinal parasitism

Cyathostomes, or small strongyles, are a group of equine nematode parasites which belong to the family strongylidae and include over 40 species (Lichtenfels, 1975). These parasites are found in the large intestine of virtually all grazing equidae. Under normal circumstances, the larval stages of cyathostomes undergo a local migration in the large intestinal wall, and commonly become arrested in their development in the caecal/colonic mucosa. At present, it is recognised that cyathostome infection is the most common cause of chronic diarrhoea in adult horses in the UK (Mair, de Westerlaken, Cripps & Love, 1990;

Love, Mair & Hillyer, 1992) and recent studies suggest that cyathostomes are an important cause of equine colic (Uhlinger, 1990). Clinical disease has been associated with the emergence of large numbers of previously inhibited fourth stage cyathostome larvae, *en masse*, from the wall of the large intestine resulting in physical disruption of the mucosal and submucosal architecture, and consequently typhlitis and/or colitis (Giles, Urquhart & Longstaffe, 1985). Other pathogenic mechanisms by which cyathostome infections produce intestinal dysfunction remain unclear. However, experimental studies have shown that infective larvae can alter both intestinal motility (Bueno, Ruckebusch & Dorchies, 1979) and mucosal permeability (Love, Escala, Duncan & MacLean, 1991). Additional mechanisms which may contribute to the occurrence of cyathostome-associated colic include intestinal mucosal oedema and/or vasoconstriction induced by local production of vasoactive substances in response to the presence of cyathostome mucosal stages (Mair & Pearson, 1995).

For many years, *Strongylus vulgaris* was considered to be the internal parasite of major concern in equine animals (Drudge, 1979) and was frequently associated with severe gastrointestinal dysfunction in adult horses (Greatorex, 1975; Merritt, Bolton & Cimprich, 1975). However, regular administration of broad-spectrum anthelmintics has been associated with a reduction in the prevalence of large strongyles in recent years (Herd, 1990), and these parasites and their associated lesions are now uncommonly encountered in necropsied horses (Lyons, Drudge, Swerczek, Crowe & Tolliver, 1981; Reinemeyer, Smith, Gabel & Herd, 1984). Similarly, recent studies have demonstrated that cyathostomes, rather than large strongyles, account for the majority of infective parasite larvae on pasture (Herd, 1986; Uhlinger, 1990). The principle pathological effect of *S. vulgaris*, arteritis and thrombosis of the cranial mesenteric artery, is caused by the extra-intestinal migration of the developing larval stages (Duncan & Pirie, 1975). Thromboembolism from such lesions was often associated with the occurrence of non-strangulating intestinal infarction. However, White (1981) reported that pathological evidence to support this theory was uncommon and proposed that the development of non-strangulating intestinal infarction was a consequence of non-occlusive restriction of blood flow to affected intestine, caused by an immune response initiated by parasitic antigens. Later, White (1985) proposed that interference with the blood supply to the gastrointestinal tract may result following the release of vasoactive substances from leucocytes or platelets. Reduced mucosal blood flow secondary to parasitic damage of the arterial blood supply has been suggested as a possible cause of small intestinal malabsorption (Murray, 1989). Experimental studies have shown that

altered gastrointestinal motility is also a feature of *S. vulgaris* infection (Sellars, Lowe, Drost, Rendano, Georgi & Roberts, 1982; Berry, Merritt, Burrows, Campbell & Drudge, 1986; Lester, Bolton, Cambridge & Thurgate, 1989). Alterations in gastrointestinal motility patterns, following either large or small strongyle infection, are generally regarded as biphasic. The first phase occurs within the first few days post-infection and is associated with larval penetration of the mucosa, whereas the second phase is associated with the induction of mesenteric arteritis by *S. vulgaris* or the emergence of cyathostome larvae from the mucosa (Bueno, Ruckebusch & Dorchies, 1979; Sellars *et al.*, 1982; Berry *et al.*, 1986; Lester *et al.*, 1989). Dyson (1983) and Mair, Hillyer & Taylor (1990) suggested that alimentary tract lesions secondary to *S. vulgaris*-induced cranial mesenteric arteritis may be a common cause of peritonitis in adult horses. In addition to the pathogenic effects of *S. vulgaris* larvae, adult stages may also cause intestinal pathology. When feeding, adult large strongyles remove plugs of mucosa or submucosa and thus damage small blood vessels resulting in incidental blood loss. Duncan & Dargie (1975) demonstrated an increase in albumin and red blood cell loss into the intestinal tract of animals which were infected with adult *S. vulgaris*.

Infection with *Parascaris equorum* is common in foals and yearlings, the incidence decreasing with age (Clayton, 1978) and it is an uncommon cause of intestinal dysfunction in the adult horse. The pathophysiological mechanisms by which *P. equorum* results in clinical disease are unclear. Histopathological lesions associated with ascaridiasis are rarely seen in the intestinal tract of affected animals. Clayton, Duncan & Dargie (1980) suggested that the decreased food intake which occurs in ascarid infections and consequent lowering of the protein intake of infected animals were important factors resulting in reduced weight gain. In addition, these authors provided experimental evidence to suggest that adult worms may take up dietary amino acids, thereby impairing amino acid utilisation by the host. Occasionally, these parasites have been associated with acute abdominal crises in heavily infected animals due to either intussusception, intestinal impaction or small intestinal rupture (Becht, 1984).

Necropsy surveys have demonstrated a high prevalence of tapeworm infection in horses, *Anoplocephala perfoliata* being the most common species encountered (Bain & Kelly, 1977; Lyons, Tolliver, Drudge, Swerczek & Crowe, 1983; Lyons, Tolliver, Drudge, Swerczek & Crowe, 1987). Occasionally, cestodiasis has been incriminated as a cause of acute intestinal dysfunction, particularly, as a possible factor in the development of intestinal intussusception and intestinal rupture (Barclay, Phillips & Foerner, 1982; Beroza, Williams,

Marcus & Mille, 1986; Edwards, 1986) and more recently as a risk factor for both spasmodic colic and ileal impaction (C.J. Proudman, personal communication). In addition, Beroza *et al.* (1986) suggested that tapeworm infection may be a cause of chronic intestinal disease which is manifest clinically as unthriftiness, intermittent colic, or diarrhoea. The proposed mechanisms by which tapeworms are hypothesised to cause intestinal disease are numerous and unproven. The predilection site for attachment of *A. perfoliata* to the intestinal mucosa is in the region of the ileocaecal valve and the pathological changes associated with these infections include mucosal thickening, oedema, ulceration, eosinophilic infiltration and diphtheresis (Pearson, Davies, White & O'Brien, 1993; Nilsson, Ljungstrom, Hoglund, Lundquist & Uggla, 1995). In addition, Lyons, Drudge, Tolliver, Swerczek & Crowe (1984) described pathological evidence of ileal hypertrophy in a horse with a heavy tapeworm burden, although there was no known evidence of intestinal disease before euthanasia. Intussusceptions, which have been associated with *A. perfoliata* infestation, are thought to result from either a disruption to the normal pattern of intestinal motility or variations in muscle contractility due to oedema, ulceration and granulation tissue at the site of tapeworm attachment (French & Chapman, 1992). Severe ulceration of the intestinal mucosa may progress to intestinal rupture (Beroza, Barclay, Phillips, Foerner & Donawick, 1983).

Although rarely documented, other parasitic agents incriminated as possible causes of chronic gastrointestinal disease, in particular chronic diarrhoea, in the adult horse include *Giardia* spp. (Kirkpatrick & Shand, 1985), *Eimeria* spp. (Wheeldon & Greig, 1977) and Trichomonas infection (Bennett & Franco, 1969). Failure to produce gastrointestinal disease by experimental infection with either *Eimeria leukarti* (Laufenstein-Duffy, 1969) or *Trichomonas* spp. (Barker & Remmler, 1970) and the demonstration of these organisms in the faeces of healthy equine animals (Damron, 1976; Lyons, Drudge & Tolliver, 1988; Xiao & Herd, 1994) suggest that these parasites are of little pathogenic significance in horses.

1.2.2 Neoplasia

Although rare, squamous cell carcinoma of the stomach is considered to be the most common primary neoplasm of the equine gastrointestinal tract (Boulton, 1987). The tumour appears to originate from the oesophageal region of the stomach and metastatic spread to other organs within the abdomen, particularly the liver, is a common feature (Meagher, Wheat, Tennant & Osburn, 1974; Tennant, Keirn, White, Bentinck-Smith & King, 1982; Olsen, 1992). The aetiology of gastric squamous cell carcinoma in the horse is

unknown. In cattle, a direct relationship has been noted between the density of bracken fern infestation of pasture, the incidence of viral papilloma of the intestinal tract and the prevalence of squamous cell carcinoma of the upper alimentary tract (Jarrett, 1980). In addition, oesophageal carcinoma in man has a higher incidence in certain geographical locations (Doll, 1969). Based on these facts it has been suggested that dietary and/or environmental factors may be important in the aetiology of gastric squamous cell carcinoma in the horse (Tennant *et al.*, 1982).

Lymphosarcoma is characterised by lymphatic tissue enlargement or invasion of non-lymphatic tissue by cells of lymphoblastic character. In the horse, lymphosarcoma is the most common neoplastic condition affecting the haemopoetic system (Gupta, Keahey & Ellis, 1972) and is classified, according to origin, into four different forms; multicentric, alimentary, thoracic and cutaneous (van den Hoven & Fraken, 1983; Mair & Hillyer, 1991). Alimentary lymphosarcoma occurs with a greater frequency than the other forms and was reported to be the second most commonly diagnosed form of intestinal tract neoplasia (Boulton, 1987). In a review of 77 cases of equine lymphosarcoma seen by Mair & Hillyer (1991), 42 animals were affected by an intestinal form of lymphosarcoma which was further categorised as either diffuse intestinal (n=15) or focal intestinal and/or mesenteric (n=27). In most cases, the small intestine and/or associated lymph nodes are infiltrated by neoplastic cells. Platt (1987) described, in detail, the pathology of alimentary lymphosarcoma in nine cases and reported that the wall of the large intestine was rarely infiltrated. Other authors have described individual cases where both small and large intestine were affected (Wiseman, Petrie & Murray, 1974; Roberts & Pinsent, 1975). The role of viruses in the aetiology of feline lymphosarcoma (Jarrett, Crawford, Martin & Davie, 1964) and enzootic bovine leukosis (Ferrer, 1980) are well recognised. However, although virus-like particles have been detected in a foal with lymphosarcoma (Tomlinson, Doster & Wright, 1979), the cause of this condition remains obscure. Interestingly, human patients with malabsorption due to coeliac disease are predisposed to lymphoreticular neoplasia of the gut (Spencer, MacDonald, Diss, Walker-Smith, Ciclitira & Isaacson, 1989).

Other tumours of the gastrointestinal tract which have been documented include adenocarcinoma (Wright & Edwards, 1984; Honnas, Snyder, Olander & Wheat, 1987; Fulton, Brown & Yamini, 1990), leiomyoma (Hanes & Robertson, 1983; Livesey, Hulland & Yovich, 1986) and myxosarcoma (Edens, Taylor, Murray, Spurlock & Anver, 1992).

1.2.3 Chronic inflammatory bowel disease

The term chronic inflammatory bowel disease (CIBD) is used to describe chronic conditions of the intestine which are characterised by severe focal or diffuse inflammatory cell infiltrates into the lamina propria, submucosa or entire wall of either a region or throughout the whole intestine (Roberts, 1984). The infiltrate may be composed of predominantly one or a mixture of cell types, which may include eosinophils, plasma cells, lymphocytes, macrophages, occasional multinucleate giant cells, neutrophils and epitheliod cells. These conditions are characterised histologically to give classifications such as granulomatous enteritis (GE), eosinophilic gastroenteritis (EGE), chronic eosinophilic granulomatous enteritis and lymphocytic-plasmacytic enteritis. Frequently the inflammatory infiltrate spreads to regional lymph nodes and/or associated organs e.g. stomach, liver and pancreas (Breider, Kiely & Edwards, 1985; Lindberg, Persson, Jones, Thoren-Tolling & Ederoth, 1985; Platt, 1986). Transmural extension of the disease process may give rise to a focal peritonitis (Dyson, 1983; Lindberg, 1984). Occasionally, as seen in some cases of EGE, distant structures such as the skin may be infiltrated with inflammatory cells (Pass & Bolton, 1982; Lindberg *et al.*, 1985). In addition the condition described as multisystemic eosinophilic epitheliotrophic disease may have gastrointestinal involvement (Hillyer & Mair, 1992).

The aetiopathogenesis of equine chronic inflammatory bowel disease is unknown. Equine GE has been compared to regional enteritis (Crohn's Disease) in humans (Merritt, Cimprich & Beech, 1976). A familial prevalence for regional enteritis is recognised in man (Kirsner, 1984) and although little evidence exists to suggest that this is the case in horses, Sweeney, Sweeney, Saik & Lichtensteiger (1986) described GE in three siblings and Lindberg *et al.* (1985) confirmed either GE or EGE in five pairs of siblings in a series of 36 cases. Solid evidence implicating an infectious agent as a cause of regional enteritis is lacking and it is generally accepted that the condition is associated with a basic defect in the patients immune response (Lindberg, 1984). Lindberg (1984) proposed that the lesions of equine GE may represent an abnormal host response to gut flora. However, parasitism (Breider, Kiely & Edwards, 1985; Platt, 1986; Cohen, Loy, Lay, Craig & McMullan, 1992) and mycobacterial infections (Merritt, Merkal, Skye & Selway, 1975; Merritt, Cimprich & Beech, 1976; Platt, 1986; Buergelt, Green, Mayhew, Wilson & Merritt, 1988; Cline, Schlafer, Callihan, Vanderwall & Drazek, 1991) have also been implicated in the pathogenesis of these lesions. In addition, small intestinal granulomatous lesions have developed in young horses experimentally infected with either *Mycobacterium johnei*

(Larsen, Moon & Merkal, 1972) or *Rhodococcus equi* (Johnson, Prescott & Markham, 1983) and granulomatous colitis has been described in horses with naturally occurring *Histoplasma capsulatum* infection (Dade, Lickfeldt & McAllister, 1973; Goetz & Coffman, 1984).

Secondary features of infiltrative conditions (either inflammatory or neoplastic) of the bowel include villous atrophy, mucosal oedema, mucosal ulceration, crypt abscessation, peri-lymphatic and transmural inflammation and lymphangiectasia (Lindberg, 1984). Interestingly, lymphangiectasia has been reported as a cause of chronic diarrhoea in an aged horse in the absence of an identifiable predisposing lesion (Milne, Woodman, Rowland, Patrick & Arthur, 1994). Similarly, weight loss and carbohydrate malabsorption due to idiopathic villous atrophy and idiopathic mucosal oedema has been described (Bolton, Merritt, Cimprich, Ramberg & Streett, 1976).

1.2.4 Gastric ulceration

Although information is sparse with regard to the prevalence of gastric ulceration in populations of adult horses, Hammond, Mason & Watkins (1986) reported the presence of gastric ulcers in 128 of 195 horses (65.6 %) necropsied over a two year period. Sixteen (8.2 %) of these animals were described as having severe gastric lesions, although no suggestion was made that any of these animals were suffering from clinical signs related to the lesions. Murray (1992a) demonstrated a causal association between gastric ulceration and signs of abdominal pain in horses: Gastric ulceration was present in 91 of 111 horses investigated because of abdominal discomfort of variable duration and severity, and was determined to be the primary cause of colic in 31 animals based on failure to identify other abnormalities, clinical response to treatment with histamine type-2 receptor antagonists, and confirmation of improvement or resolution of gastric ulceration via endoscopy. Gastric ulceration was the suspected cause of colic in 26 additional horses based on lack of other identifiable abnormalities and clinical response to treatment with histamine type-2 receptor antagonists (Murray, 1992a). The aetiopathogenesis of equine gastroduodenal ulceration is still a topic of much debate. Furr & Murray (1989) reported 55 cases of spontaneous chronic gastroduodenal ulceration, but failed to identify any specific causative agents. However, ulceration of the gastrointestinal tract due to phenylbutazone toxicity has been described (Snow, Bogan, Douglas & Thompson, 1979), and it is also generally accepted that 'stress' may be an important factor in the aetiology of gastric ulcers in horses. In some reports it has been concluded that there is a greater prevalence and severity of gastric

lesions in racehorses in-training when compared to retired or pleasure horses (Hammond, Mason & Watkins, 1986; Murray, Grodinsky, Anderson, Radue & Schmidt, 1989). In addition, Murray & Eichorn (1996) demonstrated that, compared with being at pasture, stable confinement alone appeared to be an important factor in the development of gastric ulcers in horses and that this was probably as a result of altered feeding behaviour. Although an infectious agent (*Helicobacter pylori*) has been identified as a cause of gastric ulceration in man, to date no similar type pathogens have been reported to cause gastric ulceration in adult horses (Murray, 1992b). Irrespective of the specific cause, ulceration is likely to develop because of disruption of the gastric mucosal barrier that protects the gastric mucosa from the potentially harmful effects of gastric acid and pepsin. Disruption of the gastric mucosal barrier allows the diffusion of hydrogen ions and pepsin into the mucosa resulting in auto-digestion and ulcer formation (Murray, 1992b). Although pyloric obstruction and gastric retention is an uncommon sequel to gastric ulceration in equine animals it has been reported in mature horses on a number of occasions (Church, Baker & May, 1986; Laing & Hutchins, 1992; Murphy, Howie & Love, 1994). The primary pathology in each case was extensive ulceration of the gastric pyloric region resulting in dramatic fibrous tissue development within the pyloric wall and consequently a reduction in the pyloric lumen diameter. Pyloric outflow obstruction due to congenital pyloric stenosis has been described in a yearling (Munroe, 1984) and a single case of partial gastric obstruction (gastric stenosis), which was assumed to occur secondary to chronic gastric irritation (Peterson, Donawick, Merritt, Raker, Reid & Rooney, 1972).

1.2.5 Gastric impaction

Primary gastric impaction, defined as a chronic abnormal accumulation of dry, non-swelling, poorly fermentable material, may occur due to a variety of reasons in the horse which include ingestion of dry fibrous grass, irregular watering, irregular feeding, poor mastication, defective gastric secretion, idiopathic gastric atony and pyloric stenosis (Owen, Jagger & Jagger, 1987).

1.2.6 Non-steroidal anti-inflammatory drug toxicity

The pathogenic potential of non-steroidal anti-inflammatory drugs (NSAID), particularly phenylbutazone, has been demonstrated both experimentally (Snow *et al.*, 1979; Snow, Douglas, Thompson, Parkins & Holmes, 1981; Lees, Creed, Gerring, Gould, Humphreys, Maitho, Michell & Taylor, 1983; Collins & Tyler, 1985) and in clinical reports (Roberts,

1981; Behm & Berg, 1987). The mechanism of mucosal damage is not well defined but appears to be associated with a loss of prostaglandin cytoprotective effects which results in microvascular injury (Meschter, Gilbert, Krook, Maylin & Corradino, 1990). A variety of pathological effects may occur as a consequence of NSAID toxicity and include oral, gastric and duodenal ulceration, colonic necrosis, renal crest necrosis, hepatotoxicity and vasculitis (Snow *et al.*, 1979, 1981; Gunson & Soma, 1983; Collins & Tyler, 1985; Meschter *et al.*, 1990). A discrete syndrome of right dorsal colitis has been described in adult horses, which in most cases was attributed to the administration of NSAIDs to dehydrated animals (Karcher, Dill, Anderson & King, 1990; Cohen, Mealey, Chaffin & Carter, 1995). These authors proposed that the right dorsal colon is the only segment of the large intestine with a net water flux in a secretory direction suggesting some intrinsic mucosal membrane differences which might explain the localised nature of the colitis.

1.2.7 Sand enteropathy

Chronic gastrointestinal dysfunction associated with the accumulation of sand in the gastrointestinal tract of horses occurs occasionally in the UK (Love, Mair & Hillyer, 1992) but is well recognised in certain regions of the USA (Bertone, Traub-Dargatz, Wrigley, Bennett & Williams, 1988). The common sites of sand deposition in the intestinal tract are the right colons, pelvic flexure and transverse colon (Colahan, 1987) and the mechanisms by which this causes clinical disease include colonic irritation and/or physical obstruction of the intestinal lumen.

1.2.8 Ileal hypertrophy

Muscular hypertrophy of the ileum is recognised as an infrequent cause of partial intestinal obstruction in the horse (Chaffin, Fuenteabla, Schumacher, Welch & Edwards, 1992). Compensatory (secondary) intestine muscular hypertrophy occurs in response to chronic, caudal intestinal stenosis (e.g. impaction, intussusception, neoplasia, adhesions). Muscular hypertrophy which develops in the absence of such a lesion is referred to as idiopathic (primary) and it has been proposed that neurological abnormalities producing uncontrolled peristalsis or prolonged spastic contraction may be involved in the pathogenesis of idiopathic muscular hypertrophy of the small intestine (Robertson, 1990; 1991). Hypertrophied muscle reduces the diameter of the intestinal lumen creating a partial, or in rare cases a complete, obstruction and, consequently, gaseous distension and/or feed impaction may occur cranial to the lesion.

1.2.9 Idiopathic enteropathy

Occasionally, gastrointestinal dysfunction occurs in the horse without any predisposing pathological lesion. Manahan (1970) described a chronic diarrhoea syndrome in horses which, in the majority of cases, was associated with stress and the administration of antibiotics. The diarrhoea, in these cases, was considered to be caused by a disruption to the normal balance of caecal/colonic flora. Argenzio, Southworth & Lowe (1977) reported that volatile fatty acids (VFA), by-products of colonic fermentation, contributed to the control of water fluxes across the large intestinal mucosa. Analysis of VFA concentrations in the faeces of horses with chronic idiopathic diarrhoea indicated that fermentation disorders do exist in many of these cases and may contribute to the increased intra-luminal water load (Merritt & Smith, 1980; Minder, Merritt & Chalupa, 1980). However, it has not been determined whether the altered fermentative activity was the cause or effect of increased water in the faeces. Other possible mechanisms which contribute to the intra-luminal retention of water in cases of idiopathic chronic diarrhoea remain to be elucidated.

1.3 Differential Diagnosis

Only in rare cases of chronic gastrointestinal tract disease will the clinical features be suggestive of a specific aetiological diagnosis. Indeed, horses may present without manifesting overt signs of gastrointestinal dysfunction. Furthermore, clinical signs of gastrointestinal dysfunction (i.e. diarrhoea and/or colic) may be features of a variety of conditions which are not primary gastrointestinal diseases and exclusion of these disorders is often necessary before the clinician can focus attention to the investigation of a primary gastrointestinal disorder.

1.3.1 Equine dysautonomias

Grass sickness is a disease of unknown aetiology associated with dysfunction of the autonomic nervous system, the principle manifestation of which is reduced gastrointestinal motility. The disease occurs in three overlapping forms acute, subacute and chronic: acute and subacute cases invariably die within days of presentation but, chronic cases may live for weeks/months and a proportion may survive the disease (Milne, Woodman & Doxey, 1994). Obel (1955) observed characteristic histopathological lesions in the paravertebral and prevertebral ganglia of grass sickness cases which included chromatolytic ganglion cells, pyknotic nuclei and vacuolation of cytoplasm. Similar histopathological changes have been observed in central brain stem nuclei (Barlow, 1969) and the plexuses of the enteric

nervous system (Pogson, Doxey, Gilmour, Milne & Chisholm, 1992). The epidemiology of grass sickness, particularly the association with grazing, or change in pasture, suggests that the disease is caused by a toxin ingested with the herbage (Gilmour & Jolly, 1974; Wood, Doxey & Milne, 1994). In experimental studies, Gilmour (1973) and Gilmour & Mould (1977) reproduced the neurological lesions of grass sickness by injecting normal ponies with serum and plasma from acute grass sickness cases, confirming that a neurotoxin was present within the blood of affected animals. Griffiths, Smith, Doxey, Whitwell & Love (1994) reported that serum from cases of acute grass sickness, injected into the parotid salivary gland of healthy ponies, had a specific effect on the post-ganglionic neurone supplying the tissue and that there was retrograde axonal transport to the cell body. Although there have been recent reports of a grass sickness-like syndrome ('mal seco') in Argentina (Uzal, Robles & Olaechea, 1992), it is generally accepted that grass sickness does not occur outside Europe, its incidence being highest in the UK.

Other reports of equine enteric neuropathy are limited to congenital intestinal aganglionosis/hypoganglionosis in foals (Dyke, Laing & Hutchins, 1990) and a single case of chronic intestinal pseudo-obstruction due to small colon myenteric ganglionitis in a 4-year-old mare (Burns, Karcher & Cummings, 1990).

1.3.2 Hepatopathies

Occasionally, horses with chronic liver disease may present with clinical signs suggestive of gastrointestinal disease. In the horse, chronic liver failure may occur for a variety of reasons, but is most commonly associated with prolonged ingestion of hepatotoxic plants containing pyrrolizine alkaloids (e.g. senecio jacobea) (O'Brien, 1985; West, 1996; McGorum, Milne & Love, 1997). Chronic liver disease may occur in aged horses with no identifiable precipitating cause and can be considered to be 'senile cirrhosis'. The pathophysiological mechanisms which result in gastrointestinal dysfunction in cases of chronic liver disease are complex and poorly understood. It is suggested that diarrhoea may occur consequent upon portal vein hypertension and intestinal oedema (Pearson, 1990). In addition, Milne, Pogson & Doxey (1990) reported three cases of secondary impaction associated with ragwort poisoning and proposed that altered gastrointestinal motility may be a result of the production of neurotoxic products in cases of chronic liver failure. It is possible, therefore, that altered intestinal motility may become apparent clinically as diarrhoea and/or colic. Similarly, Murray (1989) suggested that increased hydrostatic

pressure within the colonic microvasculature secondary to congestive heart failure and associated hepatic fibrosis may result in colonic dysfunction manifest clinically as diarrhoea.

1.3.3 Hyperlipaemia

Chronic diarrhoea has been recorded as a clinical feature in cases of equine hyperlipaemia (Love, Mair & Hillyer, 1992; Watson, Murphy & Love, 1992). Hyperlipaemia is a disorder of lipid metabolism commonly observed in pony breeds where fatty acids are mobilised from adipose tissue in response to reduced food intake (caused by malnutrition, stress or underlying illness) or to meet the demands of pregnancy and lactation (Naylor, 1987). The fatty acids are subsequently deposited in body tissues, including liver, kidney and muscle, frequently resulting in organ failure. The pathophysiological mechanism which results in diarrhoea is unclear in many cases but may reflect either underlying illness (e.g. parasitism) or prolonged anorexia or liver dysfunction.

1.3.4 Peritonitis

In the horse, peritonitis is usually acute and diffuse (Semrad, 1990), but it has also been reported as a cause of chronic gastrointestinal dysfunction in this species (Dyson, 1983; Mair, Hillyer & Taylor, 1990). The mechanisms by which chronic peritonitis may affect gastrointestinal function include direct constriction of intestine resulting in decreased lumen diameter which can impair ingesta flow, intestine vascular compromise secondary to adhesion-induced mesenteric contraction and by placing tension on normally mobile bowel which can cause functional impairment of motility secondary to pain (Trent, 1995). Factors, apart from abdominal surgery, which predispose horses to the development of peritonitis remain undetermined in many cases. The most commonly identified source of infection of the abdominal cavity is the intestinal tract. Intestinal lesions which may be involved in the pathogenesis of peritonitis include intestinal perforations, foreign body penetration, inflammatory bowel disease (Dyson, 1983), and neoplasia. Mair, Hillyer & Taylor (1990) reported that intestinal lesions attributed to cranial mesenteric arteritis, induced by *S. vulgaris* larvae migration, were an important cause of peritonitis and it has also been suggested that peritonitis may occur secondary to larval migration of *S. edentatus* (Love, 1992). Other possible causes of peritonitis include uterine trauma/infection, complications following castration and septicaemia (Semrad, 1990). Abdominal abscessation involving lymph nodes and/or abdominal organs is a reported cause of weight loss and intermittent colic in the horse (Rumbaugh, Smith & Carlson, 1978). The most

commonly isolated causal organisms are *Streptococcus equi*, *Streptococcus zooepidemicus* and *Corynebacterium pseudotuberculosis*, and affected animals will often have a history of upper respiratory tract infection/lymphadenitis (Rumbaugh, Smith & Carlson, 1978).

1.4 Investigation of Chronic Gastrointestinal Dysfunction

1.4.1 History

Although usually non-specific, historical information may, in certain cases of chronic gastrointestinal disease, suggest a particular diagnosis. Therefore, a detailed history is an important prerequisite to clinical examination and further investigation. The history should establish the duration of illness, rapidity of onset, numbers of animals affected, previous or intercurrent disorders, changes in feed and water intake and faecal consistency, dietary/grazing management, recent management changes, anthelmintic and health care programmes and recently administered medications.

Reid, Mair, Hillyer & Love (1995) reported that history alone may be useful in the differentiation of cyathostomosis from other causes of diarrhoea, with age, season and period since last receiving anthelmintics identified as important risk factors. Affected animals are usually young adults (i.e. less than 6 years old) and present in late winter or spring. Gibson (1953) and Smith (1976) suggested that the removal of adult worms from the intestinal lumen, either by natural ageing or anthelmintic treatments, stimulate inhibited stages to develop. This effect has been demonstrated in experimental studies (Smith, 1976) and may account for the apparent induction of naturally occurring clinical disease in some horses following prophylactic anthelmintic treatments (Mair, 1993; Reid *et al.*, 1995). However, cases of cyathostomosis have been reported to occur at other times of the year and the condition has been described in aged ponies (Mair, 1993). In addition, the majority of disease conditions which result in chronic gastrointestinal tract dysfunction in the adult horse occur sporadically in individual animals, but it is known that cyathostome-related illnesses may present as an outbreak in a group of animals (Kelly & Fogarty, 1993; Mair, 1994). The long-term deworming history of individual clinical cases is of limited diagnostic value in suspected cases of cyathostomosis. Although regular prophylactic anthelmintic therapies have been associated with a reduction in the prevalence of *S. vulgaris* infection in the horse (Herd, 1990), substantial cyathostome burdens may be present as inhibited stages within the large intestinal mucosa of some horses despite the regular administration of anthelmintics. Love (1992) suggested that historical information concerning the parasite prophylaxis program used on the farm (including worming strategy, pasture hygiene and

stocking density) is more appropriate since it may give some indication of the possible level of larval challenge.

An accurate knowledge of management factors and previous drug therapies may be of value for including/excluding other possible diagnoses in suspected cases of chronic gastrointestinal tract dysfunction. Horses at risk of developing sand enteropathy are those grazing paddocks of sandy soil with little or no grass cover (Byars, 1990). For these reasons, the condition is seldom detected in the UK (Love, Mair & Hillyer, 1992), but is well recognised in certain regions of the USA (Bertone *et al.*, 1988; Byars, 1990) where the environmental/managemental conditions necessary for the ingestion of large amounts of sand, with feed, are present. Non-steroidal anti-inflammatory drug toxicity should be considered in animals which have a history of recent treatment with drugs of this class: Toxicity may occur when NSAIDs are administered at doses exceeding those recommended, when used in combination with other NSAIDs or when administered to dehydrated animals or to animals with liver dysfunction (Murray, 1990). Manahan (1970) described a chronic diarrhoea syndrome in adult horses which, in the majority of cases, was associated with stress and the administration of antibiotics. No underlying pathological process was identified and the diarrhoea in these animals was considered to have been caused by a disruption to the normal balance of caecal/colonic flora. Interestingly, Mair (1993) observed an apparent association between the onset of diarrhoea in some cases of cyathostomosis and recent management changes and suggested that stress-induced immunosuppression may precipitate clinical disease.

Lindberg *et al.* (1985) indicated that young Standardbreds may be predisposed to develop chronic inflammatory bowel diseases: Of 36 confirmed cases of either GE or EGE, all but one were standardbreds and 34 cases were in animals four years of age or younger. A similar breed and age incidence was reported by Merritt, Cimprich & Beech (1976) when describing nine cases of GE. However, these conditions have been described in older animals and in other breeds of horse (Pass & Bolton, 1982; Platt, 1986) and ponies (Howie, 1995). Conversely, neoplasms of the gastrointestinal tract generally affect older animals. Tennant *et al.* (1982) reviewed clinical information available from 21 reported cases of gastric squamous cell carcinoma with a mean age of 10.7 years (range 6-16 years). Similarly, the mean age of 42 cases of alimentary lymphosarcoma reported by Mair & Hillyer (1991) was 9-10 years. However, animals in that series ranged in age from 2 to 20 years and Platt (1987) described the pathology of alimentary lymphosarcoma in nine horses with an age range of 2-6 years.

1.4.2 Clinical examination

Case history and clinical presentation may enable the clinician to focus attention to the gastrointestinal tract. However, horses with gastropathies/enteropathies may not present with clinical signs directly attributable to gastrointestinal disease, and conversely, it is possible that diseases of other body systems affect normal gastrointestinal tract function (Love, Mair & Hillyer, 1992), such that further investigations are frequently required to confirm gastrointestinal involvement/exclude other possible diagnoses.

In horses with chronic gastrointestinal tract disease, the clinical features may include poor body condition and/or diarrhoea and/or peripheral oedema and/or colic and/or pyrexia, none of which are specific for a particular disease process. Poor body condition is often the predominant clinical feature in animals with a chronic pathological lesion(s) of the gastrointestinal tract and may be due to one or a number of factors such as inappetance, maldigestion, small and/or large intestinal malabsorption, enteric protein loss and cachexia. The occurrence of diarrhoea in the adult horse is indicative of colonic dysfunction due to pathological and/or physiological disturbances in that organ (Murray, 1989): Therefore, diarrhoea is usually absent in animals with lesions limited to the stomach or small intestine. Peripheral oedema, associated with decreased plasma oncotic pressure, is a relatively common feature of chronic gastropathies/enteropathies and may develop secondary to enteric protein loss. The pathophysiological mechanisms involved in the development of colic in animals with chronic gastrointestinal disease are numerous: It may occur due to intense intestinal inflammation, altered intestinal motility, physical obstruction of the gastrointestinal tract, non-strangulating intestinal infarction, and abnormal tension on serosal surfaces and/or mesentry. Pyrexia is a non-specific host response to infection, inflammation or neoplasia (Mair, Taylor & Pinsent, 1989) and is associated with the production of an endogenous pyrogen (monokine interleukin-1) which stimulates prostaglandin synthesis in the hypothalmic thermoregulatory centre (Haskins, 1995).

Occasionally, an animal may exhibit clinical features which are suggestive of a particular diagnosis. Although the majority of chronic gastrointestinal diseases have an insidious onset, the classical presentation in cases of cyathostomosis is sudden onset diarrhoea and rapid weight loss (Giles, Urquhart & Longstaffe, 1985). However, recent reports indicate that cyathostome-related disease is not limited to the classical presentation of acute larval cyathostomosis and that clinical manifestations may range from failure to thrive to life-threatening gastrointestinal disorders (Mair, 1993, 1994; Lyons, Swerczek,

Tolliver, Drudge, Stamper, Granstorm & Holland, 1994; Mair & Pearson, 1995; Matthews & Morris, 1995).

Horses with chronic gastropathies may exhibit dysphagia and/or ptyalism (Tennant *et al.*, 1982; Olsen, 1992; Murphy, Howie & Love, 1994). In addition, in a proportion of these cases, behaviour at feeding time can be characterised by initial interest in food and ingestion of a small quantity before becoming disinterested and refusing to eat and/or exhibiting signs of abdominal discomfort (Tennant *et al.*, 1982). Oral/tongue ulceration, associated with mucosal ischemia, has been observed in experimental NSAID toxicity (Snow *et al.*, 1979; Gunson & Soma, 1983) and therefore may be a feature of inadvertent toxicity. In animals, where the history may indicate a possible diagnosis of sand enteropathy, auscultation of the ventral abdomen has been advocated as a useful diagnostic aid in that it may be possible to appreciate the sound of pouring sand in the colon of affected animals (Ragle & Meagher, 1987).

A number of conditions which primarily affect the gastrointestinal tract may progress to involve extraintestinal organs. Pass & Bolton (1982) and Lindberg *et al.* (1985) reported severe skin lesions, characterised by excessive scaling, patchy alopecia, lichenification and excoriations, and ulcerative coronitis in a proportion of cases with EGE. The aetiology of the skin lesions was unknown, but suggested to be immune-mediated with a common antigen responsible for both gastrointestinal and skin lesions (Pass & Bolton, 1982). Interestingly, the condition described as multi-systemic eosinophilic epitheliotrophic disease may have gastrointestinal involvement (Nimmo-Wilkie, Yager, Nation, Clark, Townsend & Baird, 1985; Sanford, 1989; Hillyer & Mair, 1992). Furthermore, Nimmo-Wilkie *et al.* (1985) suggested that chronic eosinophilic dermatitis and chronic EGE are variants of a multi-systemic eosinophilic epitheliotrophic disease. Both ulcerative coronitis (Wilson, Sutton, Groenendyk & Seawright, 1985) and polysynovitis (Platt, 1987) have been reported in association with alimentary lymphosarcoma and, again, were assumed to be immune mediated. Occasionally, tumours of the gastrointestinal tract may metastasise to involve other organ systems and affected animals may present with clinical signs referable to secondary organ involvement. Wrigley, Gay, Lording & Haywood (1981) described a case of gastric squamous cell carcinoma where extensive metastases to the pleural cavity resulted in pleural effusion and signs of respiratory distress. Similarly, paraneoplastic syndromes have been documented in association with equine alimentary tract tumours and may result in secondary clinical features. Hypercalcaemia of malignancy (pseudohyperparathyroidism) is the most common paraneoplastic syndrome in horses (Turrel, 1995) and has been described

in animals with gastric carcinoma (Meuten, Price, Seiler & Krook, 1978b; Miller, 1983) and alimentary lymphosarcoma (Mair, Yeo & Lucke, 1990). Extrapolating from human medicine, it has been proposed that, in animals, tumours produce parathyroid hormone-related protein, which results in increased osteoclastic bone resorption and renal resorption of calcium. Soft tissue mineralisation and calcium-induced nephropathy leading to renal failure, are complications of this syndrome (Marr, Love & Pirie, 1989; Mair, Yeo & Lucke, 1990).

1.4.3 Rectal findings

Rectal examination is an important component of the initial investigation of any case suspected of having chronic gastrointestinal disease. Occasionally, in animals with gastrointestinal neoplasia and CIBD enlarged mesenteric lymph nodes and/or abnormal abdominal masses are palpable *per rectum*. Mair & Hillyer (1991) reported that, of 27 horses with focal intestinal and/or mesenteric lymphosarcoma, abnormalities were palpated in 12 animals. Interestingly, many cases of gastric squamous cell carcinoma which have been described in the literature had abnormal findings on rectal examination: Of 21 cases reported up to 1982, 11 were examined rectally and, of these, eight had abnormal anterior abdominal masses and/or adhesions palpable *per rectum* (Tennant *et al.*, 1982). Similarly, Olsen (1992) reported that abnormal masses were palpable in all of four cases of gastric squamous cell carcinoma examined by this means. Palpation of masses in the mesentery or associated with the gut wall was a common feature in one series of horses with GE (Merritt, Cimprich & Beech, 1976). This observation is supported by other single case reports of that condition (Hodgson & Allen, 1982; Roberts, 1983). It has been suggested that palpation of the cranial mesenteric artery *per rectum* may permit a diagnosis of *S. vulgaris* infection (Merritt, Bolton & Cimprich, 1975; Meuten, Butler, Thomson & Lumsden, 1978a), but White (1981) demonstrated little correlation between impressions of the size of the cranial mesenteric artery acquired *per rectum* and the presence of cranial mesenteric arteritis.

Chaffin *et al.* (1992) suggested that muscular hypertrophy of the ileum should be a differential diagnosis when a thickened, rigid, tubular mass is palpated *per rectum* in the right dorsal quadrant of the abdomen: These authors reported that this feature was evident in two of their series of 10 cases of small intestinal muscular hypertrophy. Similarly, abnormal rectal findings were described in a case of small intestinal muscular hypertrophy reported by Schneider, Kennedy & Leipoid (1979).

Although no abnormalities are palpated during rectal examination of cases of cyathostomosis, a frequent incidental finding is the presence of large numbers of cyathostome larvae on the rectal sleeve thereby effecting a diagnosis. Cyathostome larvae were grossly visible, following rectal examination, in five of the 15 cases of cyathostomosis described by Giles, Urquhart & Longstaffe (1985).

1.4.4 Haematological features

Haematological abnormalities in animals with chronic gastrointestinal tract disease are usually non-specific, the most frequent abnormalities being anaemia and/or neutrophilia. The commonest causes of anaemia in such cases are the normocytic, normochromic anaemia of chronic inflammatory disease and chronic blood loss secondary to ulcerative lesions of the gastrointestinal tract. Interestingly, immune-mediated haemolytic anaemia and thrombocytopaenia were features of two cases of alimentary lymphosarcoma reported by Reef, Dyson & Beech (1984). In addition, Mair & Hillyer (1991) confirmed immune-mediated haemolytic anaemia in two of 42 cases with alimentary lymphosarcoma. Although the mechanism by which these occur is unclear, factors which may be involved in their development include tumour antibody production, tumour stimulation of antibodies that cross react with red blood cells and multisystem immunological incompetence (Reef, Dyson & Beech, 1984). Leukaemia may be rarely encountered in cases of equine lymphosarcoma (Madewell, Carlson, MacLaughlin & Feldman, 1982).

Neutrophilia is a non-specific feature that is a common response to infection, inflammation or neoplasia in the horse (Mair, Taylor & Pinsent, 1989) and therefore may be detected in animals with any chronic pathological lesion of the gastrointestinal tract. Eosinophilia is popularly attributed to intestinal parasitism, in particular strongylosis. However, reports of both naturally occurring clinical cases (Giles, Urquhart & Longstaffe 1985; Uhlinger, 1991; Love, Mair & Hillyer, 1992) and also experimental infections (Love *et al.*, 1991) suggest that this is an inconsistent feature of strongyle-associated disease.

Hyperfibrinogenaemia is, again, non-specific and indicates infection, inflammation or neoplasia (Mair, Taylor & Pinsent, 1989). Allen & Kold (1988) indicated that plasma fibrinogen is a sensitive indicator of inflammation in the horse becoming elevated within 48 hours of an inflammatory process and Jain (1986) suggested that this parameter is a better monitor of recovery than leucocyte count.

1.4.5 Blood biochemical features

Apart from eliminating liver disease as a possible differential diagnosis, blood biochemical analysis provides little/no specific diagnostic information when investigating suspected cases of chronic gastro-/enteropathy, but it is often valuable in the overall assessment of these conditions. The most common biochemical abnormalities in animals with chronic gastrointestinal disease are non-specific and include hypoalbuminaemia, hypo- or hyperglobulinaemia and increased serum AP concentrations.

Hypoalbuminaemia and/or hypoproteinaemia is a common phenomenon which has been demonstrated in association with numerous chronic gastrointestinal disorders (Knox, Reid, Love, Murray & Gettinby, 1997) and is assumed to be a primary consequence of enteric protein loss. Enteric protein loss may be exacerbated by concurrent decreased absorption due to inappetance and/or small intestinal malabsorption. Abnormal loss of serum proteins into the gastrointestinal tract may occur secondary to either obstruction of the gastrointestinal lymphatics with loss of lymph into the intestinal lumen or exudation through an inflamed/ulcerated mucosa or a disorder of mucosal cell metabolism (Scrutchfield, 1975). In patients with protein-losing enteropathy all serum proteins are lost into the intestinal tract at the same rate. However, the amount of reduction in serum protein concentration is not the same for the different classes of proteins with the concentration of albumin and gammaglobulin, proteins with the longest half-life, usually the most severely depressed. In addition, increased total plasma globulin concentration, a non-specific indicator of chronic inflammation/immune stimulation (Coffmann, 1979), may accompany hypoalbuminaemia and maintain total protein concentrations within normal limits or, in certain cases, result in an increase in total protein concentrations.

Alkaline phosphatase has a wide tissue distribution, therefore increases in serum AP concentrations may occur in association with physiological/disease processes in a variety of organs. The development of analytical techniques for the quantification of tissue-specific AP isoenzymes has improved the diagnostic specificity of this variable such that, in human medicine, it is widely accepted as an indicator of bone disease, cholestatic liver disease, pregnancy complications and as a tumour marker (Moss, 1987). Increases in total serum AP is a common biochemical feature in horses with chronic gastrointestinal disease (Lindberg *et al.*, 1985; Love, Mair & Hillyer, 1992). In addition, Blackmore & Palmer (1977) reported that, in horses with intestinal parasitism and/or diarrhoea, increases in total serum AP were due to an increase in the intestinal isoenzyme which was differentiated from other isoenzymes by L-phenylalanine inhibition. However, subsequent studies, using

different biochemical methods, failed to support the findings of Blackmore & Palmer (1977) and there is a general consensus that normal horse sera does not contain significant levels of intestinal derived AP and that intestinal AP is not likely to appear in equine serum even when gastrointestinal disease is present (Hoffmann, Dorner & Morris, 1983; Trueman, Lumsden & McSherry, 1983; Ellison & Jacobs, 1990; Hank, Hoffmann, Sanecki, Schaeffer & Dorner, 1993). Interestingly, Lindberg *et al.* (1985) reported that, although intestinal disease was generally severe and widespread in horses with either GE or EGE, the intestinal isoenzyme of AP was not identified in serum from any case. Further analysis indicated that increases in total serum AP were due to isoenzymes originating from bile ducts or liver and reflected cholangitis or cholangiohepatitis which most often occurred in association with eosinophilic gastroenteritis (Lindberg *et al.*, 1985). In addition, extension of the intestinal disease process to involve the liver often resulted in mild to moderate elevations in GGT concentrations (Lindberg *et al.*, 1985). In the majority of horses with chronic gastrointestinal tract disease and increases in serum AP, the origin of this increase is still unclear.

In animals with diarrhoea, biochemical assessment of electrolyte status (sodium, chloride, potassium), renal function (urea and creatinine) and protein status is diagnostically non-specific, but is useful in the overall assessment of the case and may influence approach to therapy. Disorders of calcium metabolism/homeostasis are rarely reported in animals with chronic gastrointestinal tract disease. Hypocalcaemia may occur in association with enteric protein loss, but is unlikely to be of clinical significance, with the lost calcium carried by plasma proteins in a bound, biologically inactive form. In addition, tumour-associated pseudohyperparathyroidism may result in hypercalcaemia.

Mair *et al.* (1990) indicated that the results of both haematological and blood biochemical tests may provide useful prognostic information in animals with diarrhoea. They demonstrated that the packed cell volumes, neutrophil counts and AP concentrations were higher and the total protein and albumin concentrations were significantly lower in horses that died when compared to those that survived. However, these authors concluded that, owing to the wide variety of their results, those for an individual horse could not be used to predict the outcome of that case (Mair *et al.* 1990).

1.4.6 Serum protein electrophoresis

Although the determination of total serum protein and albumin concentrations provides useful information with regard to protein loss/inflammatory responses in animals with

chronic alimentary tract disease, these biochemical parameters are limited in their definition of protein response to specific diseases. In addition, measurement of serum albumin concentrations by the chemical dye-method assays used routinely in many biochemical profiles may provide inaccurate results, particularly when the concentration of albumin is low and globulin concentrations are increased (Blackmore, Henley & Mapp, 1983). Because of this serum protein electrophoresis (SPE) is the preferred test for determining serum albumin and globulin concentrations. In addition, Coffmann & Hammond (1979) suggested that electrophoretic separation of serum globulins enhances the diagnostic potential of serum protein analysis. In particular, it has been suggested that serum protein electrophoresis is a useful aid in the diagnosis of strongylosis with increases in β-globulin fraction having been demonstrated in horses experimentally infected with both *Strongylus vulgaris* (Round, 1970; Duncan & Pirie, 1975; Patton, Mock, Drudge & Morgan, 1978; Kent, 1987) and also cyathostome larvae (Round, 1970). However, Love *et al.* (1991) observed no notable changes in values of any of the serum globulin fractions in ponies experimentally infected with either two or 11.5 million cyathostome larvae. In addition, although hyperbetaglobulinaemia has been described as a characteristic of acute larval cyathostomosis (Giles, Urquhart & Longstaffe, 1985), this was not a consistent feature of other reports of naturally-acquired cyathostome infections (Mair, Cripps & Ricketts, 1993) and should not be considered pathognomic of this condition. Furthermore, Matthews (1982) detected considerable variation in serum globulin components among apparently healthy animals casting doubt upon the use of quantitative electrophoresis in individual clinical cases. Similarly, in a study of serum protein changes in ponies on different parasite control programmes, Herd & Kent (1986) observed marked variation between individuals and no correlation between protein responses and increasing levels of pasture infectivity (and therefore, by inference, no correlation with infection rate of grazing animals).

Mair, Cripps & Ricketts (1993) investigated the diagnostic potential of SPE for differentiating cyathostomosis from other causes of chronic diarrhoea in the adult horse: These authors demonstrated that animals with cyathostomosis had significantly higher values for β-1-globulin than animals with diarrhoea due to other causes and that the sensitivity and specificity of β-1-globulin estimation for the diagnosis of cyathostomosis were 45.5 % and 86.0 %, respectively. They concluded that, in individual horses with diarrhoea, the identification of high β-1-globulin concentrations is suggestive of cyathostomosis, whereas the presence of normal β-1-globulin concentrations should not be considered as an indicator of the absence of the disease (Mair, Cripps & Ricketts, 1993).

1.4.7 Faecal analysis

Faecal analysis is an important component of the laboratory investigation of animals with chronic gastrointestinal disease. Perhaps the most useful and readily attainable diagnostic feature in cases with cyathostome-associated illnesses is identification of large numbers of $4^{th}/5^{th}$ stage cyathostome larvae on the rectal sleeve following rectal examination or in wet faecal preparations. However, in a proportion of cyathostomosis cases either small numbers or no larvae will be demonstrable making confirmation of the diagnosis difficult: Love, Mair & Hillyer (1992) documented that an ante mortem diagnosis was achieved in only seven of 14 cases of chronic diarrhoea due to cyathostome infection. In cyathostome-associated diseases faecal worm egg counts are usually low or negative, due to the fact that infection is often prepatent when clinical signs occur (Mair *et al.*, 1990). Similarly, in animals with large strongyle-associated disease, the major pathogenic parasite stages are immature and migratory (Duncan & Pirie, 1975), and consequently, eggs and larvae are not detected in faeces. Currently, it is considered that estimation of faecal worm egg counts in grazing cohorts, rather than in an individual case suspected of having strongyle-associated disease, is more beneficial, in that, it may provide some indirect indication of the probable level of larval challenge on a grazing area.

Although rarely documented, other parasitological agents have been incriminated as possible causes of gastrointestinal tract dysfunction, based on their identification in faeces, and include *Giardia* spp., *Eimeria leukarti* and trichomonads. Kirkpatrick & Shand (1985) suggested that giardia infection of horses may be underdiagnosed due to the infrequent use of the zinc sulphate flotation technique, the preferred diagnostic method for *Giardia* spp., in the routine parasitological examination of faeces. Similarly, standard faecal flotation methods are often inadequate for detecting *E. leukarti* oocysts which are most often readily identified using saturated sodium nitrate solutions or Sheather's sugar solution (Klei, 1986). However, both *Giardia* spp. and *E. leukarti* have been identified in the faeces of clinically healthy animals suggesting that, in general, they are of minor pathogenic significance in the adult horse. It is now recognised that, rather than being a cause of chronic diarrhoea in the horse, trichomonas populations are part of the normal equine colonic microflora (Damron, 1976) and their presence in large numbers may, as suggested by Manahan (1970), reflect a disruption in the intra-luminal environment. However, Love (1992) suggested that when protozoal agents are detected in the faeces of animals with chronic diarrhoea, empirical treatment may be justified if other causes of diarrhoea cannot be identified.

Salmonellosis is frequently incriminated as a cause of chronic diarrhoea in adult horses when cultured from diarrhoeic faeces (Merritt, Bolton & Cimprich, 1975). However, faecal bacteriological results from cases of chronic diarrhoea should be interpreted with caution: *Salmonella* spp. can be shed in the faeces secondary to another disease of the alimentary tract, such as cyathostomosis (Giles, Urquhart & Longstaffe, 1985) and alimentary lymphosarcoma (Love, Mair & Hillyer, 1992). In these cases, the salmonella shedding was considered a consequence of concurrent illness and/or alteration in the intestinal environment. In the horse, chronic diarrhoea has not been induced following experimental salmonella infection, such that serious doubt exists about this entity (Smith, Reina-Guerra, Hardy & Habasha, 1979). Other bacteria which might be of significance if isolated from faecal samples include non-haemolytic *Escherichia coli*, *Clostridium perfringens* type A, other clostridia, *Actinobacillus equuli*, *Bacteroides vulgatus*, *Pseudomonas putrefaciens*, *Aeromonas hydrophila*, *Campylobacter jejuni* and *Campylobacter coli* (Al-Mashat & Taylor, 1986). However, none of these bacteria are recognised as a primary cause of chronic intestinal disease in the horse and, as with *Salmonella* spp., their presence may reflect altered colonic environment secondary to another disease process. *Mycobacterium* spp., in particular *Mycobacterium avium-intercellularae* complex, have been implicated as a cause of chronic intestinal granulomatous lesions in the horse and the diagnosis of intestinal mycobacteriosis may be achieved by faecal culture using specific media, but it takes several weeks for colonies to grow (Buergelt *et al.*, 1988).

Demonstration of large amounts of sand in faeces, by means of the 'glove'/'swirl' test, is suggestive of sand-induced enteropathy. The test is based on the fact that when faeces is added to water in a rectal sleeve sand, if present in significant amounts, will become visible as it settles to the bottom. However, failure to detect sand in faeces by this means should not rule out sand enteropathy as a possible diagnosis, whereas the presence of large amounts of sand indicates that more extensive efforts should be undertaken to determine the role of sand in the pathogenesis of the enteropathy (Merritt, 1994).

1.4.8 Peritoneal fluid analysis

Gross, cytological, biochemical and bacteriological analyses of peritoneal fluid, obtained by abdominocentesis, are commonly employed for the investigation of acute abdominal crises in the horse (Nelson, 1979). However, apart from eliminating a possible diagnosis of peritonitis/abdominal abscess, it is generally considered that these analyses are of little diagnostic value in the investigation of chronic alimentary tract disease. Occasionally,

cytological examination of peritoneal fluid may yield a specific diagnosis in cases of gastrointestinal tract neoplasia: Neoplastic cells were detected in the peritoneal fluid of three of the five cases of gastric squamous cell carcinoma described by Olsen (1992) and a case of intestinal adenocarcinoma reported by Fulton, Brown & Yamini (1990). However, exfoliated malignant cells were rarely identified in peritoneal fluid samples obtained from horses with alimentary lymphosarcoma (Mair & Hillyer, 1991). Therefore, while identification of neoplastic cells in peritoneal fluid provides a certain diagnosis, the absence of such cells cannot be interpreted as absence of a neoplastic process. Moreover, in chronic inflammatory lesions of the peritoneum mesothelial cells may become hyperplastic and, as such, mimic neoplastic cells (Nelson, 1979).

Although analysis of peritoneal fluid in cases of CIBD is often normal, Merritt, Cimprich & Beech (1976) reported that reduced phagocytic activity of cells in peritoneal fluid was a consistent feature in horses with GE and was possibly an indication of altered immune response. However, this is a subjective evaluation which may be difficult to document in individual clinical cases and, consequently, this technique has not become established in the investigation of suspected GE cases (Murray, 1989). Increased numbers of peritoneal fluid eosinophils have been recorded in a case of chronic EGE (Gibson & Alders, 1987), but is not a consistent feature of this condition. Similarly, it has been suggested that increased numbers of eosinophils in the peritoneal fluid are suggestive of parasitic mesenteric arteritis (Bach & Ricketts, 1974; Sweeney *et al.*, 1986), but Dyson (1983) indicated that the presence of eosinophils in peritoneal fluid is an unreliable indicator of strongyle-associated disease.

Occasionally, in animals with pathological lesions of the gastrointestinal tract, the disease process may progress to involve the serosal surface of the intestine and/or other abdominal structures resulting in peritonitis and, consequently, non-specific increases in inflammatory cells and protein content in abdominal fluid samples. This progression has been reported to occur in association with EGE (Dyson, 1983), abdominal neoplasia (Mair & Hillyer, 1991; Olsen, 1992) and non-strangulating intestinal infarction due to *Strongylus vulgaris* infection (Dyson, 1983) and cyathostomosis (Mair & Pearson, 1995).

1.4.9 Intestinal function tests

Although the findings of investigative techniques detailed above may provide a diagnosis in certain cases of chronic gastrointestinal disease, they are generally non-specific and provide no/little information regarding gastrointestinal function. For this purpose a variety of

techniques have been developed to assess specific functions such as small intestinal absorptive capacity, mucosal integrity and intestinal transit.

1.4.9.1 Carbohydrate absorption studies

Four carbohydrate absorption tests have been developed for assessment of small intestinal function in the horse: the starch tolerance test (Loeb, McKenzie & Hoffsis, 1972), the OGTT (Roberts & Hill, 1973), the oral xylose tolerance test (Roberts, 1974) and the oral lactose tolerance test (Breukink, 1974; Roberts, 1975). Although the lactose tolerance test may allow detection of lactase deficiency/small intestinal mucosal damage in diarrhoeic foals, it is recognised that as horses mature they lose the ability to hydrolyse lactose (Breukink, 1974: Roberts, 1975) and therefore this test of no diagnostic relevance in the adult equine animal. Of the remainder, the OGTT is the most frequently employed for assessing small intestine absorptive capacity. Following the administration of anhydrous glucose (1 g/kg bwt, 20 % w/v solution) to healthy animals, plasma glucose concentrations increase by approximately 100 % above basal glucose concentrations (BGC) and subsequently decline to pretest values within the six hour sampling period (Roberts & Hill, 1973).

Although the test is simple to perform and assay, repeat blood sampling over a six hour period is often impractical in a practice situation. In addition, numerous extraintestinal factors are known to influence plasma glucose response following oral glucose loading (Roberts, 1985) and only limited information is available with regard to the reliability of the OGTT to detect small intestinal pathology. Mair, Hillyer, Taylor & Pearson (1991) reported that a 'total' malabsorption (defined as tests where plasma glucose concentration at 60 and 120 minutes (min) showed a lower than 15 % increase above BGC) was 100 % specific for detecting small intestinal pathology; however, a report published at roughly the same time questioned the ability of the OGTT to differentiate between large and small intestinal disease processes based on the fact that several horses with confirmed cyathostomosis exhibited carbohydrate malabsorption (Love, Mair & Hillyer, 1992).

1.4.9.2 Intestinal permeability tests

Increased intestinal permeability is a well recognised feature of numerous gastrointestinal diseases in man and the dog. Although it is generally accepted that increased intestinal permeability is secondary to a disruption of mucosal integrity, it has also been hypothesised that a primary permeability disorder could permit passage of intraluminal molecules across

the intestinal mucosa and thereby initiate a pathological process, possibly by an immune mediated mechanism (Bjarnason, Peters & Veall, 1983). Therefore, techniques which are used to assess intestinal barrier function serve not only as indicators of intestinal disease but may also advance the understanding of the pathogenesis of certain intestinal disorders.

Intestinal permeability is assessed non-invasively by measuring urinary excretion of orally administered test substrates. The desired physicochemical properties of a marker molecule include water solubility, first-order kinetics of permeation, non-toxic, non-degradable, and that it remains in extracellular compartments and is not metabolised before, during or after permeating the intestine. In addition, it is preferable that the marker molecule(s) is not naturally present in urine and that its measurement is easy, sensitive and accurate (Bjarnason, MacPherson & Hollander, 1995). The marker molecules used in intestinal permeability studies in human gastroenterology include lactulose, L-rhamnose, mannitol, polyethylene glycol, ^{51}Chromium-ethylenediaminetetraacetic (^{51}Cr-EDTA) and ^{99m}Technetium-diethylenetriaminopentaacetate (^{99m}Tc-DTPA) (Bjarnason, MacPherson & Hollander, 1995). In human gastroenterology, it is now generally accepted that measurement of permeability to a single marker molecule may be influenced by a variety of factors, apart from intestinal permeability itself, including rate of gastric emptying, small intestinal transit, renal excretion and sampling technique. This has led to formulation of the principle of dual probe permeability studies, where differential urinary excretion of two test substrates (e.g. lactulose and L-rhamnose) is calculated, eliminating the potential for error and providing a specific index of intestinal permeability. Indeed, it is possible to extend this principle further and devise ways of assessing specific intestinal functions. For example, simultaneous oral administration of lactulose and ^{51}Cr-EDTA has been used to distinguish between small intestinal and colonic disease in man (Jenkins, Nukajam, Menzies & Creamer, 1992). This is based on the principle that increased urinary excretion of both marker molecules indicates small intestinal permeability, whereas increased urinary excretion of ^{51}Cr-EDTA (which is not degraded by colonic bacteria) in the absence of a similar increase in urinary excretion of lactulose (which is rapidly fermented by colonic bacteria) provides an index of colonic permeability.

In veterinary medicine, assessment of intestinal permeability using a single marker molecule (^{51}Cr-EDTA) has been applied to the investigation of experimental parasite infections of gerbils (Sinski, MacLean & Holmes, 1987), rats (Ramage, Stanisz, Scicchitano, Hunt & Perdue, 1988), lambs (MacLean, Sinski & Holmes, 1989) and ponies (Love, 1990; Love *et al.*, 1991). Hall & Batt (1990) demonstrated that this technique

reliably detected small intestinal disease in the dog and could be a useful means of monitoring the efficacy of treatment. Love (1990) investigated the suitability of ^{51}Cr-EDTA as a test of intestinal permeability in ponies and applied this technique to monitor the pathogenic effects of experimental cyathostome infection. A significant difference in the urinary excretion of ^{51}Cr-EDTA between control and infected animals was not detected and it was concluded that the integrity of the intestinal mucosa was not compromised by cyathostome infection (Love, 1990). However, further work on experimental cyathostome infections demonstrated an increase in urinary excretion of ^{51}Cr-EDTA (i.e. increased intestinal permeability) following experimental infection of ponies with 11.5 million cyathostome larvae (Love *et al.*, 1991).

Application of these techniques to the investigation of clinical disease in the horse has not been reported, but further work in this area may assist the detection and understanding of chronic equine enteropathies.

Hypoalbuminaemia/hypoproteinaemia is commonly recorded in association with numerous causes of chronic enteropathy in the horse. Although it is considered to be the result of a protein 'leak' through mucosa of increased permeability, absolute confirmation of protein losing enteropathy requires demonstration of the loss of plasma protein into the gastrointestinal tract. This can be shown by the use of radiolabelled tracer techniques. For this purpose, the use of different radioisotopes has been described, but ^{51}Cr is the label of choice because it is not degraded and is not reabsorbed from the intestinal lumen (Love, 1990). This isotope can be injected intravenously as $^{51}CrCl_3$ after which it labels, *in vivo*, endogenous plasma proteins, particularly plasma albumin. By dividing the total ^{51}Cr radioactivity counted in 24 hour faecal collections by the activity per ml of plasma at the start of the collection period, it is possible to calculate the faecal clearance of the plasma and so the rate of plasma protein loss into the intestine (Love, 1990). A variety of techniques, using this principle, have been used in the horse to investigate altered protein metabolism/enteric protein loss in cases of inflammatory bowel disease (Merritt, Cimprich & Beech, 1976; Merritt, Kohn, Ramberg, Cimprich, Reid & Bolton, 1977; Meuten *et al.*, 1978b), phenylbutazone toxicity (Snow *et al.*, 1981) and strongyle infections (Duncan & Dargie, 1975; Love, 1990). Similarly, radiolabelled tracer techniques have also been used to study red blood cell metabolism/blood loss into the gastrointestinal tract in experimental parasitic infections (Duncan & Dargie, 1975; Clayton, Duncan & Dargie, 1980; Love, 1990).

1.4.9.3 Breath hydrogen tests

Breath hydrogen measurement is routinely used in human medicine to investigate gastrointestinal function. The technique is based on the fact that, when carbohydrate comes in contact with bacteria in the gastrointestinal tract, it is fermented and hydrogen is produced as a by-product of that process. A proportion of this hydrogen diffuses from the intestinal lumen into the portal circulation and is subsequently exhaled in breath. Because relatively few bacteria are present in the stomach and small intestine of healthy humans, hydrogen excreted in breath originates, almost entirely, from the large intestine (Levitt, 1969). Based on this knowledge, breath hydrogen measurement has been applied successfully to the clinical investigation of small intestinal carbohydrate malabsorption (Levitt & Donaldson, 1970; Bond & Levitt, 1976), small intestinal bacterial overgrowth (Metz, Gassull, Drasar, Jenkins & Blendis, 1976a) and for assessment of mouth-to-caecum transit time (Bond & Levitt, 1975). Breath hydrogen measurement is non-invasive, safe, simple and relatively inexpensive to perform and, for these reasons, is an attractive alternative to other commonly used methods for the investigation of gastrointestinal function. Recently, the technique has been applied to the clinical investigation of gastrointestinal disorders in calves (Holland, Herdt & Refsal, 1986, 1989), dogs (Washabau, Strombeck, Buffington & Harrold, 1986a) and cats (Muir, Papasouliotis, Gruffydd-Jones, Cripps & Harbour, 1991).

To date, little is known about hydrogen production in the gastrointestinal tract and the patterns of breath hydrogen excretion in equidae. Zentek (1992) reported that hydrogen was excreted in the breath of adult horses following the ingestion of either hay and/or oats and Bracher, Ousey, Fazeli, Murgatroyd & Rossdale (1995) used the technique to evaluate developmental changes in alimentary tract function of young foals. In addition, Murphy, Howie & Love (1994) reported significant and transient increases in breath hydrogen concentration which was assumed to be a consequence of gastric fermentation, one hour after the administration of xylose and lactulose to a horse with pyloric stenosis. Although the breath hydrogen test has not yet been fully validated for use in the horse, these reports indicate that further studies into the diagnostic potential of this technique in equine gastroenterology are justified.

1.4.10 Diagnostic imaging

1.4.10.1 Ultrasonography

Ultrasonography, using low frequency (2 - 3.5 MHz) linear array or sector scanners, has become an important diagnostic aid in the investigation of internal disorders in the horse (Byars & Halley, 1986) and has proved useful in the diagnosis of chronic alimentary tract disease, in particular, neoplasia (Hillyer, 1994). Hillyer (1994) indicated that, while random abdominal ultrasonography occasionally reveals a specific abnormality, it is usually unrewarding. Therefore, this technique should only be employed when indicated on the basis of either clinical and/or rectal examinations.

Abdominal neoplasia can be expected to alter the size, shape, position and internal architecture of an affected abdominal organ. However, occasionally specific organ involvement may not be detected by ultrasonography and there may only be evidence of the secondary effects of abdominal neoplasia, such as displacement of other organs from their normal anatomical position or changes to the volume or echogenicity of peritoneal fluid (Hillyer, 1994).

In the normal horse, the greater curvature of the stomach can be visualised by ultrasound on the left side of the cranial abdomen, in close apposition to the spleen (Rantanen, 1986). Ultrasonographic recognition of thickening and abnormal echogenicity of the stomach wall is consistent with a diagnosis of gastric squamous cell carcinoma (Traub, Bayly, Reed, Modransky & Rantanen, 1983; Rantanen, 1986). In addition, increased peritoneal fluid volume and/or splenic displacement and/or metastatic spread to other structures including the liver, spleen, intestines, omentum or diaphragm, may occur in association with this neoplasm.

Normal intestine is difficult to identify and evaluate ultrasonographically. However, Byars & Halley (1986) indicated that gross thickening of the intestinal wall may be a recognisable feature in alimentary lymphosarcoma. In addition, ultrasonography was considered to be a useful tool in the evaluation of a case of intestinal adenocarcinoma, but only the secondary effects of the neoplasm (increased peritoneal fluid volume and metastatic spread to the liver and diaphragm) were identified (Traub *et al.*, 1983).

Although these reports confirm that ultrasonography may be useful in the investigation of animals with gastrointestinal neoplasia, the definitive diagnosis in these cases must still be considered dependant on the cytological appearance of peritoneal fluid or histopathological examination of tissue samples. In this regard, ultrasonography may play a role in certain cases because ultrasound-guided paracentesis or needle biopsy may facilitate the collection

of required samples (Modransky, 1986). However, ultrasound-guided biopsy is only likely to be feasible where lesions are in apposition/adhered to the abdominal wall. Although information is sparse with regard to the benefits of ultrasonography when investigating other than neoplastic causes of gastrointestinal tract disease, excessive peritoneal fluid and caudal displacement of the spleen have been recorded in a case of gastric distension due to pyloric stenosis (Murphy, Howie & Love, 1994).

1.4.10.2 Endoscopy

Ante mortem diagnosis of gastric disease in the adult horse is difficult due to non-specific clinical features and the lack of specific diagnostic techniques to evaluate the equine stomach. Fibreoptic endoscopic examination of the stomach and small intestine is a well established, valuable diagnostic aid in human, small animal and foal medicine, but the length of commonly available endoscopes (usually less than two metres) limits the use of this technique in mature horses. In addition, although several authors have described the diagnosis of gastric squamous cell carcinoma based on endoscopic visualisation via a midcervical eosophagostomy (Keirn, White, King & Tennant, 1982; Fischer, Kent Lloyd, Carlson & Madigan, 1986), this is an invasive procedure and carries a high risk of complications, in particular wound dehiscence.

Where instruments of sufficient length are available (i.e. 275-300 cm), conventional endoscopy, following a 24-48 hour fast, allows easy visualisation of gastric structures in the mature horse and has aided the diagnosis of gastric ulceration, gastric parasitism, pyloric stenosis and gastric neoplasia (Brown, Slocombe & Derksen, 1985; Murray, 1992a). In addition, Brown, Slocombe & Derksen (1985) described the use of such an instrument to perform duodenoscopy and obtain duodenal mucosal biopsies and suggested that this technique could be a valuable, relatively non-invasive tool for the investigation/diagnosis of chronic small intestinal disease.

1.4.10.3 Radiography

Radiography was proposed by Dik & Kalsbeek (1985) as a valuable tool in the diagnosis of gastric disease in the horse. These authors reported that pneumogastrography enabled visualisation of gastric tumours and gastric parasitism (Dik & Kalsbeek, 1985). Similarly, contrast radiography has been reported to be of use for the diagnosis of gastric retention secondary to pyloric stenosis/mass (McGill & Bolton, 1984; Church, Baker & May, 1986). In addition to its diagnostic potential in animals with chronic gastric disease, Bertone *et al.*

(1988) indicated that abdominal radiography may be diagnostic in animals with sand enteropathy: In four animals, faecal evaluation for sand was negative, but all horses had radiographic evidence of apparently large quantities of sand in the ventral and/or dorsal colons.

Although the examinations described for radiographic investigation of the gastrointestinal tract are simple, the technique requires powerful radiographic equipment and experienced interpretation, both of which will limit the availability of this technique.

1.4.10.4 Scintigraphy

In man, abdominal scintigraphy using radiolabelled leucocytes has been used successfully for the diagnosis of abdominal abscessation (Weldon, Joseph, French, Saverymuttu & Maxwell, 1995) and for the diagnosis and assessment of disease extent in inflammatory bowel disease (Weldon, Masoomi, Britten, Gane, Finlayson, Joseph & Maxwell, 1995). The technique is based on the fact that neutrophils migrate to the site of inflammation and increased uptake of the radiolabelled marker is detected by means of gamma camera imaging. Thakur, Coleman, Mayhall & Welch (1976) first described the use of ^{111}Indium-labelled autologous leucocytes to localise abscesses in dogs and this work was subsequently performed on human patients (Segal, Thakur, Arnot & Lavender, 1976). These studies, using ^{111}Indium (^{111}In), indicated that intense, focal increasing activity on serial images up to 24 hours was diagnostic of an abscess due to continued recruitment of labelled cells (Segal *et al.*, 1976; Thakur *et al.*, 1976). On the other hand, bowel inflammation results in rapid uptake and decreasing activity with time due to shedding of labelled cells into the bowel lumen and distal transit.

While abdominal scintigraphy is recognised as being a simple and safe diagnostic tool in human medicine, diagnosis/interpretation tends to be subjective and may be complicated by overlapping activity in other organs such as bone marrow, liver and spleen, which is a normal phenomenon. Therefore, experienced interpretation of scintigrams is essential in order for this technique to be accurate. Information with regard to the use of scintigraphy for the investigation of abdominal disorders in the horse is limited. Koblik, Lofstedt, Jakowski & Johnson (1985) described the use of abdominal scintigraphy as a diagnostic aid in a horse with chronic colic and an abdominal mass palpable *per rectum*: Forty-five hours after the intravenous administration of ^{111}In labelled leucocytes, a localised area of increased radioactivity was recognisable in the same region as the palpated mass. Similar localisation of radioactivity was not detected in scintigrams obtained from two clinically normal horses

(Koblik *et al.*, 1985), providing further evidence that the focal area identified in the horse represented leucocyte migration into an abscess.

To date, the ability of abdominal scintigraphy to diagnose CIBD in the horse has been evaluated in only a small number of clinical cases: While the procedure was a technical success, the investigator found that accurate interpretation of test results was often difficult (Celia Marr, personal communication). However, based on the successful application of this technique to the diagnosis/assessment of inflammatory bowel disease in man and an increase in the availability of gamma cameras in equine hospitals, further research into the potential of this technique for detecting chronic gastrointestinal disorders in the horse is justified.

1.4.10.5 Laparoscopy

Despite its widespread acceptance in human medicine, reports which document the use of laparoscopy in the adult horse are limited. While earlier reports provided information on laparoscopic technique and basic anatomical landmarks (Witherspoon, Kraemer & Seager, 1980; Fischer *et al.*, 1986), Galuppo, Snyder & Pascoe (1995) and Galuppo, Snyder, Pascoe, Stover & Morgan (1996) were first to provide a comprehensive description of the normal laparoscopic anatomy of the abdomen in the horse. The primary indications for laparoscopic examination of the equine abdomen is in clinical cases with non-specific colic and/or suspected abdominal neoplasia/abcessation. To date, laparoscopy has proved useful in the diagnosis of gastric squamous cell carcinoma (Fischer *et al.*, 1986) and intestinal adenocarcinoma (Fulton, Brown & Yamini, 1990). The disadvantages of the technique are that an extensive knowledge of anatomical orientation is required and lesions, if present, may remain undetected, for example, when situated in the ventral abdomen. In addition, in order to enhance visualisation of abdominal structures, it is recommended that animals be fasted for 24-36 hours before examination to reduce the bulk of ingesta and to minimise gaseous distension of the bowel (Fischer *et al.*, 1986; Galuppo, Snyder & Pascoe, 1995). Furthermore, inadvertent viscus rupture (Fischer *et al.*, 1986) and subcutaneous emphysema (Galuppo, Snyder & Pascoe, 1995) are possible complications when performing this investigation. However, compared with exploratory laparotomy, wound healing following a laproscopic procedure is rapid and post-operative care is minimal. These advantages outweigh the potential disadvantages and it is likely that, as instrumentation and surgical experience continue to develop, more abdominal procedures will be performed via laparoscopy.

1.4.11 Intestinal biopsy

Given the relatively low ante mortem diagnostic rate reported in a review of 51 cases of chronic diarrhoea and the fact that a diagnosis was achieved in 22 of these animals at post mortem examination, the authors suggested a potentially valuable role for intestinal biopsy in the clinical evaluation of such cases (Love, Mair & Hillyer, 1992). It is generally regarded that biopsy features should allow detection and characterisation of, at least, the unequivocal, severe inflammatory diseases, should distinguish inflammation from neoplasia, or should identify when the mucosal histology falls within the broad range of normal. In most cases, obtaining diagnostic intestinal biopsies necessitates exploratory laparotomy which may be of limited practical value because of financial considerations and the increased risks associated with abdominal surgery in debilitated and/or hypoalbuminaemic animals. However, in conditions where there is extensive pathological change in the large intestine, rectal biopsy will, occasionally, provide histopathological evidence of a specific diagnosis. Despite the fact that numerous authors have advocated the use of rectal biopsy as a diagnostic tool (Merritt, Cimprich & Beech, 1976; Meuten *et al.*, 1978a; Traver & Thacker, 1978; Gibson & Alders, 1987), there are relatively few studies which evaluate the diagnostic potential of this technique when investigating cases of chronic intestinal disease in the horse. Of the 51 cases of chronic diarrhoea investigated by Love, Mair & Hillyer (1992), rectal biopsies had been obtained from 27 and histopathological abnormalities were identified in specimens from 13 of these animals. However, the usefulness of rectal biopsy in the evaluation of these cases was generally disappointing in that two biopsies contained cyathostome larvae and 11 had non-specific mixed inflammatory cell infiltrates. No histopathological evidence of neoplasia was detected in rectal biopsies from three cases of alimentary lymphosarcoma (Love, Mair & Hillyer, 1992). Lindberg, Nygren & Persson (1996) provided the first comprehensive study of rectal biopsy diagnoses in horses with clinical signs of gastrointestinal disease. In the same paper, these authors described, in detail, the histopathological appearance of the mucosa and submucosa of the rectum in clinically normal horses. They noted that attaching clinical significance to histopathological features in animals with clinical disease can be difficult because of the subjective nature of biopsy interpretation and the individual variation evident in the microscopic appearance of rectal tissue from clinically normal horses. Therefore, it was suggested that reference to well defined controls is important in order to overcome diagnostic difficulties. Compared to rectal tissue from clinically normal horses, histopathological examination of biopsy specimens from 116 horses presented for investigation of enteric disease allowed detection

of pathological changes in specimens from 60 horses. Although the majority of specimens were classified as having non-specific inflammatory changes, histopathological features were occasionally indicative of a specific disease entity. When correlated with post mortem findings in 40 horses, it was shown that rectal biopsy specimens permitted a definitive diagnosis in 6 of 12 cases of EGE, 4 of 9 cases of GE and 1 of 7 cases of alimentary lymphosarcoma. These results suggest that, despite its limitations, rectal biopsy is a useful adjunct for the investigation of chronic intestinal disease in the horse.

Proctoscopy may be helpful in evaluating involvement of rectal mucosa and is easily accomplished using a tube speculum (Merritt, Cimprich & Beech, 1976). However, Lindberg *et al.* (1985) reported that diagnostic histopathological changes may be present in rectal mucosal tissue even though the rectum appears grossly normal.

1.5 Summary and Project Aims

Reaching a definitive diagnosis in cases of equine chronic gastrointestinal disease is often difficult because, regardless of the aetiology, animals often present with similar historical and clinical features. In addition, although many tests are available/advocated for the investigation of such cases, an extensive investigative 'work-up' may fail to differentiate between disease entities, thereby precluding a rational approach to therapy or management. Factors which contribute to the difficulties of diagnosis are numerous and include i) a paucity of reports which detail the relative prevalence of the different gastrointestinal diseases and their associated clinical and clinicopathological features, ii) a limited knowledge of the pathogenesis of most chronic gastrointestinal diseases, and iii) few specific diagnostic tests.

The present study was carried out against this background with the overall aim of improving objective assessment of cases of equine chronic enteropathies; The specific aims were as follows,

- To review the historical, clinical and clinicopathological features of confirmed cases of equine chronic gastro-/enteropathies, presented to a referral clinic, with a view to highlighting possible features which may help differentiate between disease entities.
- To validate a fructosamine assay for use with equine serum or plasma.
- To evaluate the use of fructosamine measurement to monitor alterations in protein metabolism in ponies experimentally infected with cyathostome larvae.
- To evaluate the effect of some extraintestinal factors on the oral glucose tolerance test in healthy ponies.
- To assess the ability of a 'modified' oral glucose tolerance test to detect/exclude a diagnosis of small intestinal pathology.
- To validate the technique of breath hydrogen measurement for use as a diagnostic/research tool for the investigation of pathological and/or physiological alterations in gastrointestinal tract function.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Animals

2.1.1 Clinical material

The principal case material included in Chapter 3 and Chapter 5 comprised a series of horses referred by veterinary practitioners to the University of Glasgow Veterinary School for investigation of chronic weight loss and/or chronic diarrhoea and/or chronic colic. Using the Jarrogate data-base system (University of Glasgow), each horse was assigned a case number with the inclusion of date of presentation, breed, sex, age and owner details. Relevant historical details were recorded and a full physical examination was carried out. In all cases, blood was obtained by jugular venepuncture. The blood was collected into potassium ethylenediaminetetraacetic acid (EDTA) tubes (Vacutainer®, Becton Dickinson) for haematological examinations and lithium heparin tubes (Vacutainer®, Becton Dickinson) for biochemical analysis. Haematological and blood biochemical analyses were performed on the same day as blood collection. All or a number of the following diagnostic procedures were performed on each animal; faecal parasitological analysis, peritoneal fluid analysis, SPE, an OGTT and rectal mucosal biopsy. A definitive diagnosis was reached in all cases either based on historical, clinical and clinicopathological findings and/or post mortem examination.

2.1.2 Experimental animals

A total of 25 British native-breed ponies were used in studies on fructosamine measurement, OGTTs and breath hydrogen measurement (**Table 2.1**).

A foal/yearling group was comprised of 17 animals (7 male, 10 female) aged between 6 and 18 months when included in the studies. These ponies were reared indoors with their dams. Following weaning at 4 months of age, eight animals (Pony Nos. 114 to 121, inclusive) were maintained on a hay and coarse mix (Spillers® Original Mix, Spillers Horse Feeds) diet and bedded on straw, whereas the other nine ponies (Pony Nos. 101 to 106, inclusive and 111, 112 and 113) received a commercially-produced pelleted ration (Spillers® High-Fibre Cubes, Spillers Horse Feeds) and were bedded on wood-shavings.

An adult group, comprising of eight ponies (1 male, 7 female), were aged between 5 and 13 years when included in the studies. Three of this group (Pony Nos. 122, 126 and 129) were reared indoors and had been stabled since birth, whereas the other five (Pony Nos. 123,

124, 125, 127 and 128) had been maintained at grass. All individuals in this group had been stabled for at least one month prior to inclusion in any investigations reported in this thesis. The management of animals in this group varied depending on the particular study: They were either maintained on a hay only diet and bedded on straw or maintained on a commercially-produced pelleted ration (Spillers® High-Fibre Cubes, Spillers Horse Feeds) and bedded on wood-shavings.

Study	2	3	4	6	7	8	9
Pony No.							
101	X	X	X				
102	X	X	X				
103	X	X	X				
104	X	X					
105	X	X	X				
106	X	X	X				
111	X	X					
112	X	X	X				
113	X	X	X				
114	X						X
115	X						X
116	X						X
117	X						X
118	X						X
119	X						X
120	X						
121	X						
122						X	
123	X		X	X	X		X
124	X		X	X	X		X
125	X		X	X	X		X
126	X		X	X	X	X	
127	X		X	X	X		X
128	X		X	X	X		X
129	X		X	X	X	X	X

Table 2.1: Experimental animals used in individual studies; **2** = fructosamine assay validation, **3** = alterations in plasma fructosamine concentration following experimental cyathostome infection, **4** = effect of age and diet on the oral glucose tolerance test, **6** = breath hydrogen excretion following ingestion/administration of a variety of test meals, **7** = effect of diet on breath hydrogen excretion patterns, **8** = effect of cisapride and codeine phosphate on breath hydrogen excretion patterns, **9** = pharmacokenetics of lactulose in ponies.

Prior to inclusion in these studies, all animals were considered to be healthy based on clinical examinations. All experimental ponies were housed in individual loose boxes which were cleaned out daily. Animals were fed twice daily at 08:00 and 16:00 hours and had *ad libitum* access to water. They were regularly turned out to exercise in concrete yards.

2.2 Investigative Techniques

2.2.1 Haematological analysis

A complete blood count was obtained using an ABX Minos ST Vet (ABX) haematology analyser. Isotonic saline was used as a diluent and potassium cyanide as a lysing agent as described by the manufacturer. The parameters evaluated included red and white cell and platelet counts, haemaglobin concentration and haematocrit. For the differential white cell count, blood films were fixed and stained with a standard May-Grunwald Giemsa stain, and 200 cells counted under oil immersion microscopy.

2.2.2 Biochemical analyses

Plasma concentrations of urea, creatinine, bilirubin, AP, GGT, glutamyl dehydrogenase (GLDH), albumin, total protein, sodium, potassium, chloride, calcium and magnesium were measured on discrete clinical chemistry analysers. The commercial reagent kits, manufacturers and their methods are listed in **Table 2.2**. Plasma globulin concentrations were estimated by subtraction of albumin from total protein concentrations.

Quality assurance was guaranteed using commercially available control sera (Pathonorm™ H, Nycomed). Quality control was monitored by the Scottish Quality Assessment Scheme and UKEQAS for general clinical chemistry.

Fructosamine measurement in equine serum and plasma was performed as outlined in Chapter 4.

2.2.3 Oral glucose tolerance test

Following a 14-16 hour fast, 1g anhydrous glucose (Sigma-Aldrich Company Ltd) per kg bodyweight (bwt) was administered, as a 20% w/v warm solution, by stomach tube (Roberts & Hill, 1973). Blood, obtained by jugular venepuncture at zero min (i.e. pre-test) and at times 30, 60, 90, 120, 150, 180, 240, 300 and 360 min, was collected into fluoride oxalate glass vacutainers (Vacutainer®, Becton Dickinson). Animals under test had access to water at all times and access to food was denied until the end of the sampling period. All samples were analysed on the day of collection for glucose concentration.

Parameter	Reagent Kit	Method
Albumin[1]	T01-1377-A4, Bayer Diagnostics	Bromocresol green method
AP[1]	T01-3349-A4, Bayer Diagnostics	Enzymatic colorimetric test with p-nitrophenyl phosphate using DEA buffer
Bilirubin[1]	T01-1963-A4, Bayer Diagnostics	Colorimetric test using a blanked diazo
Calcium[1]	T01-1476-A8, Bayer Diagnostics	Colorimetric test using cresolphthalein
Chloride[1]	T01-1474-A4, Bayer Diagnostics	Colorimetric test using mercuric thiocyanate
Creatinine[1]	T01-1927-A4, Bayer Diagnostics	Colorimetric test with alkaline picrate
GGT[1]	T01-1916-A4, Bayer Diagnostics	Enzymatic colorimetric test using γ-glutamyl-p-nitroanalide
GLDH[2]	GL441, Randox Laboratories	Enzymatic colorimetric test using α–oxogluterate
Glucose[1]	T01-1833-A4, Bayer Diagnostics	Colorimetric test using the combined hexokinase and glucose-6-dehydrogenase method
Magnesium[1]	T01-2878-02, Bayer Diagnostics	Colorimetric test using xylidyl blue
Potassium[1]	-	Indirect ion-selective electrode
Sodium[1]	-	Indirect ion-selective electrode
Total protein[1]	T01-1301-A4, Bayer Diagnostics	Modified Biuret method
Urea[1]	T01 1823-A4, Bayer Diagnostics	Enzymatic UV test with urease and glutamate-dehydrogenase

Table 2.2: Commercial reagent kits, manufacturers and methods used for analysing biochemical parameters on the Technicon AXON (Bayer Diagnostics)[1] and Cobas MIRA (Roche)[2] clinical chemistry analysers. DEA = Diethanolamine, UV = ultraviolet.

2.2.4 Breath tests

2.2.4.1 Protocol and breath collection

Following an overnight fast (14-16 hours), the pattern of hydrogen excretion in the breath of animals under test was determined after the administration/ingestion of a test meal/substrate, the specific details of which are outlined in the relevant sections. End-expiratory breath samples were collected, in duplicate, at zero minutes (0 min) and at regular intervals thereafter for either 8 or 10 hours. Breath samples were collected using a plastic tube (nasal tube) (40 cm long, internal diameter 12 mm) with a valve attached at one end, two centimeters from which was situated a side exit port (8FG Dog Catheter, Rocket of London) (**Fig. 2.1**). For breath collection, the nasal tube was inserted through the nostril into the ventral nasal meatus of the pony (**Fig. 2.2**). The animal was allowed to breath through the tube on a number of occasions and the valve at its distal end served as an indicator of the breathing pattern, closing during inspiration and opening during expiration. Samples of breath were collected towards the end of expiration by aspirating, from the side exit port, into a 20 ml syringe (Plastipak, Becton Dickinson), via a three-way tap (Rocket of London). The breath samples were sealed in the syringes by closing the three-way tap and were stored in this manner at room temperature.

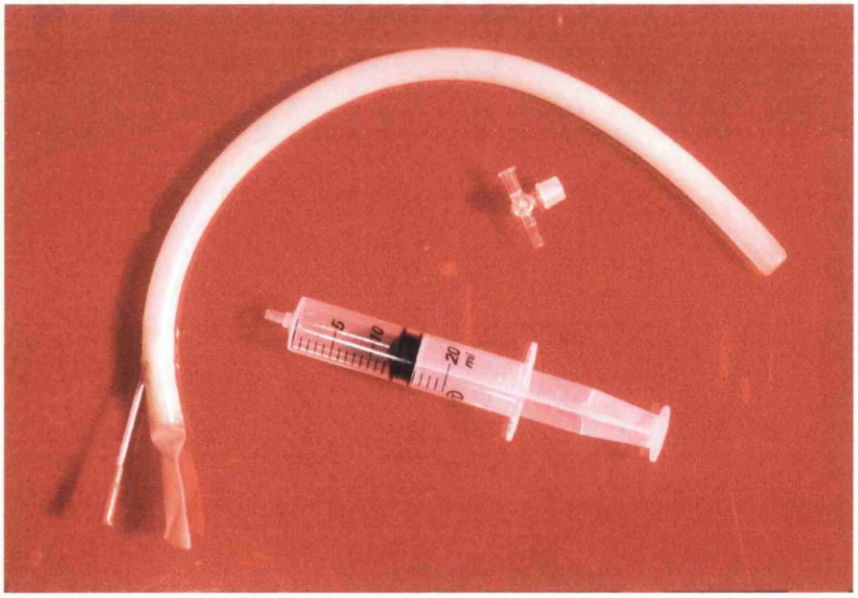


Fig. 2.1: Equipment for breath collection

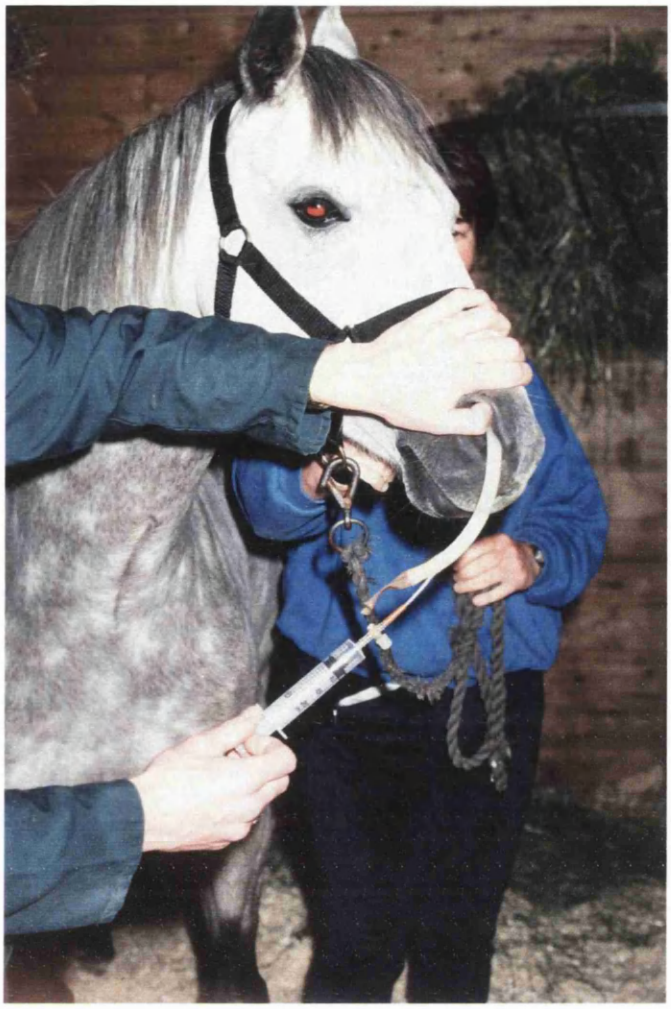


Fig. 2.2: Breath collection procedure

2.2.4.2 Breath hydrogen analysis

All samples were analysed within 30 minutes of collection using an Exhaled Hydrogen Monitor (GMI Medical Ltd) (**Fig. 2.3**). This instrument measures hydrogen concentration by means of a sensitive, selective electrochemical cell. This consists of a metallised membrane working electrode, which is held at a constant potential with reference to a reference electrode. Hydrogen diffuses through the metallised membrane and yields electrons to the electrode. This generates an electric current, the magnitude of which is proportional to the partial pressure of hydrogen, which can be read in parts per million (ppm) on a digital display. With this instrument, it is possible to obtain a direct measurement of breath hydrogen concentration within 10 seconds. The response of the electrode is reproducible and linear over a range from 1 to 250 ppm, can detect changes in hydrogen concentration of as little as 1 ppm and the method correlates well with hydrogen measurement by thermal conductivity detectors. Although the electrodes are highly

selective for hydrogen they also respond to changes in the oxygen and carbon monoxide tension in expired air. However, the differences of oxygen and carbon monoxide normally encountered in expired air should not account for more than 3 ppm in the output of the instrument (Corbett, Thomas, Read, Hobson, Bergman & Hodsworth, 1981).

Fig. 2.3: Exhaled Hydrogen Monitor (GMI Medical Ltd).

2.3 Data Organisation and Statistical Analysis

Data organisation and presentation were facilitated by use of a number of computer software packages including: Microsoft Word for Windows 95 version 7.0, Microsoft Excel for Windows 95 version 7.0 and Microsoft Powerpoint for Windows 95 version 7.0 (Microsoft Corporation) and Harvard Graphics 2.0 for Windows (Software Publishing Corporation).

Statistical analyses were performed using Microsoft Excel for Windows 95 version 7.0 (Microsoft Corporation) and MINITAB for Windows, release 9 (Minitab Incorporated).

CHAPTER 3

CHRONIC GASTROINTESTINAL DISEASE IN THE HORSE: HISTORICAL, CLINICAL AND CLINICOPATHOLOGICAL FEATURES OF 29 CASES

3.1 Introduction

The known causes of chronic gastrointestinal disease in the horse are both numerous and diverse and, as summarised within Chapter 1, many reports have been published which document the clinical, clinicopathological and pathological features of specific disease entities. Surprisingly, there are relatively few reports which provide details about series of such cases, therefore there is only limited information available on the relative prevalence of these conditions and the relative occurrence of clinical features which may help differentiate between them.

STUDY 1

3.2 Aim of Study

The aim of the study was to review historical, clinical and clinicopathological features of 29 confirmed cases of chronic gastro-/enteropathy in the horse, with a view to highlighting possible features which may aid diagnosis.

3.3 Materials and Methods

3.3.1 Control population

In order to determine whether either breed and/or age and/or gender were important factors predisposing to the development of specific chronic diseases of the gastrointestinal tract, it was necessary to generate a control population with which comparisons could be made. This control population was established by selecting all new equine cases (excluding confirmed cases of chronic gastrointestinal disease) presented to the University of Glasgow Veterinary School between 3/1/96 and 3/1/97 and, for this population, the overall breed/type distribution, age distribution and gender distribution were determined. Because of the variety of breed/type categories and the potential for overlap between categories, these descriptions were further simplified to record either horse, pony or donkey. Subsequently, it was necessary to determine if the control population was uniform, with respect to age, breed/type and gender, across referral groups (i.e. medical referrals versus surgery (orthopaedic) referrals) or whether referral groups were sources of bias in the control population. Using Yate's corrected Chi-square analysis (r x c contingency tables), both referral groups were investigated for differences in age, breed/type and gender

distribution: No significant differences were found and it was concluded that the overall group data were not markedly biased by a particular referral group.

Where appropriate, this same method of statistical analysis was used to test for associations between the presence of a factor (e.g. age <5 years) and a disease classification (e.g. cyathostomosis). Two-by-two contingency tables were used to test for the effects of either gender (i.e. either female or male (including both entire and gelded)) or type (i.e. horse or pony). Similarly, 2 x 2 contingency tables were used to test for an association between age and a particular disease. Age was divided into the categories 0-5, 6-10, 11-15 and >15 years and depending on the disease being investigated different age categories were collapsed: For example, animals may have been grouped as i) either 0-5 years or >5 years or ii) either 0-10 years or >10 years. Significance levels of less than 5 % were considered statistically significant.

3.3.2 Chronic gastro-/enteropathies

The principal clinical cases included in this study were adult (> one year old) horses and ponies presented for investigation of weight loss and/or diarrhoea and/or colic (minimum duration 7 days). A definitive diagnosis of a primary gastrointestinal disease was reached in all cases on the basis of ante mortem clinicopathological findings and/or necropsy findings.

Detailed clinical histories were obtained from the owner of each animal and a full clinical examination was performed at admission, then at least daily thereafter. In all cases, blood samples were submitted on one or several occasions for haematological and biochemical analyses. Faeces from all animals were examined for worm eggs by the modified McMaster technique and samples diluted in water were examined under the microscope for parasitic larvae. In addition, faeces from eight animals were submitted for bacteriological examination. Serum protein electrophoresis (Blackmore, Henley & Mapp, 1983) was performed on blood obtained from 15 cases. Peritoneal fluid, obtained by abdominocentesis from 20 animals, was submitted for cytological and biochemical analysis and an OGTT was performed on 20 animals. Rectal biopsy samples were obtained, by means of a sow uterine biopsy instrument, from 16 animals and submitted, in 10 % formalised saline, for histopathological examination. Multiple intestinal biopsies were obtained, via laparotomy, from one individual.

The horses were either discharged following full recovery or euthanased on humane grounds or at the owners' requests. One case died naturally. Full post mortem and histopathological examinations were performed on 23 animals.

3.4 Results

3.4.1 Control population

The total number of animals presented, for the first time, to the University of Glasgow Veterinary School for the period 3/1/96 to 3/1/97 was 290 (excluding confirmed cases of chronic gastrointestinal disease). The distribution of the general type classifications (i.e. horse, pony or donkey), together with the age and gender distribution for all animals are shown in **Table 3.1**. These data and the hospital case numbers assigned to individual animals are presented in detail in Appendix 3.

		Reasons for referral	
	Overall (n=290)	**Medicine** (n=115)	**Surgery** (n=175)
Age			
Mean	9.51	11.08	8.45
SD	6.04	7.23	4.83
Median	8	8	8
Range	1 month - 33 years	4 months - 33 years	1 month - 21 years
Not recorded	6	1	5
Sex			
Female	124	55	69
Male (gelded)	149	56	93
Male (entire)	16	3	13
Not recorded	1	1	0
Type			
Horse	184	66	118
Pony	98	44	54
Donkey	3	3	0
Not recorded	5	2	3

Table 3.1: Age, type and sex distribution of new equine cases (excluding confirmed cases of chronic gastrointestinal disease) presented to the University of Glasgow Veterinary School between 3/1/96 and 3/1/97.

3.4.2 Chronic gastro-/enteropathies

3.4.2.1 Diagnoses

Historical, clinical and laboratory data were obtained from 29 cases with confirmed chronic gastrointestinal disease. These animals ranged in age from 1.5 to 20+ years and comprised 21 mares, 6 geldings and 2 entire males. There were 15 ponies and 14 horses. The hospital case numbers assigned to individual animals are listed in Appendix 2.

The diagnoses reached were CIBD (11 cases), cyathostomosis (9 cases), alimentary lymphosarcoma (7 cases), gastric ulceration/pyloric stenosis (1 case) and *Eimeria leukarti* enteropathy (1 case) (**Table 3.2**). Five cases of cyathostomosis were discharged following full recovery. A definitive diagnosis was achieved *ante mortem* in 8 cases of cyathostomosis by demonstrating the presence of large numbers of cyathostome larvae in faeces (in five cases, larvae were grossly visible on the rectal glove following rectal examination). Two cases of lymphosarcoma were diagnosed by demonstrating characteristic pathological changes in the rectal tissue obtained by biopsy. A diagnosis of CIBD was made in one animal based on histopathological examination of intestinal biopsies. In all other cases (n=18, 62.1 %), the diagnosis was reached at post mortem examination.

Diagnosis	Number of animals	Identification numbers
Cyathostomosis	9	1-9
Alimentary Lymphosarcoma	7	10-16
Chronic inflammatory bowel disease	11	17-27
Eosinophilic enteritis	(2)	
Granulomatous enteritis	(2)	
Lymphocytic-plasmacytic enteritis	(2)	
Lymphocytic enteritis	(1)	
No specific classification	(4)	
Eimeria	1	28
Pyloric stenosis	1	29

Table 3.2: Post mortem diagnoses in 29 confirmed cases of chronic gastrointestinal tract disease in adult horses.

3.4.2.2 Pathological features

3.4.2.2.1 Cyathostomosis

The gross pathological features of cyathostomosis included typhilitis and/or colitis of variable severity. In the patients which died or those that were euthanased after a short duration of illness, there was marked mucosal congestion, haemorrhage, ulceration and/or necrosis; whereas, in the case which was euthanased after a protracted illness, intestinal pathology was not as marked and included mucosal oedema and irregular areas of congestion. In all cases, careful inspection of the mucosa allowed detection of numerous small strongyle larvae in the mucosa, which often gave the mucosa a 'peppered' appearance. One animal with cyathostomosis, euthanased because of the development of

severe colic associated with abdominal distension had a markedly dilated caecum. The histopathological features of cyathostomosis cases included mucosal distortion, mucosal oedema, dilatation of crypts and a diffuse cellular infiltrate (mononuclear and/or eosinophilic) in the submucosa, which was very intense around the larvae in the submucosa.

3.4.2.2.2 Alimentary lymphosarcoma

In five of the seven cases confirmed as alimentary lymphosarcoma, pathological changes were evident in both the large and small intestine. In one case, there was involvement of the small intestine only and in the other, the primary pathology was detected in the large intestine. In all cases, the intestinal lesions were considered to be diffuse. Typical gross pathological features included mucosal oedema/thickening, raised mucosal plaques and mucosal ulceration. In some cases serosal plaques were present, which on occasion were associated with transmural extension of the pathological process. Enlarged/oedematous ileocaecocolic lymph nodes were common. In all cases, microscopic examination at numerous sites of the intestinal tract showed diffuse infiltration by lymphoreticular cells resulting in distortion of the normal mucosal architecture, characterised by villus stunting, obliteration of crypts and mucosal oedema. At the different sites, in each individual, there was variation in the density of infiltration and in the extent of invasion of the deeper layers. On occasion, the neoplastic infiltrate was noted to invade the epithelium. The neoplastic infiltrate was often accompanied by a pronounced fibrous reaction, particularly in the submucosa. In one case, lymphosarcomatous spread to the liver was detected.

3.4.2.2.3 Chronic inflammatory bowel disease

The term CIBD is used to describe the intestinal pathology detected in 11 cases in this series. The typical gross pathological features of which were similar to those described for alimentary lymphosarcoma and histopathology was necessary in all cases to differentiate between inflammatory and neoplastic lesions. In cases of CIBD, the histopathological appearance of the lesions was characterised by severe focal or diffuse inflammatory cell infiltrates, of predominantly one or a mixture of cell types, into the lamina propria, submucosa or entire wall of a region or throughout the whole intestine. In a number of cases, the gross and histopathological features were consistent with reported descriptions of recognised pathological entities such as GE (2 cases) (Merritt, Cimprich & Beech, 1976; Lindberg, 1984), EGE (2 cases) (Pass & Bolton, 1982), lymphocytic-plasmacytic enteritis (2 cases) (MacAllister, Mosier, Qualls & Cowell, 1990) and lymphocytic enteritis (one case)

(Clark, Morris, Allen & Tyler, 1988). In the other cases, the mixed inflammatory infiltrates (lymphocytes and/or plasma cells and/or macrophages and/or eosinophils and/or globule leukocytes) lacked a precise pattern and/or features were inconsistent with recognised entities. As with alimentary lymphosarcoma, involvement of ileocaecocolic lymph nodes was common in animals with CIBD. In addition, in both animals in which the intestinal lesions were categorised as EGE, eosinophilic granulomata were present in the pancreas and there was dilatation and fibrosis of both the bile and pancreatic ducts. In the two cases classified as GE, the serosal surface of the intestine was affected by the pathological process and there were associated omental adhesions.

3.4.2.2.4 Other diagnoses

One animal in the present series was diagnosed at post mortem examination as a case of coccidial enteropathy. In this case, the small intestinal mucosa, particularly the ileum, was grossly thickened throughout and large numbers of microgametocytes and macrogametes were detected, by microscopy, in the lamina propria. There was evidence of villus stunting and, while a marked cellular reaction to the presence of these parasites was not evident, increased numbers of macrophages were apparent in the region of the villus tips.

Only one animal in this series had primary gastric disease. In this case, the stomach was grossly dilated. On opening into the stomach, it was apparent that the pyloric outlet was narrowed due to extensive thickening of the pyloric wall associated with a large ulcer (6 cm x 8 cm) proximal to the pyloric sphincter. There was a second large, deep ulcer (15 cm x 7 cm) in the squamous zone adjacent to the *margo plicatus* with additional smaller ulcers present in both the squamous and glandular regions. Histopathological examination of the pyloric lesion revealed extensive granulation tissue with proteinaceous and cellular debris adherent to the surface of the ulcer.

3.4.2.3 Historical features

The historical features of the 29 cases are summarised in **Table 3.3**. All animals were presented for investigation of weight loss and/or diarrhoea and/or colic. All cases of cyathostomosis were presented between November and March and young animals (0-5 years) were significantly over-represented within this disease category when compared to the control population ($p<0.01$) (seven of the animals with cyathostomosis were three years or less). By contrast, the age distribution of animals in the CIBD ($p<0.01$) and alimentary

lymphosarcoma ($p<0.01$) disease groups were significantly older when compared to the control population and cases were seen throughout the year.

Seventy five percent of all animals diagnosed as cyathostomosis or alimentary lymphosarcoma were ponies, compared with the fact that ponies accounted for only 33.8 % of the total equine control population (i.e. ponies were significantly over-represented within each of these two disease categories ($p<0.05$)). A significant association between animal type and a diagnosis of CIBD was not detected.

Compared to the control population, females were significantly over-represented ($p<0.05$) within the CIBD disease group and the association between being female and a diagnosis of alimentary lymphosarcoma approached significance ($p=0.06$): 85 % of animals diagnosed as either CIBD or alimentary lymphosarcoma were female compared with the statistic that the majority of animals in the control population were male (56.9 %).

Three of the animals diagnosed as cyathostomosis had received anthelmintics, as part of a parasite prophylaxis programme, within the ten days prior to the onset of illness. No similar historical feature was recorded for any animal in the other two major disease categories.

3.4.2.4 Clinical features

Generally, the time course of cyathostomosis was relatively short, with 66 % of cases exhibiting signs for 2 weeks or less prior to presentation, compared with the time-course for CIBD and alimentary lymphosarcoma, with 66 % of these cases exhibiting signs for 4 weeks or more (**Table 3.4**). At the time of presentation, poor body condition was a feature of all cases included in this study, and varied from mild to marked in all major disease categories. Diarrhoea was most commonly associated with cyathostomosis (89 %) and alimentary lymphosarcoma cases (86 %), but was not a totally consistent feature of either disease entity. Similarly, peripheral oedema occurred with equal frequency in cases with cyathostomosis (44 %) and alimentary lymphosarcoma (43 %) and was less common in animals with CIBD. Only one animal with CIBD exhibited signs of abdominal discomfort at initial presentation but during hospitalisation, recurrent low grade abdominal pain was observed in a number of other animals in each disease category. One case of cyathostomosis developed severe colic associated with caecal tympany/dilatation and was euthanased on humane grounds. Five cases of cyathostomosis were pyrexic (rectal temperature greater than 38.3^{0}C) when initially presented and two additional cases were pyrexic at some stage during their hospitalisation. By contrast, pyrexia was not a feature of

Case No.	Age (years)	Breed	Sex	Month of presentation	Duration of illness* (weeks)	Reason for presentation	Length of hospitalisation	Outcome
1	13	P	F	Nov.	1	rapid weight loss, diarrhoea	2 days	D
2	2	P	M	March	12	poor weight gain, lethargy	18 days	D
3	3	P	F	March	6	weight loss, diarrhoea	20 days	S
4	1.5	ID	MN	March	2	watery diarrhoea, weight loss	17 days	S
5	2	C	F	Dec.	3	weight loss, diarrhoea	2 days	D
6	2	High.	M	March	2	weight loss, diarrhoea	60 days	S
7	1.5	Dales	F	Dec.	1	watery diarrhoea, rapid weight loss	25 days	S
8	3	P	F	Feb.	1	rapid weight loss	14 days	S
9	20	H	MN	Dec.	1	watery diarrhoea	8 days	D
10	11	P	F	June	8	weight loss, diarrhoea	14 days	D
11	14	RH	F	Feb.	3	diarrhoea, one episode mild colic	28 days	D
12	16	Fell	F	April	2	weight loss diarrhoea	14 days	D
13	13	P	MN	April	>12	weight loss, semi-formed faeces	120 days	D
14	15	P	F	Aug.	>12	weight loss, semi-formed faeces	21 days	D
15	15	P	F	Feb.	8	weight loss, diarrhoea	18 days	D
16	20	Fell	F	Dec.	4	weight loss	2 days	D
17	15	P	MN	Jan.	6	weight loss, diarrhoea	7 days	D
18	11	A	F	Aug.	>12	intermittent diarrhoea, weight loss	210 days	D#
19	14	TB	F	June	>12	weight loss	21 days	D
20	8	P	F	Dec.	4	diarrhoea, weight loss	9 days	D
21	20	H	F	Feb.	2	diarrhoea	56 days	D#
22	6	STB	F	April	4	weight loss, intermittent mild colic	24 days	D
23	13	TBx	F	Oct.	3	weight loss	14 days	D
24	20	P	F	June	-	weight loss	2 days	D
25	13	A	F	Nov.	8	recurrent colic, mild weight loss	14 days	D
26	14	TB	MN	July	>12	weight loss, peripheral oedema	14 days	D
27	20	TB	F	Oct.	3	rapid weight loss, one bout of colic	6 days	D
28	15	H	MN	Aug.	>12	weight loss	21 days	D
29	4	TB	F	June	>12	weight loss, ptyalism, intermittent colic	29 days	D

Table 3.3: Historical data from 29 cases of confirmed chronic gastrointestinal disease in adult horses. P=pony, ID=Irish Draught, C=Cob, A=Arab, H=Hunter type, Sh=Shire, STB= Standardbred, TB=Thoroughbred, F=female, M=entire male, MN=gelding, S=survived, D=died/destroyed, D#=experienced a transient improvement on therapy, but subsequent deterioration and were destroyed. *represents duration of illness prior to presentation to GUVS.

Case No.	Physical Findings at Initial Presentation						Clinical Progression
	Weight loss	Faecal consistency	Colic	Peripheral oedema	Pyrexia	Other	
1	++	watery	no	yes	yes	-	rapid deterioration; died
2	+++	sloppy	no	no	yes	-	intermittent, mild colic; watery diarrhoea; euthanased
3	++	watery	no	no	yes	-	gradual improvement; discharged
4	++	watery	no	yes	yes	weakness	persistent pyrexia; intermittent, mild colic; gradual improvement; discharged
5	++	watery	no	yes	yes	weakness	rapid deterioration; euthanased
6	+++	watery	no	no	no	-	intermittent pyrexia; preputial oedema; gradual improvement; discharged
7	++	watery	no	no	no	-	intermittent pyrexia; improvement; relapse; improvement; discharged
8	++	normal	no	no	no	-	gradual improvement; discharged
9	++	watery	no	yes	no	-	developed moderate to severe colic; euthanased
10	+++	sloppy	no	no	no	-	transient response to symptomatic therapy; euthanased
11	+/-	watery	no	yes	no	-	transient response to symptomatic therapy; occasional colic; euthanased
12	++	watery	no	yes	no	-	euthanased
13	+++	sloppy	no	no	no	-	transient response to therapy; peripheral oedema; colic; euthanased
14	+	sloppy	no	no	no	-	euthanased
15	++	watery	no	no	no	-	euthanased
16	++	normal	no	yes	no	-	euthanased
17	+++	watery	no	no	yes	-	persistent pyrexia; peripheral oedema; euthanased
18	+++	watery	no	no	no	-	transient response to symptomatic therapy; euthanased
19	+++	normal	no	no	no	-	intermittent loose faeces; euthanased
20	+++	watery	no	no	no	weakness	euthanased
21	+++	watery	no	yes	no	weakness	intermittent pyrexia; transient response to symptomatic therapy; euthanased
22	++	normal	mild	no	no	-	exploratory laparotomy; wound dehiscence; euthanasia
23	+	normal	no	no	no	-	exploratory laparotomy; euthanasia
24	+++	sloppy	no	no	no	-	euthanased
25	+	normal	no	no	no	-	intermittent colic; exploratory laparotomy; euthanasia
26	++	normal	no	yes	no	-	euthanased
27	+++	normal	no	yes	no	-	euthanased
28	++	normal	no	no	no	-	euthanased
29	+	normal	no	no	no	ptyalism	intermittent, low grade abdominal pain

Table 3.4: Clinical data from 29 cases of confirmed chronic gastrointestinal disease in adult horses. +++=severe, ++=moderate, +=mild, +/- not evident at time of presentation, but reported by owner.

alimentary lymphosarcoma cases and was only infrequently detected in animals with CIBD. Five of the 29 animals in this study did not exhibit any abnormality apart from weight loss at initial presentation. Ptyalism and intermittent, low grade colic were the principle clinical features of one case with gastric disease.

No palpable abnormalities were detected, *per rectum*, in any of the cases included in this study. However, an incidental finding following rectal examination was visualisation of cyathostome larvae on the rectal sleeve in 5 cases (56 %) of cyathostomosis.

3.4.2.5 Clinicopathological features

The significant laboratory findings for the 29 cases are summarised in **Table 3.5**. The haematological and biochemical abnormalities commonly identified were hypoalbuminaemia (< 30 g/L) (24 cases), neutrophilia (> 6.8 x 10^9/L) (20 cases) and increased serum AP concentrations (> 310 U/L) (22 cases). Although these abnormalities were not specific for a particular diagnosis, all cases of cyathostomosis had a neutrophilia which in the majority of cases was marked (median neutrophil count - 17.4 x 10^9/L). The only other haematological abnormality detected was a mild anaemia in five cases (two of cyathostomosis and three of CIBD). All cases of cyathostomosis and alimentary lymphosarcoma were hypoalbuminaemic. Increases in plasma urea and creatinine concentrations and decreases in electrolyte concentrations associated with dehydration were detected in three cases of cyathostomosis and one case of CIBD. Increases in GGT concentration were detected in two cases of alimentary lymphosarcoma, both of which had evidence of liver pathology at post mortem examination.

Faeces submitted for parasitological evaluation yielded faecal worm egg counts ranging from 50 to 300 eggs per gram in 6 cases. Faeces from all cases of cyathostomosis were negative for worm eggs, but large numbers of cyathostome larvae were present in faeces from 8 cases. *Campylobacter* spp. and *Salmonella typhimurium* were cultured from the faeces of three cases and one individual case, respectively.

Bacteriological analysis of peritoneal fluid yielded no significant abnormalities in any of the cases from which peritoneal fluid was obtained. However, increases in white cell count and total protein were detected in the peritoneal fluid of three horses, two of which were subsequently confirmed as GE with serosal involvement. The cause of the increase in the total protein concentration of peritoneal fluid in the other case with CIBD was not identified at post mortem examination.

Case No.	WCC (x 10^9/L)	Neut. count (x 10^9/L)	Albumin (g/L)	Globulin (g/L)	AP (IU/L)	Other	Serum Protein Electrophoresis	OGTT	Rectal Biopsy
1	30.2	27.2	17	31	440	↑ urea, ↑ creatinine	NAD	-	-
2	22.4	17.4	20	31	346	↓ RCC	↑ α2	N	-
3	13.1	7.8	18	48	359	-	↑ α2, β	N	-
4	26.4	19.9	22	23	804	↓ RCC, ↓ K^+	-	P	-
5	29.8	24.1	18	34	853	↑ urea, ↑ creatinine, ↓ K^+	↑ α2	-	-
6	12.6	9.8	11	49	440	-	↑ α, β	-	-
7	28.1	23.6	21	32	695	-	↑ α2	-	-
8	13.9	9.73	29	39	705	-	NAD	-	-
9	17.4	12.4	21	35	667	↑ urea, ↑ creatinine, ↓ K^+	NAD	-	-
10	15.5	11.7	18	41	502	-	↑ β1	P	lymphocytic enteritis
11	4.8	2.4	17	24	935	-	↑ β2	T	lymphosarcoma
12	10.2	7.9	14	19	779	↓ K^+	-	T	lymphosarcoma
13	8.0	3.4	17	20	181	-	NAD	P	NAD
14	24.8	18.4	20	60	663	↑ GGT	-	N	NAD
15	10.2	7.0	19	31	290	-	-	P	NAD
16	8.4	6.7	14	34	3009	↓ urea, ↑↑ GGT	-	-	-
17	6.6	4.6	16	28	1662	↓ RCC, ↓ K^+	-	U	NAD
18	11.7	9.9	31	37	503	-	-	N	enteropathy
19	8.9	7.4	28	31	225	-	-	P	enteropathy
20	22.8	19.6	23	30	339	↓ K^+	-	-	-
21	18.1	13.9	12	25	480	↑ urea, ↑ creatinine, ↓ K^+	-	N	NAD
22	9.7	7.2	33	28	224	↓ RCC	-	P	proctitis
23	8.3	6.1	35	30	346	↓ RCC	NAD	N	NAD
24	10.7	6.7	22	64	484	-	-	-	-
25	9.8	6.3	27	34	184	-	-	P	-
26	6.9	4.5	23	27	440	↓ K^+	NAD	P	NAD
27	8.8	7.3	19	35	999	-	NAD	P	NAD
28	5.9	3.7	35	33	151	-	NAD	P	proctitis
29	10.5	7.7	32	29	169	-	-	P	NAD

Table 3.5: Laboratory data from 29 cases of confirmed chronic gastrointestinal disease in adult horses. AP=alkaline phosphatase, OGTT=oral glucose tolerance test, NAD=no abnormality detected, GGT=gamma glutamyl transferase, K^+=plasma potassium, RCC=red cell count, WCC=white cell count, Neut.=neutrophil, N=normal, P=partial, T=total, U=uninterpretable.

Abnormal serum globulin profiles were detected, by SPE, in five of eight cases diagnosed as cyathostomosis and two of three cases with alimentary lymphosarcoma. In two animals with cyathostomosis, marked increases in the relative proportions of both α– and β–globulin fractions were evident, whereas in the other three cases moderate increases in α2-globulin fraction only were detected. In the two cases of alimentary lymphosarcoma, abnormalities of either the β1- (markedly increased) or β2- (moderately increased) globulin fractions only were apparent. Serum globulin profiles in three animals with cyathostomosis and three animals with CIBD were normal.

The results of OGTT's were recorded as either normal (6 cases), partial malabsorption (11 cases) or total malabsorption (2 cases), as defined by Mair *et al.* (1991). The OGTT from one case was uninterpretable due to a high BGC. Two cases of cyathostomosis had normal OGTT results, the test in another case being classified as evidence of partial malabsorption (the glucose concentration at 60 min was 178 % of BGC). Both animals which had test results indicative of total malabsorption were confirmed as cases of alimentary lymphosarcoma.

Rectal biopsies were obtained from 16 animals in the present series and histopathological abnormalities were identified in seven of these specimens. Pathological change characteristic for alimentary lymphosarcoma was detected in rectal tissue obtained from two animals, and this diagnosis was subsequently confirmed at necropsy. In another case, which was confirmed as alimentary lymphosarcoma at post mortem examination, a marked lymphocytic infiltrate was evident in the rectal lamina propria. Histopathological examination of biopsied rectal tissue from another two animals detected severe inflammatory lesions: Both of these animals were subsequently diagnosed as cases of CIBD. In the remaining two cases, there was a moderate increase in cellularity in the lamina propria of the rectal tissue which was interpreted as non-specific proctitis.

3.5 Discussion

Chronic inflammatory bowel disease, cyathostomosis and alimentary lymphosarcoma accounted for the majority of confirmed cases of chronic equine gastrointestinal disease in the present series. These findings are similar to the observations of Love, Mair & Hillyer (1992) who reported that of 51 cases of chronic diarrhoea, a specific diagnosis was achieved in 37 horses, and of these, 28 (78 %) were diagnosed as either cyathostomosis (14 cases), idiopathic chronic colitis (9 cases) or alimentary lymphosarcoma (5 cases).

In the present series, the pathological features of cyathostomosis were comparable to previous descriptions of the condition (Giles, Urquhart & Longstaffe, 1985). In addition, the gross and histopathological intestinal features of the majority of cases of CIBD were consistent with reported descriptions of recognised pathological entities (Merritt, Cimprich & Beech, 1976; Pass & Bolton, 1982; Lindberg, 1984; Clark *et al.*, 1988; MacAllister *et al.*, 1990), all other cases having severe mixed inflammatory infiltrates lacking a precise pattern. No obvious cause of the chronic inflammatory change in these cases was identified. Although the histopathology of alimentary lymphosarcoma cases was similar to that described by Platt (1987), the extent of the intestinal involvement was different from previous reports: In all cases in this series, the lesions were classified as diffuse and pathological change in the large intestine was a feature in six of the seven cases. By contrast, Platt (1987) reporting on the pathological features of nine cases of alimentary lymphosarcoma, described diffuse lymphosarcomatous change in the small intestine only. Furthermore, Mair & Hillyer (1991), when describing the clinical features of lymphosarcoma in the horse, reported that in animals with intestinal lymphosarcoma, the small intestine only was involved in the majority of cases and that focal lesions were more common than diffuse intestinal lesions. The factors which influence the extent of intestinal involvement in cases of alimentary lymphosarcoma are unknown.

Both coccidial enteropathy and pyloric stenosis secondary to gastric ulceration, diagnosed in single cases in the present series, are uncommon conditions of the adult horse. *Eimeria leukarti* has been reported as a cause of equine chronic diarrhoea and the pathological features of this case are similar to those described previously (Wheeldon & Greig, 1977). However, based on a paucity of clinical reports and the failure to produce disease by experimental infections (Laufenstein-Duffy, 1969), this parasite is currently considered to be of slight pathogenic significance in horses (Love, 1992).

Interestingly, primary gastric disease was a feature of only this single case in the present series, emphasising the point that chronic disease of the equine stomach is uncommon. In fact, pyloric obstruction as a sequel to gastric ulceration has been reported in mature horses on only two previous occasions (Church, Baker & May, 1986; Laing & Hutchings, 1992). Furthermore, the results of this study indicate that in our population, alimentary lymphosarcoma was the most common neoplasm of the equine gastrointestinal tract and not gastric squamous cell carcinoma as had been suggested by Boulton (1987). The case of chronic gastric disease described in this report was characterised clinically by ptyalism and chronic, low grade colic which should be interpreted as evidence of possible gastric

dysfunction. While endoscopy may aid the ante mortem diagnosis of gastric disease in the adult horse (Murray, 1992a), an instrument of sufficient length was not available for use in the investigation of this case.

The historical features of cyathostomosis cases in the present study are similar to previous reports (Reid *et al.*, 1995) in that, typically, affected animals are young adults and present in winter or early spring. In addition, the clinical presentation is characterised by sudden onset diarrhoea and rapid weight loss. By contrast, CIBD and alimentary lymphosarcoma tended to be diagnosed in older animals and, in general, for both conditions, the duration of clinical signs prior to presentation at the referral centre was longer than that recorded for cyathostomosis. While these features may be useful for differentiating cases of cyathostomosis from other causes of chronic gastrointestinal tract disease, it is important to acknowledge that not all cases of cyathostomosis have the 'classical' history: For example, one animal in the present series was aged (20 years), another presented because of rapid weight loss in the absence of diarrhoea and another exhibited chronic ill-thrift. Similarly, neither CIBD nor alimentary lymphosarcoma are confined to the older horse (Lindberg *et al.*, 1985; Platt, 1987; Mair & Hillyer, 1991). In fact, the majority of cases of either GE or EGE reported in the literature were adult animals of 5 years of age or less (Lindberg *et al.*, 1985; Platt, 1986) and Platt (1987) described the pathological features of alimentary lymphosarcoma in nine animals all aged between 1 and 6 years.

Comparison of animal type and sex distribution data from the clinical cases with similar data from the control population yielded some interesting results: It appears that ponies are at a greater risk of developing either cyathostomosis or alimentary lymphosarcoma than horses, and that female animals are at greater risk of developing CIBD and may be at a greater risk of developing alimentary lymphosarcoma than males. Although these associations appear to be significant in the present study they are not supported by previous reports of these conditions. Gender and type of animal were not identified as important risk factors for the development of cyathostomosis (Reid *et al.*, 1995). Similarly, Mair & Hillyer (1991), providing details of a greater number of cases, reported that alimentary lymphosarcoma occurred with equal frequency in both horses and ponies and was diagnosed more often in male animals, but in that study the relative prevalence of these conditions within a gender or type group (i.e. statistical comparison to a control population) was not investigated. Certain inflammatory enteropathies (most notably GE and EGE) have been reported almost exclusively in horses, in particular in Standardbreds, but CIBD was diagnosed in three ponies in the present series. Furthermore, when compared to data from

our control population, horses were not over represented in this disease category (i.e type distribution was similar for both the control group and the CIBD category).

Anthelmintic treatments preceded the onset of illness in three cases of cyathostomosis and preceded the onset of diarrhoea in another case presented because of chronic ill-thrift. A comparable association between anthelmintic treatments and the onset of illness was not recorded for either of the other two major disease categories, supporting the hypothesis that anthelmintic treatments may precipitate clinical disease in animals with a heavy mucosal cyathostome burden (Gibson, 1953; Reid *et al.*, 1995).

The results of the present study highlight the difficulty in reaching a diagnosis in animals with chronic gastrointestinal disease based on clinical features alone: Reported weight loss was a feature of all cases and a number of animals in each of the major disease categories exhibited additional signs such as diarrhoea and/or peripheral oedema and/or colic. The non-specific nature of the clinical presentation was further emphasised by the fact that five animals exhibited no abnormality apart from poor body condition/reported weight loss at the time of initial presentation. Diarrhoea was recorded with a greater frequency in cases of alimentary lymphosarcoma in this study compared to the incidence of that clinical feature in other reports of the condition (Platt, 1987; Mair & Hillyer, 1991), and was considered to reflect the frequency of extensive pathological involvement of the large intestine. In the present series, pyrexia was identified as the most useful clinical feature for differentiating cyathostomosis from other causes of chronic gastrointestinal disease. However, the greater frequency with which pyrexia was detected in cases of alimentary lymphosarcoma (Mair & Hillyer, 1991) and CIBD (Lindberg *et al.*, 1985) in other case series indicates that the differentiating ability of this clinical feature is questionable.

An interesting aspect of the present series is the variety of clinical presentations (such as chronic weight loss, rapid weight loss in the absence of diarrhoea and severe colic) recorded for animals with cyathostomosis. In recent years, numerous reports describe a variety of cyathostome-associated diseases, with clinical presentations distinct from the 'classical' features of sudden onset diarrhoea, which becomes chronic, and rapid weight loss. Mair (1993) described a previously unrecorded syndrome of cyathostome-related recurrent diarrhoea in five aged ponies. In addition, Mair (1994), when documenting an outbreak of cyathostomosis in a group of five young horses, described rapid weight loss, severe peripheral oedema and pyrexia, without diarrhoea, in four of those animals: A similar weight loss syndrome has been increasingly observed in weanlings during the autumn

grazing period (S. Love, personal communication). Reilly, Cassidy & Taylor (1993) reported chronic weight loss/ill-thrift of several months duration prior to cyathostome-associated sudden onset diarrhoea: a history similar to that of Case 2 in the present series. Cyathostomes have also been incriminated as the cause of a seasonal (late autumn-spring) 'malaise syndrome' in adult horses which is characterised by reduced appetite, lethargy, weight loss with variable faecal consistency and which responds to anthelmintic therapy (Matthews & Morris, 1995). In addition, cyathostome infection is increasingly recognised as an important cause of equine colic: The results of a study which compared the effect of different anthelmintic programmes on the incidence of colic demonstrated a marked decrease in colic incidence on farms on which effective cyathostome control was achieved compared with that recorded on farms where cyathostome control failed (Uhlinger, 1990). Furthermore, cyathostomes have been identified as a cause of caecocaecal intussusception (Lyons *et al.*, 1994; S. Love, personal communication) and also non-strangulating intestinal infarction (Mair & Pearson, 1995). Mechanisms which may contribute to the occurrence of cyathostome-associated colic include intestinal mucosal oedema and/or vasoconstriction induced by the local production of vasoactive substances in response to the presence of cyathostome mucosal stages (Mair & Pearson, 1995). In addition, a disruption to normal intestinal motility has been observed following experimental cyathostome infection (Bueno, Ruckebusch & Dorchies, 1979) and it is possible that altered intestinal motility may contribute to the development of intussusceptions and may have resulted in caecal tympany which developed in one animal (Case 9) described in the present report.

The most useful diagnostic aid in the present series was faecal analysis: Eight cases of cyathostomosis (89 %) were diagnosed by detecting large numbers of cyathostome larvae in faeces and in five of these cases larvae were visible grossly, facilitating a rapid diagnosis. Love, Mair & Hillyer (1992) reported an ante mortem diagnosis rate of 50 % for cyathostomosis, simply by observing larvae in faeces, whereas the remaining cases could only be confirmed at post mortem examination which emphasises the fact that the absence of larvae from faeces makes conformation of a diagnosis, *ante mortem*, difficult. Although blood analyses are important for case assessment and may fashion approach to treatment, it is generally considered that such analyses have limited diagnostic value when investigating cases of chronic gastrointestinal disease in the horse. However, while neutrophilia was detected in animals in each of the three main disease categories, it was a consistent feature of cyathostomosis cases such that absence of neutrophilia in an animal presented for investigation of weight loss and/or diarrhoea would be suggestive of a diagnosis other than

cyathostomosis. This observation is supported by the results of previous reports which indicate that leucocytosis due to neutrophilia is a common feature of cyathostome-associated illness (Giles, Urquhart & Longstaffe, 1985; Uhlinger, 1991; Kelly & Fogarty, 1993; Mair, 1994). Hyperbetaglobulinaemia, which has been described as characteristic of cyathostome infection (Giles, Urquhart & Longstaffe, 1985; Kelly & Fogarty, 1993), was detected in only two of eight cases of cyathostomosis in the present series. In three other cases, moderate increases in α2-globulin fraction only were detected, whereas in the remainder (3 cases) no abnormality in serum globulin profile was detected. Moreover, a marked increase in β1-globulin was detected in one of three animals with alimentary lymphosarcoma. Consequently, the detection of hyperbetaglobulinaemia was an inconsistent feature of cyathostome infection and had poor diagnostic specificity. Mair *et al.* (1993), when investigating the usefulness of SPE to help differentiate between cyathostomosis and other causes of chronic diarrhoea, reported that a marked increase in β1-globulin fraction was suggestive of cyathostomosis, but it was considered that the absence of that feature could not preclude that diagnosis.

On the basis of the OGTT results, two animals in the present series exhibited evidence of total glucose malabsorption and both of these cases were subsequently diagnosed as alimentary lymphosarcoma. This finding supports the observation of Mair *et al.* (1991) that evidence of total malabsorption suggests a diagnosis of either alimentary lymphosarcoma or CIBD. The test results in another 11 cases were classified as evidence of partial malabsorption: Specific interpretation of these results was not possible because this category included at least two cases which did not have primary small intestinal disease (i.e. one case of cyathostomosis and the case of chronic gastric ulceration). A more detailed analysis of the OGTT as a diagnostic test is presented in Chapter 5 (**Study 5**).

Marked pathological change suggestive of a severe enteropathy was evident in biopsied tissue obtained from five of the 16 animals from which specimens were obtained (31 %), and the findings permitted a definitive diagnosis in two cases of alimentary lymphosarcoma. These results compare favourably with those of Lindberg, Nygren & Persson (1996) who reported that rectal biopsy findings were diagnostic in 11 of 40 (27.5 %) cases of chronic gastrointestinal disease which were confirmed at necropsy. The findings of the present study and that described by Lindberg, Nygren & Persson (1996) confirm that rectal biopsy is a simple, safe and useful adjunct for the investigation of chronic intestinal disease in the horse.

In conclusion, this study demonstrates that the most frequently identified causes of chronic gastrointestinal disease in the adult horse are cyathostomosis, CIBD and alimentary lymphosarcoma and that, for the latter two conditions in particular, a definitive diagnosis is often difficult to achieve. While history and certain clinical features were considered as being useful for differentiating cyathostomosis from other causes of chronic gastrointestinal disease, the only specific diagnostic tests identified in this study were either faecal analysis for parasitic larvae or histopathological examination of biopsied rectal tissue. In fact, in addition to the limited diagnostic specificity of clinicopathological findings in cases of chronic gastrointestinal disease, there were numerous opportunities for misdiagnosis in the present study; the markedly elevated plasma GGT and AP concentrations detected in one animal with alimentary lymphosarcoma may have been misinterpreted as evidence of primary hepatic disease; demonstration of increased WCC and total protein concentrations in the peritoneal fluid of two animals diagnosed as GE with serosal involvement may have led to a misdiagnosis of peritonitis as the primary disease process; and, the culture of *Salmonella* and *Campylobacter* from the faeces of a number of cases may have been incorrectly interpreted as evidence of intestinal disease with a primary bacterial aetiology. These findings emphasise the importance of cautious interpretation of clinicopathological data.

Finally, although it is recognised that chronic gastrointestinal tract diseases are uncommon in adult equine animals, they are an important category of conditions because they can be severely debilitating. Reaching a definitive diagnosis in such cases is important, in that it allows for a rational approach to therapy/case management, but is often difficult because of the apparent overlap in clinical features between disease entities and a lack of specific diagnostic tests. Therefore, it is necessary that the information base on these conditions be expanded by seeking input from colleagues in general practice and other referral institutions to furnish more detailed epidemiological, clinical and clinicopathological data. In addition, this study serves to emphasise that further study should be directed towards developing/evaluating diagnostic techniques which may provide more objective information on gastrointestinal function in the horse.

CHAPTER 4

FRUCTOSAMINE MEASUREMENT IN PONIES: VALIDATION AND RESPONSE FOLLOWING EXPERIMENTAL CYATHOSTOME INFECTION

4.1 Introduction

4.1.1 Fructosamine formation

Fructosamine is a stable ketoamine compound. It is formed when a glucose molecule in the open chain form reacts non-enzymatically with the free amino groups of a protein molecule to create an unstable aldimine compound; aldimine may either dissociate back into glucose and protein or undergo spontaneous transformation, by amadori rearrangement, into a stable (i.e. irreversible) ketoamine (Dominiczak, 1991). The fructosamine concentration in peripheral blood is a measure of the glycation of all serum proteins, approximately 80-90 % of which, in man, is attributable to glycated albumin (Johnson, Metcalf & Baker, 1982; Kennedy, 1992). Consequently, the principal factors which influence fructosamine formation are the half-life of serum albumin (which is reported to be 19 days in horses (Mattheeuws, Kaneko, Loy, Cornelius & Wheat, 1966)) and the average glucose concentration to which the albumin is exposed while in circulation. Therefore, fructosamine concentration in peripheral blood varies according to the long-term glycaemic status and/or protein metabolism of an individual.

4.1.2 Clinical applications of fructosamine measurement in human and veterinary medicine

To date, the most common application of fructosamine measurement, in human medicine, is as an indicator of long-term glycaemic status in diabetic patients (Rowe & Dominiczak, 1989). For this purpose fructosamine measurement has a major advantage over blood glucose estimation in that it reflects the average blood glucose concentration over the preceding 2-4 weeks and is unaffected by transient hyperglycaemia. Recently, a number of researchers in veterinary medicine have reported the successful use of fructosamine measurement to monitor therapeutic control of the hyperglycaemia of diabetes mellitus in dogs (Jensen, 1992; Reusch, Liehs, Hoyer & Vochezer, 1993; Graham, 1995) and cats (Kaneko, Kawamoto, Heusner, Feldman & Koizumi, 1992; Reusch *et al.*, 1993). Conversely, Cantley, Ford & Heath (1991) reported that serum fructosamine concentrations are decreased in sheep with pregnancy toxaemia, which was considered to be a consequence of prolonged hypoglycaemia. In addition to its application in the monitoring of glycaemic

status, it has been proposed that fructosamine measurement will reflect alterations in the rate of protein turnover: alterations in fructosamine concentration occur in patients with thyroid dysfunction (Lloyd & Marples, 1986; Waterson & Mills, 1988) in the absence of alterations in serum protein or glucose concentrations and are assumed to reflect changes in the rate of protein turnover.

4.1.3 Aim of studies

The aim of this study was to validate a fructosamine assay for use on equine serum and plasma and to evaluate the use of fructosamine measurement to monitor alterations in protein metabolism using, as a 'model', experimental cyathostome infections, which have been shown to result in enteric protein loss (Love *et al.*, 1991).

STUDY 2

4.2 Fructosamine Validation

4.2.1 Background

The initial stage in the evaluation of a laboratory test, before it can be applied to a clinical setting, is to define the performance characteristics of the test within the analytical laboratory. While the validity of laboratory methods for the measurement of serum/plasma fructosamine have been studied and reported both in the dog (Jensen, 1992; Graham, 1995) and in the cat (Reusch *et al.*, 1993), a fructosamine assay has not yet been validated for use on equine serum or plasma.

4.2.2 Experimental objective

The objective of the study was to validate a fructosamine assay for use on equine serum and plasma.

4.2.3 Materials and methods

4.2.3.1 Animals and sample collection

Twenty four British native-breed ponies were used in this study and were grouped according to age and diet (**Table 4.1**). All animals were stabled for the duration of the study and bedded on straw (Group A and B) or wood shavings (Group C) and were considered to be healthy based on clinical examinations. Blood was collected from these animals, into heparinised (Groups A, B and C) and plain (Groups A and B) vacutainers (Becton-Dickinson), once weekly for five weeks, by jugular venepuncture. Plasma and serum analyses were performed on the day of sample collection unless otherwise stated.

	n	Age	Sex	Diet
Group A	7	5-11 years old	1 male, 6 female	hay only
Group B	8	9-12 months old	4 male, 4 female	hay and coarse mix[a]
Group C	9	9-12 months old	3 male, 6 female	high-fibre cubes[b]

Table 4.1: Age, sex and diet of three groups of ponies used to validate the fructosamine assay. n = number of animals.
[a] Spillers® Original Mix, [b] Spillers® High-Fibre Cubes (Spillers Horse Feeds).

4.2.3.2 Fructosamine analysis

Plasma and serum fructosamine concentrations were measured using a commercial colorimetric test kit based on the ability of ketoamine to reduce nitroblue tetrazolium (NBT) in alkaline conditions (Unimate Fructosamine, Roche). The production of formazan forms of NBT is proportional to the concentration of fructosamine and was measured photometrically using an autoanalyser (Cobas Mira, Roche). Fructosamine measurements are made against a calibrator solution based on glycated polylysine supplied by the kit manufacturer (Fructosamine Calibrator, Roche).

4.2.3.3 Data analyses

Using a paired t-test and linear regression analysis, the effect of using either serum or plasma for fructosamine measurement was assessed by comparing the serum fructosamine concentrations with the plasma fructosamine concentrations which were measured in pony Groups A and B for five consecutive weeks.

The within-run variation was assessed by determining the coefficient of variation (CV) of 10 replicate measurements of fructosamine in two pony plasma samples (Group A pooled plasma, Group B pooled plasma). The between-day variation was assessed by determining the CV of five replicate measurements of fructosamine concentrations in two pony plasma samples (Group A pooled plasma, Group B pooled plasma), each stored at -20°C in five separate vials, of which one was thawed and assayed each day.

In order to determine the accuracy of the assay for measuring fructosamine at low concentrations, a plasma sample with an initial fructosamine concentration of 255 μmol/l was diluted 1/1.5, 1/2, 1/4 and 1/8 with physiological (0.9 %) saline. To evaluate the stability of fructosamine during storage, blood was collected from 13 animals (all Group A and six ponies in Group B) into heparinised vacutainers, and plasma fructosamine was measured i) immediately, ii) following storage of whole blood for 72 hours at room temperature, iii) following storage of plasma for 7 days at 4°C and iv) following storage of plasma for 28 days at -20°C.

To assess the effect of diet and age on fructosamine measurement in plasma, the means of animals (mean of five observations per animal) in Group B were compared to those of Group C and the means of Group A were compared to those of Groups B and C combined. Both factors were evaluated using the Mann Whitney U test. A probability of less than 5% ($p<0.05$) was taken to infer the presence of statistically significant differences.

A reference range for fructosamine in pony plasma was calculated based on the mean of five observations per animal (n=24) (reference range = mean ± 2SD).

4.2.4 Results

Data which contributed to the results presented in this section are detailed in Appendices 4, 5, 6, 7, 8 and 9.

Paired plasma and serum fructosamine measurements (n=75) were highly correlated ($p<0.001$) (**Fig. 4.1**). The mean serum fructosamine concentration and associated standard deviation (270.4 ±37.7 μmol/L) was greater than that calculated for plasma fructosamine concentration (263.3 ± 29.1 μmol/L). However, both sets of results were not statistically different ($p=0.20$). Because greater variation in serum fructosamine concentrations were observed, measurement of fructosamine in equine plasma was considered to be more appropriate, therefore validation results for plasma only are reported.

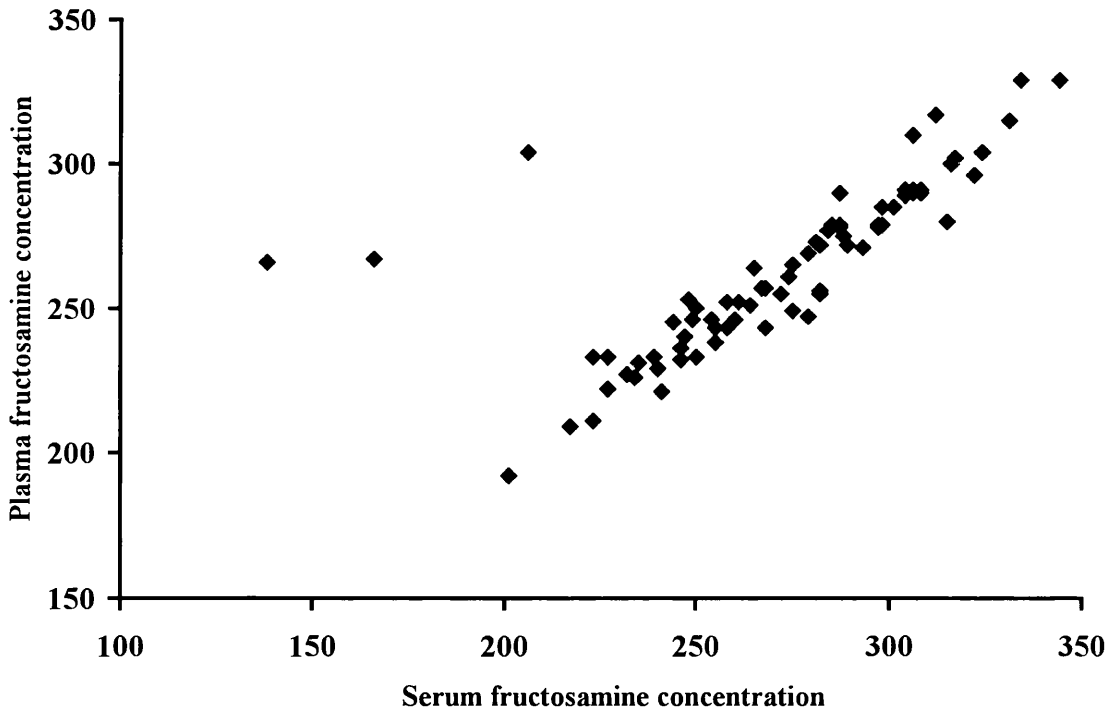


Fig. 4.1: Scatter plot demonstrating the correlation between plasma fructosamine concentration and serum fructosamine concentration based on measurements made following repeated sampling of ponies in Groups A and B. Total number of paired samples assessed was 75.

The intra-assay coefficients of variation were 1.60 and 1.52 % for Group A plasma pool and Group B plasma pool, respectively, giving a mean CV of 1.56 %. The inter-assay coefficients of variation were 4.77 and 3.94 % for Group A plasma pool and Group B

plasma pool, respectively, giving a mean CV of 4.35 % (**Table 4.2**). Linearity, expressed as a percentage of the initial fructosamine concentration in a serially diluted plasma sample, is shown in **Figure 4.2**. Deviation from linearity is observed in all diluted samples, the analysis tending to overestimate the predicted fructosamine concentration. However, the measured fructosamine concentrations did not differ markedly from the predicted fructosamine concentrations.

	Intra-assay		Inter-assay	
	Group A	Group B	Group A	Group B
n	10	10	5	5
mean	257.3	248.3	252.5	243
SD	4.11	3.77	12.03	9.57
CV %	1.6	1.52	4.77	3.94

Table 4.2: Intra-assay and inter-assay variations in equine plasma fructosamine concentrations (μmol/L). n = number of replicates, SD= standard deviation, CV = coefficient of variation.

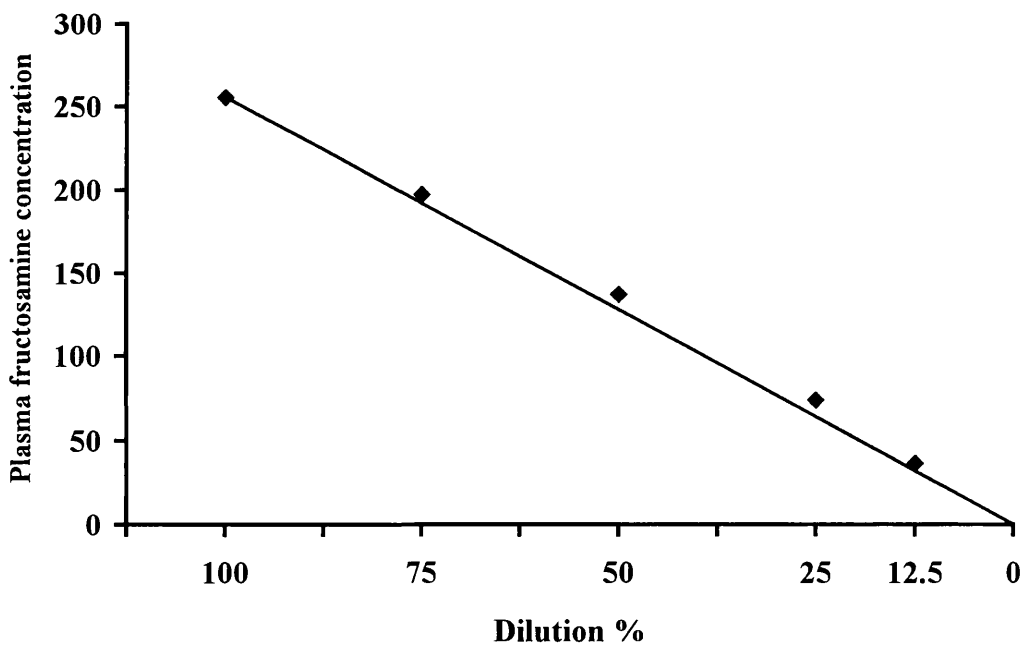


Fig. 4.2: Quantitative recovery of fructosamine in a diluted equine plasma sample.

Assessment of stability during storage (**Fig. 4.3**) indicated that fructosamine concentrations in whole blood stored for 72 hours (mean ± SD, 264 ± 18.23 μmol/L) were significantly less ($p<0.001$) than those recorded immediately following sample collection (311.85 ± 18.94 μmol/L). Although no significant difference was observed between immediate analysis and measurement of fructosamine after plasma storage for seven days at 4°C (301 ± 20.54, $p=0.21$), storage for 28 days at -20°C resulted in a significant decrease (296.3 ± 18.26, $p=0.044$).

No statistically significant differences between groups was recorded due to the effect of either diet ($p=0.6$) or age ($p=0.145$) on plasma fructosamine concentrations. The reference range for fructosamine in pony plasma, based on data from the 24 animals in this study, was calculated to be 256.88 ± 53.04 μmol/L (203-310 μmol/L).

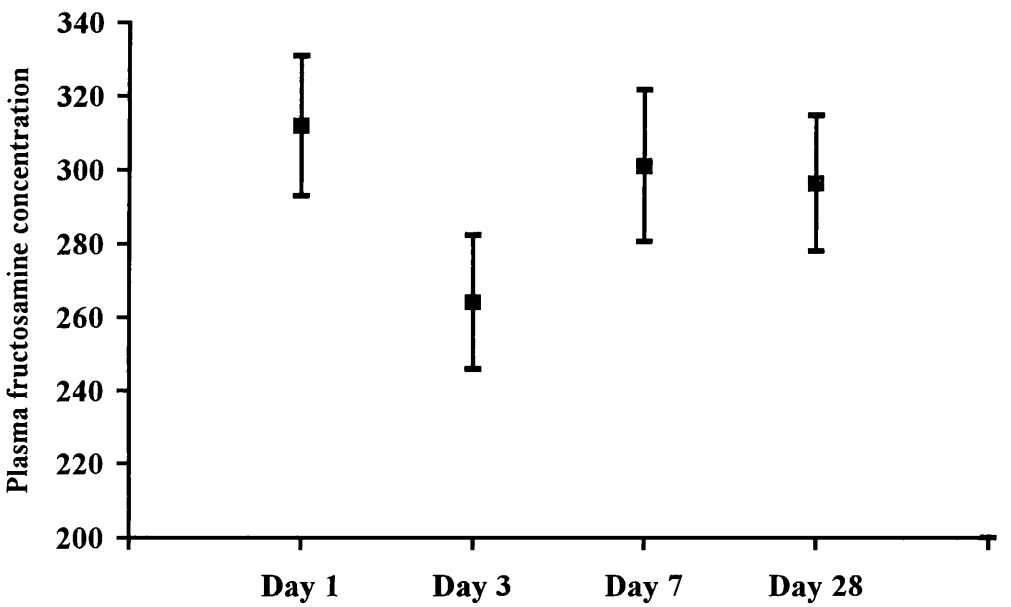


Fig. 4.3: The effect of storage on plasma fructosamine concentrations (μmol/L). Day 1 = immediate analysis, Day 3 = whole blood stored at room temperature for 72 hours, Day 7 = plasma stored at 4°C for 7 days, Day 28 = plasma stored at -20°C for 28 days.

4.2.5 Discussion

Fructosamines can be measured easily and rapidly, in equine serum or plasma, using the method outlined in this study. Simultaneous serum and plasma measurements were well correlated and not statistically different. However, because greater variation in serum fructosamine concentrations were observed (i.e. greater SD), measurement of fructosamine in equine plasma was considered to be more appropriate. The precision of the fructosamine assay, for use in equine plasma, as judged by intra- and inter-assay coefficients of variation

was similar to that previously reported in both human (Kruse-Jarres, Jarausch, Lehmann, Vogt & Rietz, 1989) and animal validation studies (Jensen, 1992; Reusch *et al.*, 1993; Graham, 1995). Based on the dilution studies (linearity), the assay method was shown to be acceptably accurate for measuring low concentrations of fructosamine in equine plasma. In addition, the detection limit for this analytical method, based on analysis of 20 samples of physiological saline, was calculated to be 7 μmol/L (mean $\pm$ 2.6SD) (Graham, 1995). Jensen (1992) reported that fructosamine was stable in canine serum when stored at 4°C and 25°C for five days and at -20°C for one month. Similarly, Graham (1995) demonstrated that fructosamine is stable in whole blood, at room temperature, for 48 hours. The results of the present study indicate that a significant and unacceptable reduction in plasma fructosamine occurs when equine whole blood is stored at room temperature for 72 hours, however, it is stable for one week when the plasma is separated and stored at 4°C. In addition, although a significant decrease in plasma fructosamine concentration was observed following sample storage for one month at -20°C, the variation from immediate analysis was less than 5 % and may, in part, be explained by inter-assay variation. Therefore, it was considered that storage of equine plasma samples for up to one week at 4°C and up to one month at -20°C is acceptable for fructosamine determination. Although possible interference by other plasma constituents was not investigated, the assay method/protocol (reaction pH and time course) ensure negligible interference by other reducing substances such as creatinine and glucose (Johnson, Metcalf & Baker, 1982). In addition, Kruse-Jarres *et al.* (1989) observed no interference due to anticoagulants and Reusch *et al.* (1993) reported that fructosamine measurement was unaffected by transient hyperglycaemia.

Analysis of data for the different groups of ponies showed no significant effect due to diet or age. However, because the effect of age approached significance ($p=0.145$), this factor should ideally be re-evaluated using larger groups of animals.

The reference range, calculated after combining data from all ponies included in this validation study (203-310 μmol/L) is 'narrower' than reference ranges reported for other species (Reusch *et al.*, 1993; Graham, 1995) and may be a reflection of the single breed type used in this study and the similar background of those animals (i.e. all stabled). Interestingly, Jensen & Aaes (1992) reported a narrow reference range of 258.6-343.8 μmol/L for fructosamine concentrations in canine serum, however, it was based on data from 29 adult Beagle dogs maintained under similar conditions.

The validation study indicates that the assay is rapid and precise and accurate for measuring low concentrations of fructosamine in equine plasma.

STUDY 3

4.3 Fructosamine Response Following Experimental Cyathostome Infection

4.3.1 Background

The detection of hypoalbuminaemia, in animals with chronic gastro-/enteropathies, indicates that severe intestinal pathology exists. In mild or subclinical disease, protein loss and/or increases in protein catabolism may be compensated for by increased synthesis such that measurement of albumin concentrations give results within the normal range. Thus, measurement of albumin concentrations alone is a relatively unreliable/crude indicator of altered protein metabolism and it is considered that measurement of the rate of protein turnover would be more informative: This has only been accurately achieved in the horse using radioisotope tracer techniques (Duncan & Dargie, 1975; Love, 1990). However, in recent years, studies in man have indicated that serum fructosamine measurement may prove to be a simple, sensitive method of assessing altered protein metabolism (Lloyd & Marples, 1986; Waterson & Mills, 1988).

4.3.2 Experimental objective

The specific objective of the study was to evaluate the use of fructosamine measurement to monitor alterations in protein metabolism using, as a 'model', experimental cyathostome infections.

4.3.3 Materials and methods

4.3.3.1 Animals, experimental infections and sampling

The animals used in this study were nine British native-breed ponies, the details of which are outlined in **Table 4.1** (Group C). All animals, reared indoors with their dams, were considered to be helminth-naïve prior to experimental infection. The ponies were randomly allocated to one of three groups; **Infected Group 1** (Pony Nos. 101,104 and 105), **Infected Group 2** (Pony Nos. 111, 112 and 113) and an **Uninfected Control Group 3** (Pony Nos. 102, 103 and 106).

Cyathostome third-stage larvae (L_3) were cultured and harvested from faeces obtained from two ponies which had naturally acquired a cyathostome infection by grazing the same pasture. The larvae were stored for 6-10 weeks at 4°C (i.e. cold-conditioned) and given as a 'trickle' infection, with doses of 150,000 L_3 administered by nasogastric tube three times weekly. Infected **Group 1** received a total of 3.9 million L_3 over a nine week period and infected **Group 2** received a total of 3.15 million L_3 over seven weeks.

From four weeks prior to infection, blood was obtained once weekly, from all animals, for blood biochemical analyses (plasma fructosamine, plasma albumin and SPE). In addition, from eight weeks prior to infection all animals were weighed weekly. Post infection, each pony was monitored daily for alterations in demeanor, appetite and faecal consistency.

4.3.3.2 Laboratory analyses

Plasma fructosamine was measured using the method outlined above (Section *4.2.3.2*). Total plasma protein and plasma albumin were analysed using a modified Biuret and bromocresol green methods, respectively. Electrophoretic separation of the serum proteins was achieved in an integrated system (Paragon Electrophoresis System, Beckman Instruments Inc.) on thin layered, buffered agrose gels (Paragon SPE kit, PN 655900, Beckman Instruments Inc.). The protein fractions were quantified by densitometry (Appraise Densitometer System, Beckman Instruments Inc.) and calculated from total protein.

4.3.3.3 Data analyses

The effects of experimental group, time and animal on weight change (kg), plasma fructosamine concentration (μmol/L), plasma albumin concentration (g/L) and serum globulin fractions (α2, β1, β2 and γ) (g/L) were assessed. Although the group sizes in this experiment are too small to comment on anything other than trends, statistical analysis using analysis of variance (ANOVA) techniques were performed to support observations. For this purpose, the data for each parameter up to the time of infection were analysed separately from data collected after infection. Where there was no significant difference among groups prior to infection, the raw post infection data were analysed. Where there was a significant difference among groups prior to infection, the post-infection data were adjusted by subtracting the mean of the pre-values from each of the post-values for each animal (i.e. the dependent variable in the ANOVA equation was the change in the measured parameter rather than the absolute value). Significance was set at the 5 % level.

4.3.4 Results

The results, presented below, are based on pre-infection data and that obtained during the first 20 weeks post-infection and are detailed in Appendices 10, 11, 12 and 13. Full clinical, haematological, blood biochemical and parasitological findings for this study are recorded elsewhere (Murphy & Love, 1997).

4.3.4.1 Clinical observations

Following initial infection, the weight gain of ponies in both infected groups was reduced, relative to the control animals. This effect was observed in all infected animals by five weeks post infection (PI) (**Fig. 4.4**).

One pony in infected Group 2 became dull, intermittently diarrhoeic and had a reduced appetite 51 days PI. Due to a gradual worsening of these clinical features and the development of colic, this pony was euthanased 66 days PI: At post mortem examination cyathostome-induced typhlitis/colitis was evident. The remaining animals in Group 1 and Group 2 remained bright, appetant and demonstrated no clinical evidence of intestinal disease during the 20 week PI period.

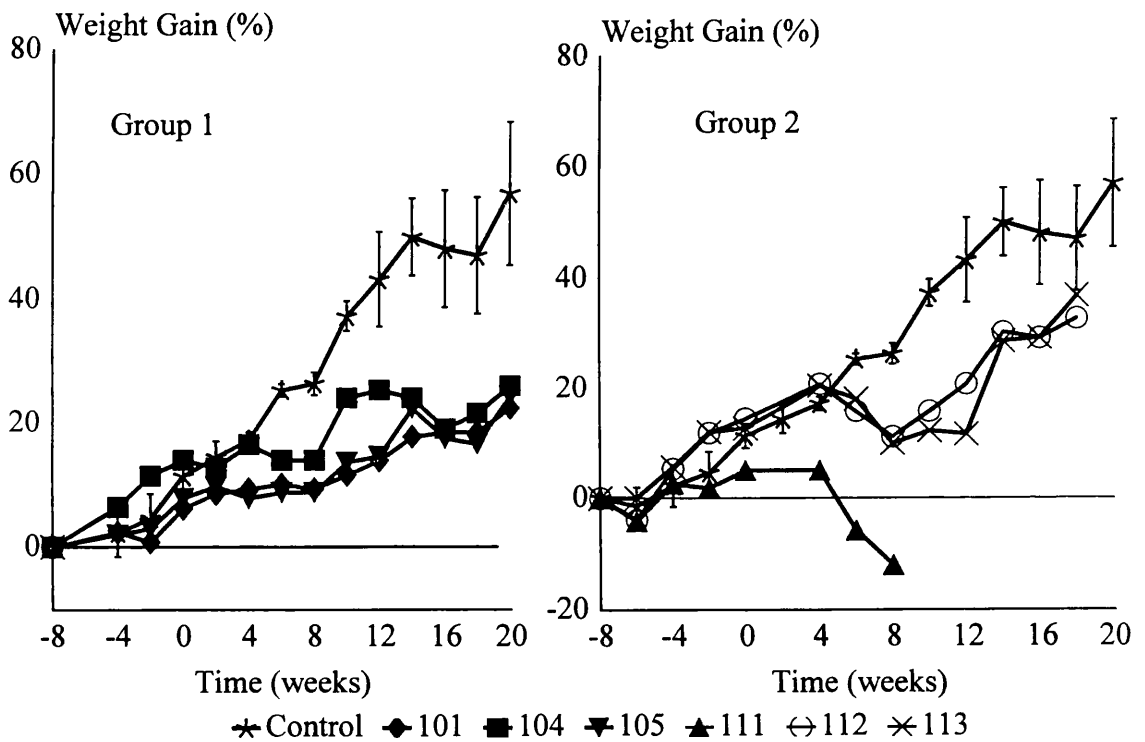


Fig. 4.4: Weight changes (expressed as a percentage of individual weights at 8 weeks pre-infection) of individual ponies following experimental cyathostome infection compared with Control Group mean (±SD).

4.3.4.2 Blood biochemistry

Plasma fructosamine concentrations increased in the control ponies during the course of this study (mean ± SD, 216 ± 32.5 μmol/L at day 0 to 312 ± 17 μmol/L at 20 weeks PI), but decreased, relative to the controls, in both infected groups (**Fig. 4.5**). In the infected groups, fructosamine concentrations, in five ponies, fell below the lowest limit of the

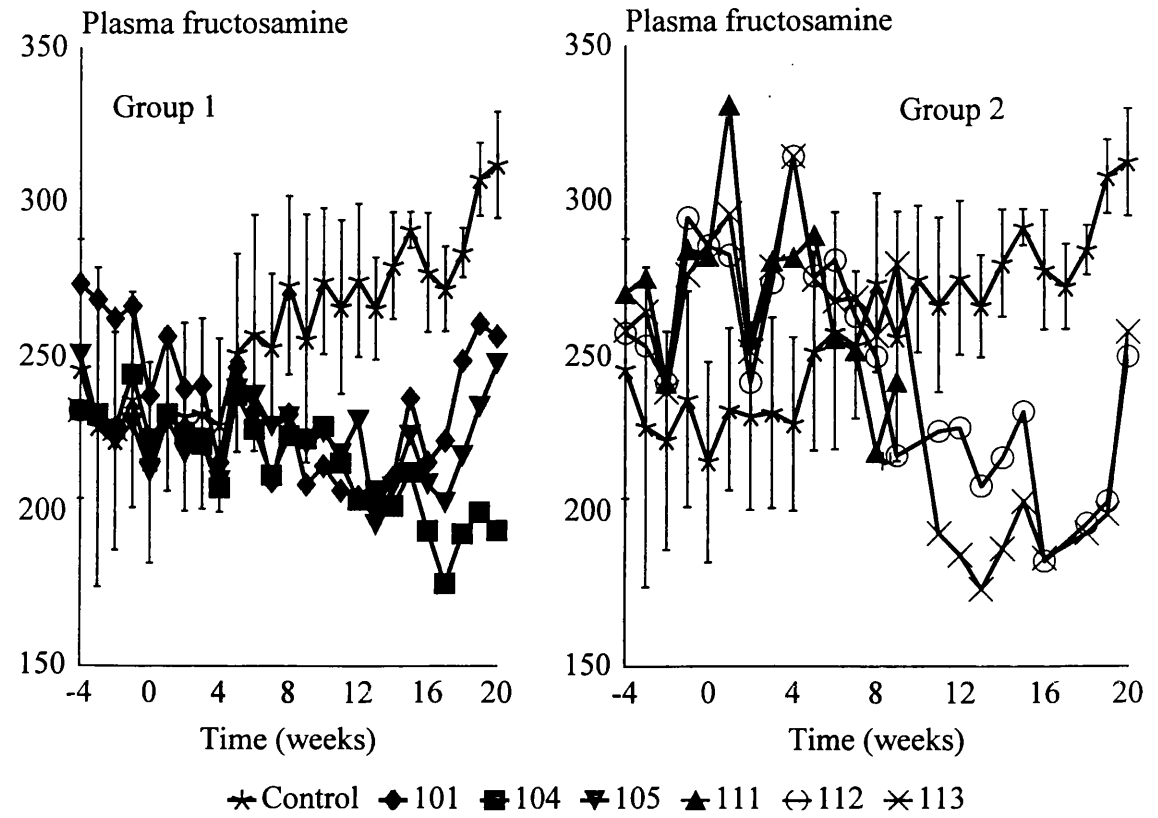


Fig. 4.5: Plasma fructosamine concentrations (μmol/L) in individual ponies following experimental cyathostome infection compared with Control Group mean (±SD).

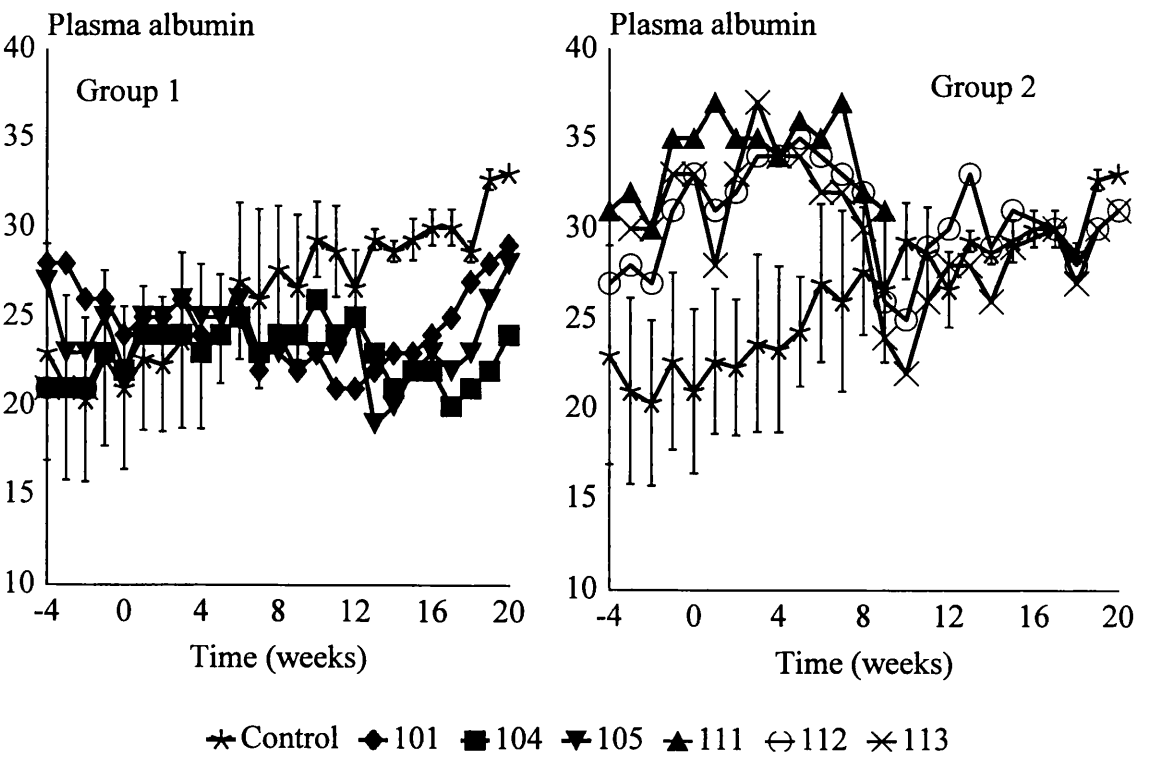


Fig. 4.6: Plasma albumin concentrations (g/L) in individual ponies following experimental cyathostome infection compared with Control Group mean (±SD).

reference range (i.e. 203.8 μmol/L) between 13 and 18 weeks PI. Similarly, plasma albumin concentrations increased in the control group during this study period (mean ± SD, 21 ± 4.6 g/L at day 0 to 33 ± 0 g/L at 20 weeks PI). Visual assessment of **Figure 4.6** shows that changes in plasma albumin concentration were more marked in **Group 1** compared with those in **Group 2**. Notably, no obvious decrease in plasma albumin concentration was detected in pony 111 prior to euthanasia (31g/L).

Increases in α2- (four ponies), β2- (five ponies) and γ–globulin fractions (six ponies) were observed from 4-6 weeks PI, whereas increases in β1-globulin were observed in five animals at a later stage (10-12 weeks PI) in the course of these infections (**Fig. 4.7**).

For all measured parameters, the observed differences in trends were supported by the statistical analyses ($p<0.001$).

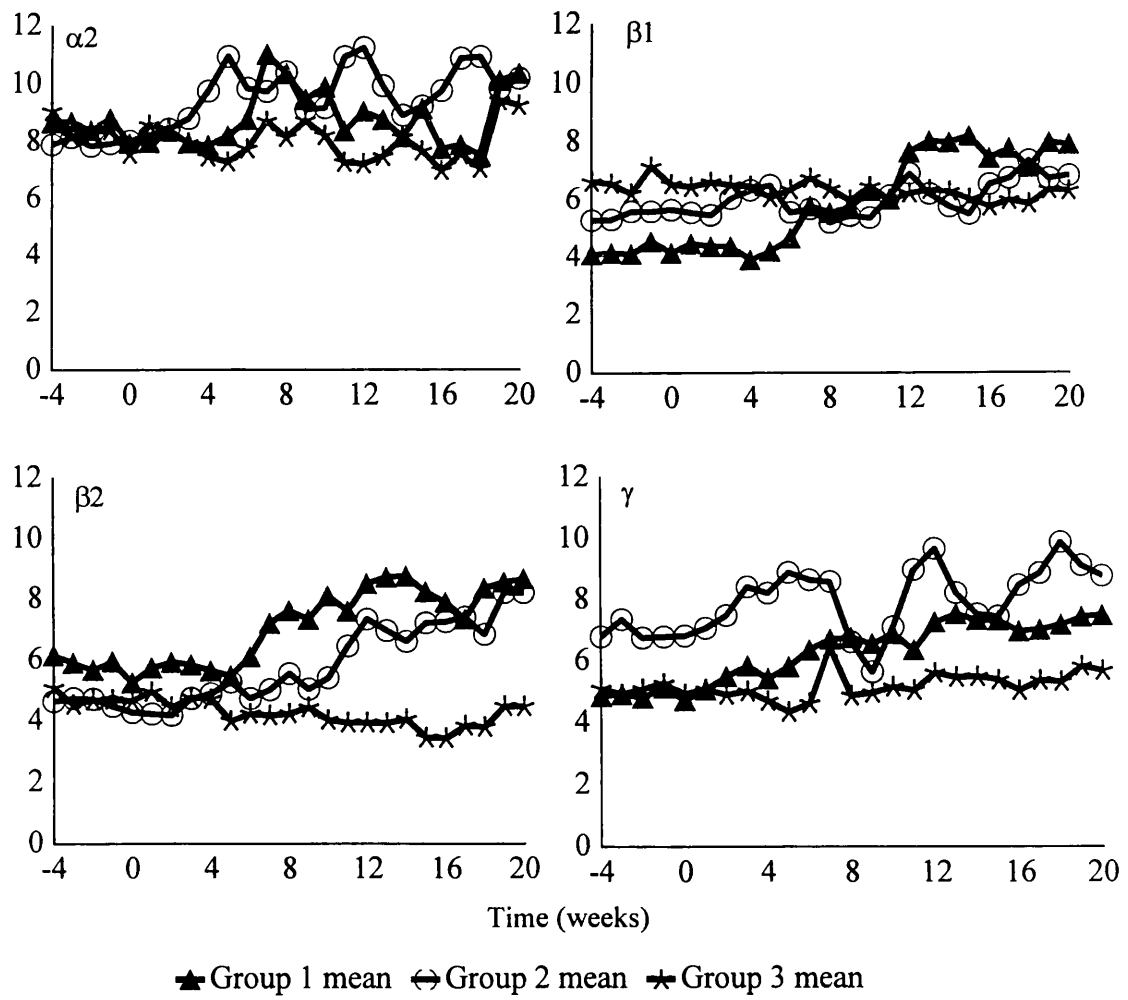


Fig. 4.7: Group mean alterations in serum globulin fractions (α, β1, β2 and γ) (g/L) following experimental cyathostome infection.

4.3.5 Discussion

Parasitism, in particular disease due to cyathostome infection, is recognised as an important cause of gastrointestinal dysfunction in equine animals, which may manifest clinically as weight loss and/or colic and/or diarrhoea (Mair *et al.*, 1990; Uhlinger, 1990; Love, Mair & Hillyer, 1992). The results of the present study demonstrate that cyathostomes are pathogenic to ponies when administered in conditions and at rates considered likely to mimic the uptake of infective larvae by the grazing animal. All infected ponies failed to gain weight at the same rate as the control animals and one pony developed clinical signs attributable to gastrointestinal disease within the 20 week PI study period. In addition, infection resulted in significant changes in numerous constituents of peripheral blood within the first 4-6 weeks of infection indicating that mucosal penetration may contribute to the pathogenic effects which have traditionally been attributed to larval emergence. Although, from the present study, it was not possible to confirm the pathophysiological mechanism of these changes, it is likely that they reflect intestinal mucosal inflammation.

A decrease in plasma fructosamine concentration was detected in both infected groups and became apparent in all animals 4-6 weeks PI. Based on our knowledge of fructosamine formation, the factors which may be responsible for a decrease in plasma fructosamine concentration include increased protein turnover and/or protein loss and/or altered composition of serum proteins and/or prolonged hypoglycaemia. Hypoalbuminaemia is a frequent, but not consistent, feature of naturally-occurring cyathostome infections (Uhlinger, 1991; Love, Mair & Hillyer, 1992), and is assumed to be a consequence of increased intestinal permeability and enteric protein loss (Love *et al.*, 1991). In the present study, there was a decrease in plasma albumin concentration in both infected groups, relative to the control animals, and this feature is likely to have been a major influencing factor on the observed decrease in fructosamine concentration.

In studies in man, alterations in fructosamine concentration occur in patients with thyroid dysfunction in the absence of alterations in serum protein or glucose concentrations and are assumed to reflect changes in the rate of protein turnover (Lloyd & Marples, 1986; Waterson & Mills, 1988). Based on this observation, Heath & Connan (1991) investigated the usefulness of fructosamine measurement as a means of monitoring alterations in protein metabolism in ovine gastrointestinal parasitism, but their findings were not conclusive and the decreases in fructosamine concentrations which they detected could have been due to factors other than increased plasma protein turnover. It is known that in ovine gastrointestinal parasitism marked increases in enteric protein loss and the rate of albumin

turnover may occur in the absence of a marked decrease in serum albumin suggesting that increases in the catabolism of albumin may be compensated for by increased synthesis (Holmes & MacLean, 1971). On this basis, the straight forward measurement of albumin concentrations can give results within normal ranges such that they are relatively unreliable indicators of underlying dynamic intestinal pathogenic processes in sheep. From the results of individual animals in the present study, it would appear that similar effects may arise in equine intestinal parasitism. For example, in pony 111 (Group 2), an obvious decrease in fructosamine concentration was observed PI, suggesting significant disturbances in protein metabolism, however, no concurrent alteration in plasma albumin concentration was detected in this animal. In addition, for the other two animals in Group 2, decreases in plasma albumin concentration were relatively mild compared with the observed decreases in plasma fructosamine concentration.

Much of the data available on changes in serum globulin fractions in cyathostome infections are conflicting. In experimental infections, Love *et al.* (1991) detected no noticeable changes in values of any serum globulin fractions in ponies which received greater numbers of infective cyathostome larvae, whereas Round (1970) detected elevations in β-globulin fraction. Similarly, in natural infections, Giles, Urquhart & Longstaffe (1985) reported increased α- and β-globulin fractions, whereas Mair, Cripps & Ricketts (1993) detected significantly higher values for β1-globulin fraction in horses with acute larval cyathostomosis when compared with horses which were diarrhoeic for other reasons. The results of this study demonstrate that marked alterations in serum globulin composition may occur in animals with cyathostome infection. This feature, presumed to be a consequence of an intestinal inflammatory response, may have an effect on fructosamine concentration, because it is known that certain classes of globulins are more potent glycators than others (Handley, Johnson & Peel, 1993).

Although fructosamine concentrations have been shown to decrease in medical conditions associated with prolonged hypoglycaemia (Cantley, Ford & Heath, 1991), no data is available on possible alterations in the glycaemic status of the infected ponies in the present study. Therefore, the influence of this potential variable on fructosamine concentrations following experimental cyathostome infection is unknown.

In conclusion, the results of this study confirm that cyathostomes are pathogenic to young ponies and that it is possible to assess these effects by sequential clinical measurements. With regard to the use of fructosamine for monitoring alterations in protein turnover, it is possible that it may be a more effective means, than albumin measurement, for

monitoring relatively subtle effects of experimental cyathostome infections which might occur after administration of relatively small inocula of larvae (i.e. low level infection). Similarly, since a proportion of animals with naturally-acquired cyathostomiasis are normoalbuminaemic, it is possible that fructosamine measurement may be a more sensitive indicator than albumin measurement for the purposes of detecting altered protein metabolism in these individuals. Finally, it is possible that fructosamine measurement could be utilised for assessment and/or clinical monitoring of other equine chronic enteropathies.

CHAPTER 5

STUDIES ON THE ORAL GLUCOSE TOLERANCE TEST IN THE HORSE

5.1 Introduction

5.1.1 The oral glucose tolerance test in equine gastroenterology

Carbohydrate absorption studies were first introduced to equine gastroenterology in the early 1970s as a means of evaluating small intestinal function. Loeb, McKenzie & Hoffsis (1972) described the use of a carbohydrate digestion/absorption test for assessment of upper gastrointestinal tract and/or pancreatic function. Following an overnight fast, animals under test were administered 2 lbs of corn starch in four litres of water. Serial blood samples were obtained pretest and hourly for four hours thereafter and analysed for glucose concentration. For normal individuals, a mean peak increase of approximately 40 % above mean basal glucose concentrations was detected one hour post starch administration. Consequently, it was hypothesised that failure to detect an increase in plasma glucose concentration was indicative of either pancreatic disease with decreased amylase secretion/activity or small intestinal malabsorption. In addition, it was suggested that performing an OGTT would facilitate differentiation between pancreatic and small intestinal disease (Loeb, McKenzie & Hoffsis, 1972). Roberts & Hill (1973) described an OGTT for use in the horse and demonstrated its potential for detecting small intestinal malabsorption. In those studies, following an overnight fast, 1 g anhydrous glucose per kg bwt was administered by stomach tube as a 20 % w/v solution. Blood samples were collected at zero, 30, 60, 90, 120, 180, 240, 300 and 360 min and analysed for glucose concentration. In normal individuals, plasma glucose concentrations increased to approximately 200 % of the BGC within 120 min of oral glucose loading and subsequently decreased to pretest concentrations within the six hours sampling period (Roberts & Hill, 1973).

The absorption of glucose by the small intestine is a highly efficient process, whereby the molecule is actively transported against a concentration gradient by the combination of a sodium dependent glucose-galactose specific carrier and an energy dependent sodium pump (Freeman & Quamme, 1986). Therefore, it is assumed that a suboptimal plasma glucose tolerance curve suggests a defect in specific nutrient absorption due to marked morphological and/or functional damage to the small intestinal mucosa. When Roberts & Hill (1973) performed an OGTT on two horses with clinical signs of gastrointestinal tract dysfunction, minimal increases in plasma glucose concentrations were detected and these

animals were subsequently confirmed, at necropsy, as having severe small intestinal pathology.

Only one report has been published which correlates the plasma glucose response in individual horses with post mortem findings (Mair *et al.*, 1991). In that study, OGTTs were performed as described by Roberts & Hill (1973) and the results were classified as either normal or evidence of either partial malabsorption or total malabsorption (Mair *et al.*, 1991). Normal glucose response was defined as tests where the plasma glucose concentration at 60 or 120 min was greater than 185 % of the BGC, whereas a total malabsorption was defined as tests where plasma glucose concentrations at 60 and 120 min showed a lower than 15 % increase above BGC. All horses which exhibited a total malabsorption had small intestinal disease (either alimentary lymphosarcoma or GE) when examined at necropsy (i.e. the test was 100 % specific). However, in a report published at roughly the same time the ability of the OGTT to differentiate between large and small intestinal disease processes was questioned, based on the fact that several horses with confirmed cyathostomosis exhibited carbohydrate malabsorption (Love, Mair & Hillyer, 1992).

5.1.2 Factors which influence the oral glucose tolerance test

It is recognised that a variety of factors, apart from malabsorption, may influence plasma glucose concentrations after oral glucose loading and compromise interpretation of test results. Breukink (1974) emphasised the need to use a standardised test protocol because quantity and concentration of glucose administered and length of starvation prior to the test affect the glucose absorption curve. Food deprivation for 24 to 36 hours was found to reduce glucose absorption in ponies (Breukink, 1974). Although the physiological mechanism(s) responsible for this are unclear, they may involve altered gastrointestinal motility, decreased efficiency of mucosal transport systems or altered insulin function (Freeman, Ferrante, Kronfeld & Chalupa, 1989). Regardless of the precise mechanism involved, it is possible that the results of carbohydrate absorption studies performed in animals deprived of food/anorexic animals, without gastrointestinal disease, could resemble the absorption curves of horses with primary malabsorption syndromes (Freeman *et al.*, 1989). Similarly, it has been demonstrated that maintenance diet may influence the glucose absorption curve. Jacobs & Bolton (1982) detected substantially lower glucose absorption curves in horses fed a stable diet comprised of hay, chaff and a commercial complete feed, when compared with those obtained for pasture-fed horses. These findings prompted the

authors to suggest that the OGTT should be further standardised by feeding a standard diet for 1 week prior to performing the test. Based on the observations of Jeffcott, Field, McLean & O'Dea (1986), it is known that breed/endocrine status will influence glucose metabolism and therefore plasma glucose concentrations post oral glucose loading. These authors noted that higher plasma glucose concentrations were achieved in normal ponies when compared to Standardbreds, with glucose concentrations for both groups returning to pretest concentrations within 360 min. When the OGTTs were performed on ponies which were obese and/or laminitic peak plasma glucose concentrations were higher than those achieved in Standardbreds and glucose levels remained elevated for the remainder of the sampling period. These findings were considered indicative of innate insulin resistance in ponies (Jeffcott *et al.*, 1986) and suggest that separate reference ranges for normal plasma glucose response in horses and ponies should be established.

5.1.3 Other carbohydrate absorption tests in equine gastroenterology

Breukink (1974) and Roberts (1975) documented studies, using a variety of oral disaccharide and monosaccharide tolerance tests, designed to evaluate the efficiency of specific disaccharidase enzymes and sugar transport mechanisms in the equine small intestine. Of these tests, it was considered that the lactose tolerance test was potentially of most clinical value in that it may allow detection of lactase deficiency/small intestinal mucosal damage in diarrhoeic foals. It is recognised that the ability to hydrolyse lactose is severely affected in humans with diffuse intestinal mucosal damage, lactase activity being depressed more than the other disaccharidases and is the last to recover (Roberts, 1975). Therefore, it has been suggested that a reduced tolerance curve may indicate the need to restrict/prevent access to milk for a period to avoid osmotic diarrhoea (Roberts, 1985). As horses mature they lose the ability to hydrolyse lactose, being lactase deficient from 2.5 to 3 years of age (Breukink, 1974: Roberts, 1975) and, therefore, this test procedure is of no diagnostic relevance in the adult equine animal.

The oral D-xylose tolerance test has also been adapted for use in the horse (Roberts, 1974). This test is based on the same principle as the OGTT, in that evidence of abnormal xylose absorption is suggestive of small intestinal pathology (Bolton *et al.*, 1976; Roberts & Norman, 1979). Compared to small intestinal transport of glucose, D-xylose has a low affinity for active transport systems in small intestinal mucosa, which become saturated at low intra-luminal concentrations of xylose (Freeman, 1993). Based on this knowledge, Freeman (1993) indicates that the xylose absorption test should be regarded as a measure of

intestinal mucosal surface area and mucosal permeability rather than as a specific measure of nutrient carbohydrate absorption. Therefore, absorption of this sugar would be expected to occur predominantly by passive mechanisms under conditions of the D-xylose absorption test. Information regarding the clinical value of the xylose absorption test is limited. Merritt, Mallonee & Merritt (1986), extrapolating from studies in other species suggested that assessment of xylose absorptive capacity was a more sensitive indicator of small intestinal disease than the OGTT. Although Brown (1992) reported that the results of the D-xylose tolerance test correlated well with necropsy findings in 11 animals (five horses with normal xylose absorption had no lesions of the small intestine, whereas five of six horses with evidence of xylose malabsorption had either lymphocytic-plasmacytic enteritis or alimentary lymphosarcoma with small intestinal involvement), no data has been published which compares the efficacy of both the OGTT and the oral xylose tolerance test for detecting small intestinal pathology. Furthermore, it has been suggested that xylose is preferable to glucose as a test substrate in carbohydrate absorption studies because it is not normally present in plasma and it is not subject to individual variation in glucose metabolism (Mair *et al.*, 1991). However, the cost of xylose is greater than that of glucose and the availability of xylose analysis is limited such that the OGTT is more commonly employed for the investigation of small intestinal malabsorption.

5.1.4 Aim of studies

The aim of the studies reported in this Chapter was to investigate some of the factors which may influence the OGTT and to assess the efficacy of this technique for detecting small intestinal pathology in the horse.

STUDY 4

5.2 The Effect of Age and Diet on the Oral Glucose Tolerance Test in Ponies

5.2.1 Background

It is generally accepted that factors other than reduced absorptive capacity, due to small intestinal pathology, may influence the plasma glucose response following oral glucose loading. A number of these factors have been investigated previously and include, glucose dose and concentration (Roberts & Hill, 1973), period of starvation prior to the test (Breukink, 1974), breed/type and endocrine events (Jeffcott *et al.*, 1986). In addition, Jacobs & Bolton (1982) reported that altering the diet, from pasture to hay and concentrate or *vice versa*, in the week prior to testing, resulted in significantly different oral glucose tolerance curves. However, for both diets assessed in that study, the plasma glucose response would have been considered 'normal' by conventional standards (i.e. peak glucose concentration > 85% increase above BGC) (Mair *et al.*, 1991).

Although xylose absorptive capacity, following oral D-xylose loading, decreases with advancing maturity in the growing foal (Merritt, Mallonee & Merritt, 1986), the effect of age on the OGTT has not been reported.

5.2.2 Experimental objectives

The objectives of this study were two fold: i) to determine whether significant differences in OGTT results exist between foals and adult ponies when maintained on the same diet and, ii) to evaluate alterations in the OGTT when adult ponies are maintained on a hay diet compared with when maintained on a 'complete' pelleted ration.

5.2.3 Materials and methods

5.2.3.1 Animals and experimental design

Fourteen British native-breed ponies were used in this study (**Table 2.1**). All animals were considered to be healthy based on history and clinical examinations. The ponies were assigned to two groups: Group A (6 - 9 months) and Group B (6 - 13 years). All ponies in Group A were reared indoors with their dams during which time they were bedded on straw and had free access to hay. Following weaning at four months, they were maintained on a high fibre pelleted ration, fed twice daily, and bedded on woodshavings. Oral glucose tolerance tests were performed on this group on three occasions over a two-week period. The animals in Group B were stabled for at least six months prior to the beginning of this study, during which time they were fed hay only (**Diet 1**) and bedded on barley straw.

Subsequently, these animals were changed to a high fibre pelleted ration (**Diet 2**), fed twice daily, and bedded on shredded paper. OGTT's were performed on all animals in Group B while maintained on Diet 1, then on Diet 2. Tests were repeated on each animal on three occasions while on each diet. A period of 4 weeks was allowed, for acclimatisation to Diet 2, before tests were carried out. Each pony was weighed on a weekly basis and these body weights were used to determine the required amount of glucose. When tests were repeated on individuals, at least 72 hours were allowed between tests.

5.2.3.2 Oral glucose tolerance tests

A standard OGTT protocol was established based on that described by Roberts & Hill (1973), and performed as outlined in *Section 2.2.3*. All samples were analysed for glucose concentration on the day of collection.

5.2.3.3 Statistical analyses

The effect of age on the OGTT was analysed by two-factor ANOVA. A repeated measures design was used with the pony treated as a random effect nested within age group. Comparison of the OGTT results for the adult ponies, when maintained on different diets, was performed using two-factor ANOVA with repeated measures with the pony, again, treated as a random effect and without nesting. In both cases, each replicate was analysed separately. Paired Student's t-tests were used to test for statistical significance between groups for the glucose concentration at each sampling time. In order to assess possible weight alterations in the adult ponies due to the change in diet, mean weights while on Diet 1 were compared to mean weights while on Diet 2 by a paired student's t-test. For all analyses, significance was set at the 5% level.

5.2.4 Results

Data which contributed to the results presented in this section are detailed in Appendices 14, 15 and 16.

When analysis of the OGTTs was performed on the foal and adult ponies, maintained on the same diet, there was a significant difference between the groups in their response over time ($p<0.05$) (i.e. a significant interaction between age and time for each replicate). No significant difference was detected between groups for BGC. However, from 30 min until 240 min post oral glucose loading, plasma glucose concentrations were consistently lower for the adults (peak glucose concentrations (Glu_{MAX}) (mean ± SD) = 6.8 ± 1.3 mmol/L at 90

min) when compared to the foals (Glu_{MAX} = 11.5 ± 1.3 mmol/L at 90 min) (**Fig. 5.1**). For adult animals, plasma glucose concentrations returned to pre-test values by 240 min, whereas, for the foals, return to pre-test concentrations occurred at 300 min. In addition, for the foal group, glucose concentrations were significantly lower at 360 min ($p<0.001$) when compared to those recorded for BGC. For 15 of the 21 OGTTs performed on the foal group, plasma glucose concentrations of 3 mmol/L or less were detected at 300 and/or 360 min.

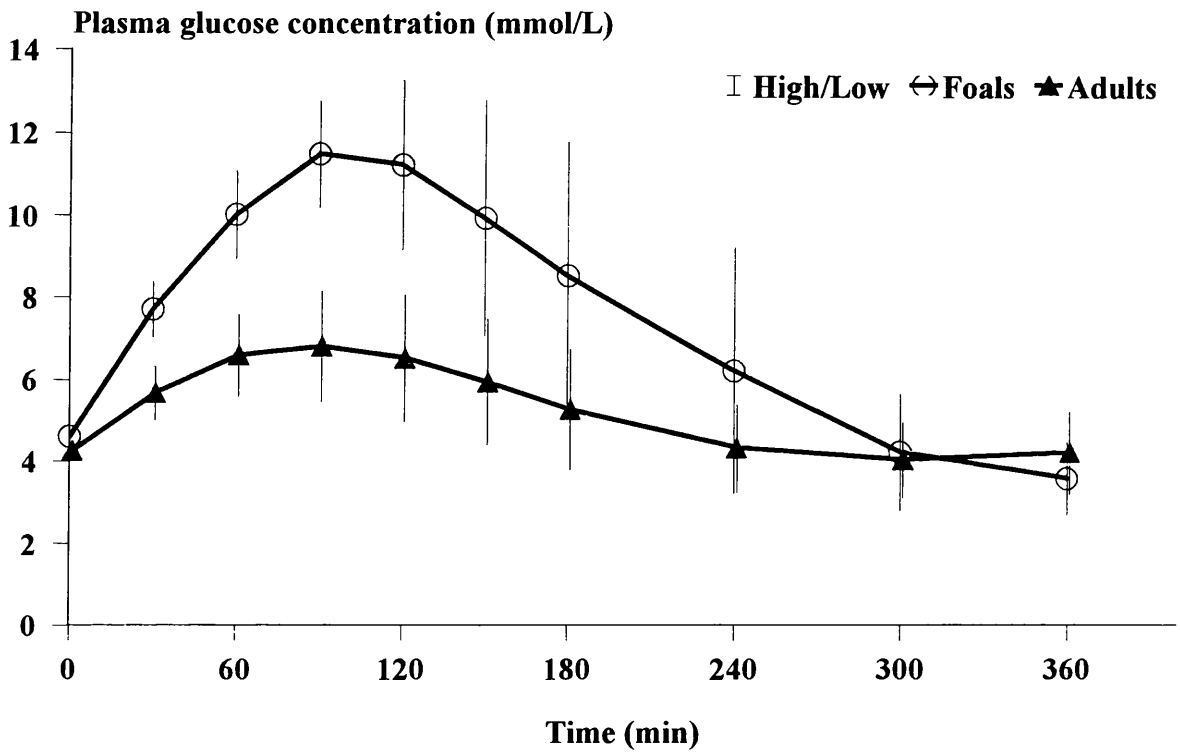


Fig. 5.1: Mean (±SD) plasma glucose concentrations, following the administration of 1g glucose/kg bwt to seven adult and seven pony foals when maintained on Diet 2 (pelleted ration).

When comparing the results of OGTT's performed on the adult ponies, when maintained on different diets, a significant difference in the plasma glucose response over time was detected ($p<0.05$) (i.e. a significant interaction between diet and time). Glucose concentrations at 0 min were significantly greater for Diet 1 ($p<0.01$) when compared to Diet 2. In general, ponies maintained on Diet 1 had a higher plasma glucose response, following oral glucose loading, and Glu_{MAX} occurred later (9.6 ± 2.1 mmol/L at 150 min) when compared to Diet 2 (6.8 ± 1.3 mmol/L at 90 min) (**Fig. 5.2**). In addition, when on Diet 1, the mean plasma glucose concentration returned to pre-test values at 360 min, compared to 240 min for Diet 2. When maintained on the hay diet, assessment of individual pony responses to oral glucose loading indicated that Glu_{MAX} exceeded 11.0 mmol/L in

three animals (two ponies on two occasions, one pony on one occasion), and on four of the 21 occasions that the test was performed plasma glucose concentrations had not decreased below 6 mmol/L by 360 min post-glucose administration.

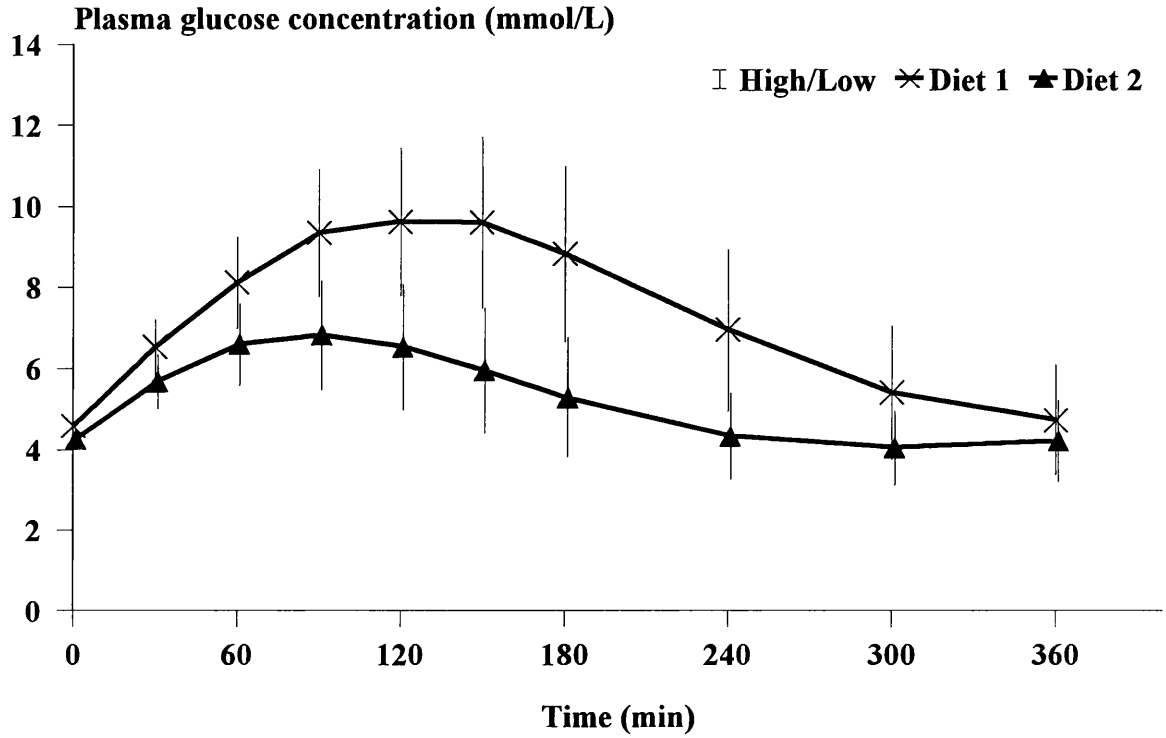


Fig. 5.2: Mean (±SD) plasma glucose concentrations, following the administration of 1g glucose/kg bwt to seven adult ponies, when maintained on Diet 1 (hay diet) compared with when maintained on Diet 2 (pelleted ration).

Analysis of adult pony weights indicated that the ponies were significantly heavier ($p<0.05$) at the time of performing the OGTT's on Diet 2 compared to Diet 1 (**Fig. 5.3**).

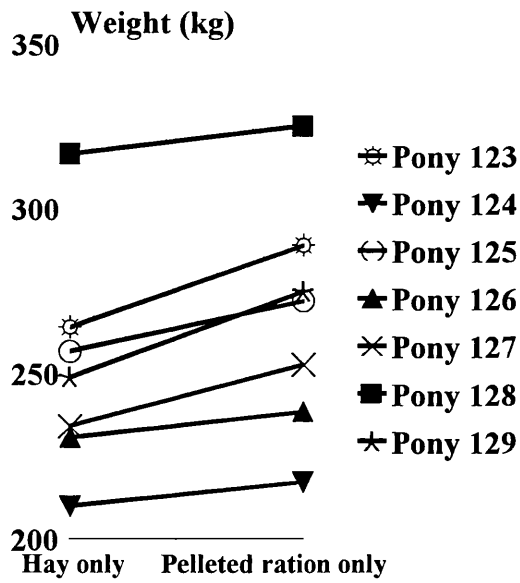


Fig. 5.3: Mean body weight of the individual adult ponies when maintained on the hay diet compared with when maintained on the pelleted ration only.

5.2.5 Discussion

The results of this study indicate that both age and diet have significant effects on plasma glucose concentrations, measured during an OGTT.

In this study there were marked effects of diet on the OGTT: The mean peak plasma glucose concentration of ponies, when maintained on the pelleted ration, represented an increase of only 68 % above basal glucose concentrations. Jacobs & Bolton (1982) were first to demonstrate an effect of diet on the OGTT. They reported that stabled horses, receiving a diet of a commercial complete feed, oats and alfalfa chaff, had significantly lower glucose tolerance curves compared to those obtained when animals were maintained at pasture, which led them to suggest that diet influenced either the amount of glucose which was absorbed or glucose metabolism (Jacobs & Bolton, 1982).

The effect of diet on glucose metabolism has been acknowledged since the early 1970's, when Argenzio & Hintz (1972) observed greater glucose availability and more efficient glucose utilisation when ponies were maintained on an oat diet compared with when receiving a high-fibre diet of alfalfa/beet pulp, despite the fact that the latter diet supplied greater digestible energy. In addition, plasma glucose concentrations while on the grain diet were less than those measured while on the roughage diet. These results demonstrated the marked effect of the type of diet on glucose metabolism and may explain, in part, the differences observed in BGC and the glucose response following oral glucose loading when maintaining ponies on different diets. The increased weight observed in these animals when fed Diet 2 was most likely due to increased energy intake, but analysis of nutrient intake by individual adult animals, while on each diet, was not performed.

Based on the classification system described by Freestone, Shoemaker, Bessin & Wolfsheimer (1992), adult ponies with peak plasma glucose concentrations exceeding 11.1 mmol/L, following oral glucose loading are defined as having impaired glucose tolerance. Using this criterion, three adult ponies in this study when maintained on the hay diet exhibited evidence of impaired glucose tolerance. In a different study, Freestone, Beadle, Shoemaker, Bessin, Wolfsheimer & Church (1992), reported that rested ponies fed a complete pelleted feed ration, *ad libitum*, lost weight and showed evidence of improved insulin sensitivity. Although animals in this study gained weight while maintained on a complete pelleted diet, fed twice daily, impaired glucose tolerance was not observed and, on all occasions, plasma glucose concentrations returned to pre-test values within the six hour sampling period (i.e. in this study, an apparent improved insulin sensitivity was observed on the pelleted versus the hay diet). Therefore, it is possible that, regardless of body condition

status, the type of diet may influence insulin response/sensitivity and might account for the observed differences in plasma glucose response. Interestingly, Smyth, Young & Hammond (1989) reported that, although postprandial insulin secretion in horses is affected by dietary composition, the exocrine pancreas readily adapts to changes in dietary composition. Their results suggest that insulin response (output) following oral loading with a particular dose and concentration of glucose should not be influenced by maintenance diet.

Other dietary factors which may affect the plasma glucose response in healthy ponies, following oral glucose loading are those which result in altered absorption of glucose, such as, changes in intestinal motility/transit time and/or changes in bacterial populations in the small intestine and/or changes in mucosal cell function/glucose transport mechanism. Clarke, Roberts & Argenzio (1990) suggested that bolus feeding of concentrate meals will result in increased small intestinal transit of ingesta, due to increased bulk of ingesta and/or alterations in the intrinsic motor activity of the gut, when compared to a steady feeding state. Consequently, if the pattern of small intestine motor activity varies with diet, this would be expected to alter the efficiency of small bowel digestion/absorption and result in a variable proportion of soluble carbohydrate reaching the large intestine. Similarly, changes in diet have been shown to alter microbial populations and the fermentation process in the equine large intestine (Kern, Slyter, Weaver, Leffel & Samuelson, 1973; Willard, Willard, Wolfram & Baker, 1977). More recently, Mackie & Wilkins (1988) demonstrated that substantial populations of bacteria are present in the small intestine of grass-fed horses and that they contribute to the digestion of carbohydrates. Therefore, it would seem reasonable to suggest that dietary alterations will also influence microflora populations in the small intestine which could, presumably, influence the amount of glucose available for absorption from the gut lumen. In addition, variation in diet may influence intestinal/mucosal function. Mathers, Kennard & James (1993), when summarising the effects of diet on gut function, concluded that the amount and composition of a diet can have a major influence on mucosal proliferation at many sites in the gastrointestinal tract, but the mechanisms involved are not apparent. Similarly, alterations in gut flora in experimental animals are reported to have a profound effect on mucosal villous shape (Creamer, 1967).

The results of this study indicate that age also influences plasma glucose response following oral glucose loading and that significant differences exist between adults and ponies 6 - 9 months of age. Previously, Merritt, Mallonee & Merritt (1986) reported that xylose absorptive capacity decreased in growing foals and was similar to that of the adult horse by three months of age. Comparing the results of that study with those of the present

study, it would appear that there may be differences in the rate of maturation of the equine intestine with regard to mucosal-glucose and mucosal-xylose interactions.

Because the principle source of energy for the suckling foal is lactose compared with enzymatic/fermentative products of complex carbohydrates in adults, differences in carbohydrate metabolism may exist between suckling foals and adults, which could contribute to the differences reported here, despite the fact that these foals had been weaned for at least three months. It was notable that, for the foal group, plasma glucose concentrations at 360 min were significantly lower than those recorded pre-test. It is possible that the greater peak plasma glucose concentrations achieved in the foals resulted in a relatively greater stimulus for insulin secretion and consequently more rapid normalisation of plasma glucose concentrations, which frequently resulted in an over-swing to relative hypoglycaemia.

Because both foals and adults were managed under the same conditions (i.e. stabled, fed the same diet twice daily) for at least one month prior to performing the repeated OGTT's, we assume that factors such as intestinal motility and luminal flora would not be dissimilar between the groups and should not be expected to account for the differences observed in glucose tolerance. The differences may be explained by age-related changes in enterocyte function/transport mechanisms which may be responsible for altered intestinal absorption with advancing maturity as proposed by Merritt, Mallonee & Merritt (1986). In both man and rats it is known that there is an age-related decline in the digestive capacity of the small intestine (Feibusch & Holt, 1982; Mathers, Kennard & James, 1993), reduction of active sugar absorption (Esposito, Faelli, Tosco, Orsenigo & Battistessa, 1985; Freeman & Quamme, 1986; Beaumont, Cobden, Sheldon, Laker & James, 1987) and decline in intestinal blood flow (Csaky & Varga, 1975; Richey & Bender, 1977). Although similar alterations may have contributed to the differences observed in glucose tolerance between the foal and adult pony groups of the current study, these features were not specifically investigated. Moreover, it should be borne in mind that age-related change in dietary function often reflects transition to a geriatric state rather than comparisons between young/adolescent and mature adult groups as were performed in the present study. In the horse, such changes have not been documented, although it is known that alterations in enzymatic digestion occur as horses mature (i.e. decline in lactase activity) (Roberts, 1975).

Although the factors responsible for age- and diet-related alterations in the OGTT cannot be clearly defined in this study, the results indicate that when performing the OGTT for assessment of gastrointestinal function in equine animals, the resulting tolerance curve

should be compared with tests for clinical healthy animals of the same age group and with a similar dietary history.

STUDY 5

5.3 A Modified Oral Glucose Tolerance Test as an Indicator of Small Intestinal Pathology in the Horse

5.3.1 Background

In the horse, diseases of the small intestine resulting in malabsorption are relatively uncommon, but when they present they can be severely debilitating, difficult to diagnose and carry a guarded to poor prognosis. Chronic inflammatory bowel diseases (e.g. GE, EGE, lymphocytic-plasmacytic enteritis) and alimentary lymphosarcoma account for the majority of true malabsorptive states (Mair *et al.*, 1991). Numerous other recognised and potential causes have been documented and include idiopathic villous atrophy, verminous arteritis, intestinal ischemia, mucosal biochemical abnormalities, mucosal oedema and lymphangiectasia (Bolton *et al.*, 1976; Roberts, 1985; Murray, 1989; Milne *et al.*, 1994). The predominant presenting clinical feature in cases of small intestinal malabsorption is chronic weight loss and, occasionally, horses may not exhibit specific physical signs of intestinal dysfunction. The OGTT is commonly used for the investigation of such cases, in particular for assessing small intestinal absorptive capacity. However, only limited information is available with regard to the reliability of the OGTT to detect small intestinal pathology and the relative incidence of different diseases that cause malabsorption in the horse. In addition, although the test is simple to perform and assay, repeat blood sampling over a six hour period is often impractical in a practice situation.

5.3.2 Study objective

The objective of this retrospective study was to assess the sensitivity and specificity of a 'modified' OGTT for detecting/excluding a diagnosis of small intestinal pathology in the horse. The 'modified' OGTT was based on a single sample taken at 120 min post glucose administration and this was compared to serial sampling for the conventional six hour test period.

5.3.3 Materials and methods

5.3.3.1 Animals

The authors reviewed the records of 50 consecutive cases which had been subjected to an OGTT, performed as described by Roberts & Hill (1973), for investigation of either chronic weight loss and/or chronic diarrhoea and/or chronic colic. These animals were a mixed population of adult (>1 year) equidae, with variable dietary histories. Data from cases

which did not have a full post mortem examination were excluded (n=27). In two individual tests BGC was ≥6 mmol/L and these were excluded from the analysis due to the difficulty interpreting subsequent plasma glucose response. The remaining OGTT results from 21 necropsied cases were correlated with post mortem findings, that is, either the presence or absence of small intestinal pathology as determined by histopathological examination using standard techniques.

5.3.3.2 Data analyses

For each of these individual tests two values were determined: peak plasma glucose concentration, regardless of the time post glucose administration (A, i.e. 'conventional' OGTT), and plasma glucose concentration at 120 min post glucose administration (B, i.e. 'modified' OGTT). Both (A) and (B) were expressed as a percentage increase above BGC. Using each of these values the sensitivity and specificity of the OGTT was assessed over a range of cut-off values from a 15 % to 50 % increase above BGC, where obtained values less than each cut-off level were taken to indicate 'total' malabsorption (**Table 5.1**).

	Small intestinal pathology present	Small intestinal pathology absent
Total malabsorption	W true positive	Y false positive
'Normal' OGTT	X false negative	Z true negative
	W + X	Y + Z

Sensitivity = W/W+X
Specificity = Z/Y+Z

Table 5.1: Determination of sensitivity and specificity.

5.3.4 Results

The characteristics of individual OGTTs included in this study are detailed in Appendix 17.

Of the 21 cases from which OGTTs were analysed, histopathological confirmation of small intestinal pathology was evident in 11 animals; CIBD and alimentary lymphosarcoma accounted for abnormal small intestinal histology in nine of these cases (**Table 5.2**). Small intestinal pathology was absent in 10 cases, two of which had no pathological abnormality detected at post mortem examination (**Table 5.2**). The sensitivities and specificities of the

OGTT over the range of cut-off values, using either the peak plasma glucose concentration (A) or the plasma glucose concentration at 120 min (B), are shown in **Table 5.3**. When total malabsorption was defined as a less than 15 % increase over BGC, the OGTT, using either of the measurements (A) or (B), was 90 % specific (i.e. one false positive of 10 cases without small intestinal pathology); however, the sensitivity of the test using method (A) was only 9 % compared to 45 % when using method (B) (i.e. use of method (B) allowed detection of a higher proportion of true positives). Using a cut-off level of a 20 % increase above BGC in conjunction with method (B) resulted in the sensitivity of the test increasing to 64 %, without a deleterious effect on specificity. Using these criteria, the causes of 'total' malabsorption which were identified included three cases of CIBD, two of alimentary lymphosarcoma, one coccidial enteropathy and one non-specific enteropathy. In one animal with a 'total' malabsorption no abnormality of the small intestine was detected (i.e. false positive): The post mortem findings in this case included, poor dentition, partial gastric impaction and a substantial caecal/colonic mucosal cyathostome burden. Although increasing the cut-off to a level greater than 20 % increase above BGC improved the sensitivity of the test there was a concurrent marked decrease in specificity.

Pathological Diagnosis	SI pathology present	SI pathology absent
Alimentary lymphosarcoma	3	1
Chronic inflammatory bowel disease	6	1
Cyathostomosis	0	1
Poor dentition/partial gastric impaction/cyathostomosis	0	1
Coccidial enteropathy	1	0
Pyloric stenosis	0	1
Peritonitis	0	1
Equine motor neuron disease	0	1
Non-specific SI enteropathy	1	0
Non-specific colitis	0	1
No post mortem diagnosis	0	2
Total	11	10

Table 5.2: Post mortem histopathological findings in 21 adult horses investigated because of chronic weight loss and/or chronic gastrointestinal dysfunction. SI = small intestine.

'cut-off' level* % increase above BGC	15	20	25	30	35	40	45	50
(A) Sensitivity %	9	18	27	54	73	73	73	73
Specificity %	90	90	80	80	80	70	50	50
(B) Sensitivity %	45	64	64	73	73	73	73	82
Specificity %	90	90	60	60	60	50	50	50

Table 5.3: The sensitivities and specificities of the conventional (A) and the modified (B) OGTT for detecting/excluding a diagnosis of small intestinal pathology over a range of cut-off levels from a 15 % to a 50 % increase above basal glucose concentration (BGC).
*Less than each cut-off level is interpreted as 'total' malabsorption.

5.3.5 Discussion

The results of the present study indicate that the 'conventional' OGTT protocol offers no advantage over the 'modified' test based on a single sample taken at 120 minutes following oral glucose administration. This finding has both practical and economic implications, because the 'modified' test is more conducive to the practice situation and the numbers of blood samples to be analysed for glucose is reduced from the conventional nine to just two. When the criteria for 'total' malabsorption is defined as failure of the plasma glucose concentration at 120 min to increase greater than 15 % above BGC, the results of this study (specificity = 90 %, sensitivity = 45 %) are similar to those reported by Mair *et al.* (1991) (specificity = 100 %, sensitivity = 40 %); however, based on the present data, increasing the cut-off to a 20 % increase above BGC allows for greater sensitivity without affecting the specificity of the modified test. In addition, Mair *et al.* (1991) stated that a total malabsorption was indicative of either alimentary lymphoma or GE. Although this was not a consistent finding in the present study, it is true that the majority of cases which exhibit total malabsorption have severe infiltrative conditions of the small bowel and, consequently, this feature is an indicator of poor prognosis.

The reason for detection of total malabsorption in one case in the present study in which small intestinal pathology was absent is unclear. It is recognised that delayed gastric emptying may interfere with the normal plasma glucose response following oral glucose loading (Roberts, 1985) and may possibly have been a factor which contributed to the abnormal OGTT results detected in this case. It is important to consider that since detection of 'total' malabsorption in a clinical setting is likely to lead to a recommendation

of euthanasia of the affected case, the necessity for a test to avoid "false positives" is of critical importance: The consequences of misclassifying an animal as negative are much less than the converse situation. In the present study, the specificity of the test was not compromised by using the modified protocol.

In conclusion, the results of the present study indicate that modification of the OGTT to a two sample test (i.e. 0 and 120 min) is appropriate and that the test, in this form, constitutes a useful, practical and economic investigative technique for assessing small intestinal absorptive capacity, where identification of total malabsorption is suggestive of extensive small intestinal pathology and a poor prognosis.

CHAPTER 6

BREATH HYDROGEN MEASUREMENT FOR ASSESSMENT OF GASTROINTESTINAL TRACT FUNCTION IN PONIES

6.1 Introduction

6.1.1 The origin of hydrogen excreted in breath

Nielsen (1961), when investigating the origin and nature of bean-related flatulence, demonstrated that after feeding baked beans to volunteers hydrogen appeared in exhaled breath and that the rise in breath hydrogen was coincident with the subjects' abdominal discomfort. Similar studies performed by Calloway (1966) indicated that breath hydrogen measurements were reproducible, substrate related and correlated to some extent with symptoms of abdominal discomfort. Formal studies of the site and rate of hydrogen production were performed by Levitt (1969). The results of these studies indicated that hydrogen production in the fasting state was negligible at all sites in the intestine, but after direct installation of a fermentable carbohydrate (lactose) into the gastrointestinal tract of healthy individuals, hydrogen concentration increased and was produced almost entirely in the colon. In addition, it was demonstrated that respiratory hydrogen excretion was linearly related to intestinal production, averaging 14 % of the total. These findings led the author to conclude that breath hydrogen measurement can be used as a reliable indicator of intestinal hydrogen production which, in the healthy human subject, is primarily dependent upon the delivery of ingested fermentable substrates to large intestinal flora. (Levitt, 1969).

6.1.2 Clinical applications of breath hydrogen measurement in human gastroenterology

Calloway, Murphy & Bauer (1969) and Levitt & Donaldson (1970) reported on the application of breath hydrogen measurement to detect intestinal lactase deficiency in man and this was the first breath test to be accepted for extensive clinical use. The results of breath hydrogen analysis after lactose ingestion have been found to correlate well with lactose tolerance tests using blood sugar analysis (Calloway, Murphy & Bauer, 1969; Levitt & Donaldson, 1970; Newcomer, McGill & Thomas, 1975) and with measurements of lactase specific activity in intestinal mucosal biopsies (Newcomer, McGill & Thomas, 1975). Furthermore, Bond & Levitt (1972) used this technique to demonstrate glucose malabsorption in partially gastrectomised patients. In addition, these authors indicated that by administering a measured dose of lactulose (1,4-b-galacto-fructose), a semi-synthetic

non-absorbable disaccharide, and determining the resulting breath hydrogen excretion in each subject and comparing this with the hydrogen produced after the glucose load, the amount of carbohydrate malabsorbed could be estimated (Bond & Levitt, 1972). Therefore, it was proposed that breath hydrogen measurement could serve as a semi-quantitative indicator of carbohydrate absorption. More recently, Casellas, Chicharro & Malagelada (1993) reported that the breath hydrogen test, using D-xylose as a test substrate, was a useful and practical test for the diagnosis and follow-up of malabsorption in man due to either non-tropical sprue, lymphoma, Whipple's disease or giardiasis: Test performance analysis indicated that the test had a sensitivity of 86 % and a specificity of 100 % for the detection/exclusion of small intestinal malabsorption.

In addition to the use of breath hydrogen measurement for assessment of carbohydrate malabsorption, the other main clinical application of this technique in man is for the investigation of small intestinal bacterial overgrowth. Metz *et al.* (1976a) assessed the patterns of breath hydrogen excretion after oral glucose administration to patients suspected of having bacterial colonisation of the small intestine and these were correlated with bacteriological examinations of jejunal juice aspirates. Rapid (i.e. within two hours of post-glucose administration) and transient increases in breath hydrogen concentration (to levels greater than 20 ppm) were considered indicative of fermentation within the small intestine since no increase in breath hydrogen excretion would be expected following the administration of glucose to normal subjects. These authors reported that the test had a sensitivity of 66 % and a specificity of 100 % for the detection/exclusion of positive jejunal bacterial cultures (Metz *et al.*, 1976a). However, subsequent reports evaluating the diagnostic potential of this technique documented wide ranging results with sensitivities and specificities as low as 36 % (Taylor, Avgerinos, Taylor, Hill & Misiewicz, 1981) and 73 % (O'Connor, Healy, Kehely, Keane, O'Moore & Weir, 1987), respectively. Using the glucose-hydrogen breath test, Corazza, Menozzi, Strocchi, Rasciti, Vaira, Lecchini, Avanzini, Chezzi & Gasbarrini (1990) obtained sensitivity and specificity values of 62 % and 83 %, respectively: These authors concluded that examination of jejunal juice was still the preferred diagnostic technique for small intestinal bacterial overgrowth in man.

6.1.3 Clinical applications of breath hydrogen measurement in veterinary medicine

In the past 10 years, breath hydrogen measurement has been applied to the clinical investigation of gastrointestinal disorders in calves, dogs and cats. Holland, Herdt & Refsal

(1986) investigated the clinical usefulness of breath hydrogen measurement as a means of assessing chloramphenicol-induced carbohydrate malabsorption in preruminating calves. They observed that, compared to base-line data, chloramphenicol administration resulted in a significant decrease in intestinal villus length and xylose absorption, with a concurrent significant increase in the concentration of hydrogen excreted in breath following ingestion of a milk feed. Based on these findings, they concluded that this technique may be useful in evaluating malabsorption in calves with naturally occurring enteric disease (Holland, Herdt & Refsal, 1986). Subsequently, this group reported that breath hydrogen measurement facilitated detection of carbohydrate malabsorption in preruminating calves experimentally infected with *Cryptosporidium* spp. (Holland, Herdt & Refsal, 1989). Similarly, Washabau, Strombeck, Buffington & Harrold (1986b) demonstrated that hydrogen concentration increased in breath following lactulose administration to healthy dogs and that there was a quantitative relationship between the amount of unabsorbable carbohydrate ingested and hydrogen excretion. In addition, these authors found that although breath hydrogen excretion after the ingestion of a commercial hypoallergenic diet did not differ significantly from that after fasting, the addition of wheat or corn flour to this diet significantly increased breath hydrogen excretion. Based on these results, it was suggested that wheat and corn appear to be incompletely assimilated in healthy dogs and they may be inappropriate dietary constituents in dogs with clinical evidence of carbohydrate malabsorption (Washabau *et al.*, 1986b). Further work by this group indicated that breath hydrogen measurement could identify carbohydrate malabsorption in dogs with pancreatic exocrine insufficiency or chronic small intestinal disease, and that this technique was a more sensitive means of identifying carbohydrate malabsorption in dogs with clinical disease than xylose absorption testing (Washabau *et al.*, 1986a). Moreover, in pancreatic exocrine insufficient dogs, pancreatic enzyme therapy partially corrected carbohydrate malassimilation, as evidenced by a significant decrease in hydrogen excretion when compared to pre-therapy data (Washabau *et al.*, 1986a). In the cat, breath hydrogen measurement has been used to evaluate carbohydrate malassimilation in healthy cats after oral antibiotic treatment (Muir, Gruffydd-Jones, Cripps & Brown, 1996) and carbohydrate malassimilation in clinical cases with intestinal disease (Muir, Gruffydd-Jones, Cripps, Papasouliotis & Brown, 1994). The results reported by Muir *et al.* (1994) indicate that, as was shown in canine studies, breath hydrogen measurement may be a more reliable indicator of carbohydrate malassimilation than the xylose tolerance test.

To date, there is only a single report which describes the application of breath hydrogen measurement to the investigation of clinical disease in the horse: Murphy, Howie & Love (1994) reported rapid and transient increases in breath hydrogen concentration which was assumed to be a consequence of gastric fermentation, following the administration of xylose and lactulose to a horse with pyloric stenosis.

6.1.4 Assessment of gastrointestinal transit by breath hydrogen measurement

Although not directly applicable to the investigation of individual clinical cases, breath hydrogen measurement following a test meal is commonly employed in physiological studies as a simple, non-invasive means of assessing mouth-to-caecum transit time (MCTT), where MCTT is defined as the time between meal ingestion and the detection of a significant and sustained increase in breath hydrogen excretion (Bond & Levitt, 1975). This technique is routinely used in human studies and has been applied to the assessment of MCTT in dogs (Papasouliotis, Gruffydd-Jones, Sparkes & Cripps, 1995) and cats (Muir *et al.*, 1991; Papasouliotis, Gruffydd-Jones, Galloway & Smerdon, 1993).

6.1.5 Aim of studies

The overall aim of the studies reported in this Chapter was to investigate the potential for use of breath hydrogen measurement as either a diagnostic and/or research technique in equine gastroenterology.

STUDY 6

6.2 Breath Hydrogen Measurement for Assessment of Small Intestinal Function in Ponies

6.2.1 Background

Information on the patterns of breath hydrogen excretion in equidae is limited. Zentek (1992) documented preliminary data which indicated that hydrogen was excreted in the breath of adult horses following the ingestion of either hay and/or oats and that the concentrations of hydrogen in breath were dependent on the quantity and quality of feed ingested. However, these studies had not been reported in full and a detailed account of the test protocol and results were not available. More recently, breath hydrogen measurement has been used to evaluate developmental changes in alimentary tract function of young foals (Bracher *et al.*, 1995). These authors suggested that repeated breath hydrogen measurements may reflect the changes in alimentary tract function occurring during the post-natal adaptation period. However, there was great variation in hydrogen excretion profiles amongst animals such that it was not possible to draw definite conclusions from these studies.

6.2.2 Experimental objective

The objective of the study was to assess the patterns of breath hydrogen excretion in adult ponies following either voluntary consumption or the administration per stomach tube of different test meals/carbohydrate substrates.

6.2.3 Materials and methods

6.2.3.1 Animals

Seven British native-breed ponies were used in this study (**Table 2.1**). This group ranged in age from six to 13 years and in weight from 210 to 320 kg. All animals were considered to be healthy based on history and clinical examinations. The ponies were maintained on a hay only diet and bedded on barley straw. All animals were weighed at least once weekly during the study period and, where appropriate, these weights were used to calculate the amount of test substrate to be administered.

6.2.3.2 Experimental design

Following an overnight fast (14-16 hours), the pattern of breath hydrogen excretion was determined, for each pony, following the administration/ingestion of either no test meal (i.e.

fasted), glucose, xylose, oats, wheat flour, lactulose or lactose (**Table 6.1**). Fasting tests were performed on all individuals on three separate occasions. A maximum of three animals were under test on any particular day and at least two days were allowed between tests on any individual. The choice of test substrate was selected randomly for each pony on a particular test day. Each test was started between 07:00 and 08:00 hours and breath samples were collected, in duplicate, at zero min (i.e. pre-test) and every 30 min thereafter for eight hours. All breath samples were analysed for hydrogen concentration within 30 min of collection. Animals under test had access to water at all times and access to food was denied until the end of the sampling period.

Test Substrate	Dose	Administration
None (i.e. fasted)	-	-
Glucose[a]	1 g/kg bwt, 20 % w/v solution	per stomach tube
Xylose[b]	0.5 g/kg bwt, 10 % w/v solution	per stomach tube
Lactose[c]	1 g/kg bwt, 20 % w/v solution	per stomach tube
Lactulose[d]	1 g/kg bwt, (0.67g lactulose/ml syrup)	per stomach tube
Wheat flour[e]	1.6 g/kg bwt, 40 % w/v solution	per stomach tube
Oats	1 kg	voluntary intake

Table 6.1: Dose, concentration and method of administration of the test substrates used for initial assessment of hydrogen excretion in ponies. bwt = bodyweight.
[a] D(+)-Glucose, Sigma-Aldrich Company Ltd.
[b] D(+)-Xylose, Sigma-Aldrich Company Ltd.
[c] D-Lactose Monohydrate, Sigma-Aldrich Company Ltd.
[d] Lactulose Solution BP, Cox Pharmaceuticals.
[e] Allinson Original 100% Wholemeal Flour, Westmills Foods Ltd.

6.2.3.3 Data analyses

Analysis of test results utilised the mean of duplicate measurements at each time point (*Appendix 18*). Breath hydrogen excretion curves following the various test meals/carbohydrate substrates were analysed individually: They were regarded as being biologically significantly different from the fasting breath hydrogen excretion curves if there was a detected increase in breath hydrogen concentration greater than 10 ppm (*Appendix 19*), sustained for at least two consecutive time points, i.e. 30 min. For each test, three

variables were calculated; the total AUC (AUC_{8hours}), the peak hydrogen concentration (ΔH_2) and the time at which a significant, sustained increase in hydrogen excretion (if any) was first noted (T_{sig}).

6.2.4 Results

Data which contributed to the results presented below are detailed in Appendices 19 and 20.

When the ponies were fasted (i.e. 21 individual tests), hydrogen concentrations excreted in breath for the eight hour sampling period were negligible (mean (±SD) hydrogen concentrations for that period ranged from 1.29 (±1.37) ppm to 3.71 (±3.26) ppm).

Sustained increases greater than 10 ppm were detected in all ponies following the ingestion/administration of oats (**Fig. 6.1**) and wheat flour, in three ponies following the administration of glucose and xylose and in two ponies following the administration of lactulose and lactose. In one animal (Pony No. 123), there were significant increases in breath hydrogen excretion following the ingestion/administration of each test substrate. The breath hydrogen responses of individual ponies, to both the oats and wheat flour test meals, were variable (**Table 6.2**). Following ingestion of oats, values for AUC_{8hours}, ΔH_2 and T_{sig} ranged from 80-343 ppm x hour, 25.5-80 ppm and 60-300 min, respectively. Similarly, following the administration of wheat flour values for AUC_{8hours}, ΔH_2 and T_{sig} ranged from 38-875 ppm x hour, 14.5-279 ppm and 150-240 min, respectively.

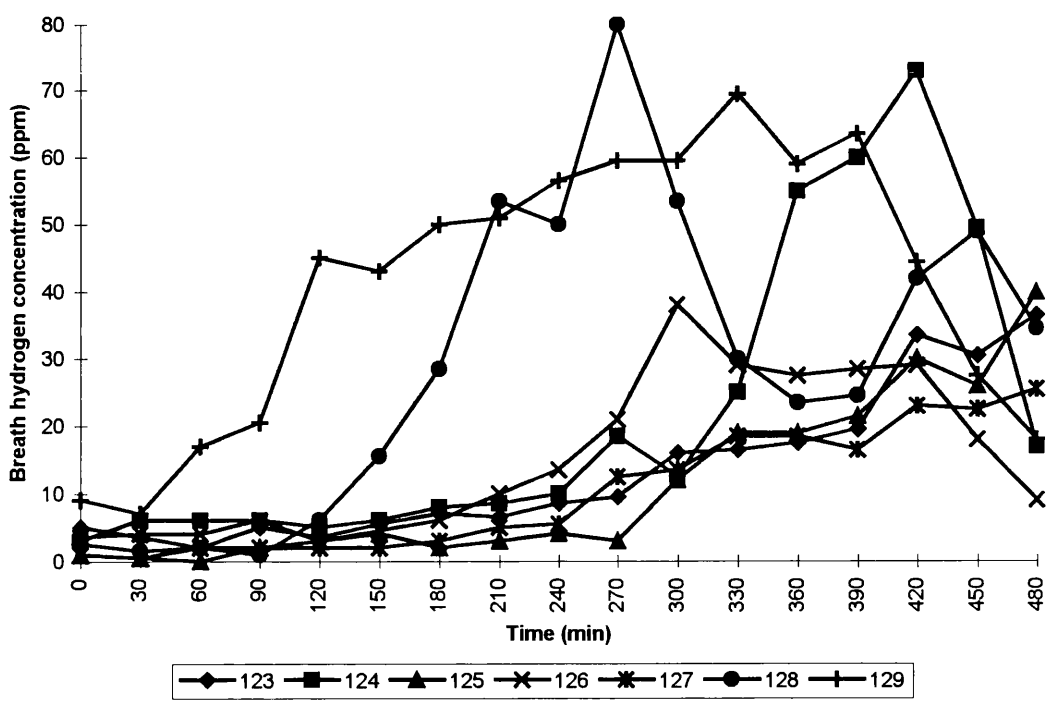


Fig. 6.1: Breath hydrogen concentrations detected following the ingestion of 1 kg oats by seven adult ponies.

Pony Procedure	Variable	123	124	125	126	127	128	129
Glucose	**T_{sig} (min)**	90	NA	NA	210	NA	NA	30
	ΔH_2 (ppm)	134.5	NA	NA	36	NA	NA	96.5
	AUC_{8hours} (ppm x hour)	437	16	16	81	15.5	20	378
Xylose	**T_{sig} (min)**	90	NA	NA	120	NA	NA	60
	ΔH_2 (ppm)	211	NA	NA	42.5	NA	NA	35.5
	AUC_{8hours} (ppm x hour)	791	20	29	132.5	16	8	135
Oats	**T_{sig} (min)**	300	240	300	210	270	150	60
	ΔH_2 (ppm)	36.5	73	40	38	25.5	80	69.5
	AUC_{8hours} (ppm x hour)	103	179.5	85	124	80	239.5	343
Flour	**T_{sig} (min)**	150	150	240	270	150	180	150
	ΔH_2 (ppm)	279	23	14.5	16	92	47	121
	AUC_{8hours} (ppm x hour)	875	83	38	57	350	107	343
Lactulose	**T_{sig} (min)**	210	NA	NA	60	NA	NA	NA
	ΔH_2 (ppm)	65	NA	NA	54.5	NA	NA	NA
	AUC_{8hours} (ppm x hour)	162	12	10	303	16	3.5	16.5
Lactose	**T_{sig} (min)**	120	NA	NA	NA	NA	NA	300
	ΔH_2 (ppm)	96	NA	NA	NA	NA	NA	20
	AUC_{8hours} (ppm x hour)	333	49	3	18	11	3.5	72

Table 6.2: Breath hydrogen excretion in individual ponies following the administration/ingestion of the different test meals. AUC_{8hours} = total area under the hydrogen excretion curve for the eight hour sampling period, ΔH_2 = the peak hydrogen concentration, T_{sig} = the time at which significant increases in hydrogen excretion (if any) were first noted. NA = not applicable.

6.2.5 Discussion

All animals excreted significant concentrations of hydrogen in breath following the ingestion/administration of both oats and also wheat flour. In human studies, Levine & Levitt (1981) reported that ingestion of flours derived from oats, corn and potatoes resulted in significant breath hydrogen excretion. In addition, Stephen, Haddad & Phillips (1983) demonstrated that 2-20 % of starch derived from different foods was not absorbed from the small intestine. Similarly, significant small intestinal malabsorption of wheat and corn flour has been reported in the dog (Washabu *et al.*, 1986b). In the present study, the breath hydrogen responses of individual ponies, as determined by T_{sig}, ΔH_2 and AUC_{8hours} were variable. These findings may be a consequence of individual differences in the rate of meal ingestion and/or gastric emptying/intestinal transit and/or small intestinal absorption and/or populations of luminal bacteria.

Interestingly, a number of animals in this study excreted hydrogen in breath following the administration of monosaccharides (glucose and xylose). In human medicine, significant increases in breath hydrogen concentration, following the administration of such carbohydrates, is indicative of small intestinal disease: a rapid and transient increase in breath hydrogen concentration, following a test substrate is indicative of bacterial fermentation within the proximal intestine (i.e. small intestinal bacterial overgrowth) (Metz *et al.*, 1976a), whereas significant increases in breath hydrogen concentration 2-5 hours after the administration of a glucose solution is suggestive of carbohydrate entry into the large intestine (i.e. small intestinal malabsorption) (Levitt & Donaldson, 1970). All animals used in this study were healthy and exhibited no evidence of gastrointestinal dysfunction, therefore significant small intestinal disease was considered unlikely. In addition, the pattern of breath hydrogen excretion in these ponies (significant increases noted between 30 and 120 minutes and were sustained for five to seven hours) does not appear to represent a rapid fermentative process, but rather, a gradual presentation of substrate to the intestinal bacteria. By contrast, Murphy, Howie & Love (1994) reported significant and transient increases in breath hydrogen concentration which was assumed to be a consequence of gastric fermentation, following the administration of xylose and lactulose to a horse with pyloric stenosis. Breukink (1974) demonstrated that, in normal horses, a proportion (15-20 %) of an oral glucose load will escape absorption from the small intestine and this fact may explain the findings in this study. Studies in man indicate that 5-10 g of unabsorbed carbohydrate entering the large intestine is sufficient to generate a detectable rise in breath hydrogen (Levitt & Donaldson, 1970). However, in healthy humans, hydrogen excretion is

negligible even when administered 100 g of glucose orally (Levitt & Donaldson, 1970; Bond & Levitt, 1972), suggesting that in man relatively large doses do not exceed the absorptive capacity of the small intestine. The fact that hydrogen was excreted in the breath of some healthy ponies following the administration of an absorbable monosaccharide solution is likely to preclude the use of breath hydrogen measurement for the investigation of small intestinal malabsorption in equine animals.

Another interesting feature of this study was that only a few animals excreted significant concentrations of hydrogen in breath following the administration of both lactose and also lactulose. Lactose is a disaccharide which is enzmatically digested in the small intestine of young animals, and its constituent monosaccharides are subsequently absorbed. Lactase activity decreases with age and Roberts (1975) reported that equine animals over 2.5 years are lactase deficient. Therefore, in this group of ponies, it was surprising that all animals did not excrete significant concentrations of hydrogen due to bacterial fermentation of lactose. Similarly, hydrogen excretion following the administration of lactulose was infrequently observed. Lactulose, a semi-synthetic non-absorbable disaccharide, is commonly used with breath hydrogen tests, in human medicine, as a marker of small intestinal transit.

Bond & Levitt (1975) suggested that failure of individuals to excrete hydrogen following lactulose ingestion is uncommon but may occur due to the inability of intestinal flora to produce hydrogen or rapid utilisation of hydrogen by hydrogen-catabolising bacteria for the production of methane. These explanations are unlikely to account for the findings in this study because all ponies excreted significant concentrations of hydrogen following the ingestion/administration of oats and wheat flour. Another possible explanation for lactose and lactulose 'false-negative' tests is that insufficient substrate is presented to the intestinal bacteria. This may be a consequence of either enzymatic digestion of the disaccharides within the intestinal lumen or possibly their absorption as undigested whole molecules from the small intestine. However, adult equine animals are recognised as being lactase deficient, as stated previously, and studies in humans (Kynaston, Fleming, Laker & Pearson, 1993) and small animal species (Papasouliotis, Gruffydd-Jones, Sparkes, Cripps & Millard, 1993) have demonstrated that lactulose is poorly absorbed from the healthy small intestine. Kotler, Holt & Rosensweig (1982) observed that preloading individuals with lactulose on the evening prior to a lactulose-breath hydrogen test significantly altered the breath hydrogen excretion pattern, indicating more rapid hydrogen production. They hypothesised that the preload produced an adaptive response by the intestinal bacteria

(increased enzymatic activity) which affected the rate of lactulose metabolism after the second dose (Kotler, Holt & Rosensweig, 1982). Therefore, it is possible that the intestinal flora in these ponies were unable to metabolise what were essentially 'novel' carbohydrates on primary exposure and may require repeated exposure to the sugar to enhance bacterial metabolic/enzymatic activity. The findings of the present study warrant further investigation, because, if lactulose is digested in and/or absorbed from the equine small intestine or if there is a delay in adaptation of intestinal bacteria to lactulose metabolism, this would have important implications with regard to the use of lactulose in the medical treatment of hepatic encephalopathy.

The results of this study indicate that hydrogen is excreted in the breath of ponies following the ingestion/administration of certain test meals, but the pattern of breath hydrogen excretion is subject to variation between individuals. The results of the present study indicate that further research must be performed before breath hydrogen measurement can be applied to the investigation of gastrointestinal dysfunction in the horse.

STUDY 7

6.3 The Fate of Oral Lactulose in Horses

6.3.1 Background

Lactulose is a synthetic disaccharide, which in healthy individuals is poorly absorbed from the small intestine and consequently the majority of it is degraded by colonic fermentation. In man, urinary excretion of lactulose following oral lactulose loading has been used for assessment of small intestinal permeability (Bjarnason, MacPherson & Hollander, 1995). In addition, lactulose is commonly used, in association with breath hydrogen measurement, for calculation of orocaecal transit time (Bond & Levitt, 1975). Furthermore, this carbohydrate is frequently used in many species for the symptomatic treatment of hepatic encephalopathy (Lanthier & Morgan, 1985): Acidification of the large intestinal contents reduces the intra-luminal production of ammonia (NH_3^-) and promotes the conversion of non-ionised (absorbable) NH_3^- to the ionised (relatively unabsorbable) ammonium ion (NH_3^+).

From the results of the previous study (**STUDY 6**) it is evident that lactulose administration infrequently results in breath hydrogen excretion. The possible reasons for this include absorption of the molecule, whole, from the small intestine or an inability of large intestinal bacteria to ferment lactulose on primary exposure. In addition, it is possible that prolonged gastrointestinal transit due to delayed gastric emptying of a hyperosmolar solution (in previous studies lactulose was administered as a syrup with 3.75 g lactulose/5 ml) may result in failure of hydrogen excretion within the eight hour sampling period.

6.3.2 Experimental objectives

The specific objectives of this study were i) to determine the effect of prolonged lactulose administration on breath hydrogen excretion patterns and ii) to the determine the proportion of an oral lactulose load, administered as a 20 % w/v solution, which is absorbed from the equine small intestine and subsequently excreted in the urine.

6.3.3 Materials and methods

6.3.3.1 The effect of prolonged lactulose administration on breath hydrogen excretion

6.3.3.1.1 Animals

Six yearling British native-breed ponies (**Table 2.1**), four female and two male ranging in weight from 162 to 236 kg, were used in this study. All were stabled, bedded on straw and maintained on a hay/coarse mix diet. All animals were considered to be healthy based on

history and clinical examinations and none of these animals had been administered lactulose prior to this study.

6.3.3.1.2 Experimental design

Day 1: Following an overnight fast (14-16 hours), lactulose at a dose of 1 g/kg bwt was administered to each individual per stomach tube. Breath samples were collected in duplicate at zero min (i.e. pre-test) and thereafter at intervals of 30 min for an eight hour period. Blood samples were collected into heparinised tubes at zero min and thereafter at intervals of 60 min for the eight hour sampling period. Breath samples were analysed for hydrogen concentration within 30 min of collection. Plasma NH_3^- concentrations were determined using a dry chemistry analyser (Vettest 8008, IDEXX Laboratories Ltd). Immediately after blood collection, samples were centrifuged at 9000 rpm for 2.5 min. The plasma was decanted, stored in sterile bijou bottles, and either placed on ice and analysed within 60 min or stored at -20°C and analysed within 12 hours.

Days 2-12: From the group of six animals, four were chosen at random and were administered 37.5 g of lactulose daily (Pony Nos. 114, 115, 117 & 119). The remaining two animals (Pony Nos. 116 &118) were used as 'untreated' controls.

Day 14: The protocol outlined above for Day 1 was repeated on all individuals (i.e. breath hydrogen measurement and plasma ammonia determination for an eight hour study period following oral lactulose loading).

Analysis of hydrogen excretion patterns utilised the mean of duplicate measurements at each time point (*Appendix 18*).

6.3.3.2 Urinary excretion of lactulose in ponies

6.3.3.2.1 Animals

Six adult female British native-breed ponies (age, 7-15 years; weight, 211-320 kg) were used in this study (**Table 2.1**). All animals were stabled, bedded on straw and maintained on a hay only diet for at least one month prior to beginning this study. All animals were considered to be healthy based on clinical examinations and the results of haematological, blood biochemical and urine analyses were within normal limits.

6.3.3.2.2 Experimental design

Following an overnight fast (14-16 hours), 250 g of lactulose was administered by nasogastric tube as a 20 % w/v solution in warm water.

Before lactulose administration a 24-F Foley catheter (Bard Limited) was inserted into the bladder and was retained in place by inflation of the balloon with 30 ml of 0.9 % sterile saline solution. In all cases, catheter placement initiated urine flow, a 20 ml sample of which (i.e. a pretest urine sample) was collected into a sterile container and stored at -20°C for use as a 'blank' in subsequent analyses. The catheter was then connected by plastic tubing to a two litre plastic bag suspended from a surcingle. Urine was allowed to flow continuously into the bag. Five hours after lactulose administration, the collection bags were removed and the bladder was completely evacuated by applying suction at the end of the urinary catheter using a 50 ml syringe. Clean collection bags were immediately attached to the urinary catheter for a further five hours with complete bladder evacuation at the end of that period. For each collection period (i.e. 0-5 hours, 5-10 hours) urine output was recorded and a 20 ml aliquot was stored in a sterile container with 0.25 ml thiomersal (1 g/L) (BDH Chemicals Ltd) as preservative, at -20^{0}C until analysed.

Following lactulose administration, breath samples were collected (see *Section 2.2.4*), in duplicate, at 30 min intervals for five hours and then hourly for the next five hours. Breath samples were analysed for hydrogen concentration within 30 min of collection. Analysis of hydrogen excretion patterns utilised the mean of duplicate measurements at each time point (*Appendix 18*).

6.3.3.2.3 Urine analysis

Quantification of lactulose in urine was performed at the Department of Clinical Chemistry, Treliske Hospital, Royal Cornwall Hospitals, Truro, Cornwall, UK, using anion-exchange high pressure liquid chromatography (HPLC). Sample preparation and the HPLC analytical technique are described in detail elsewhere (Kynaston *et al.*, 1993). Validation studies have determined that the assay is linear to 40 mg lactulose/L and has a detection limit of 0.4 mg/L in urine. In addition, analytical recovery ranged from 89.1-97.6 % and the within assay coefficient of variation was calculated to be 4.9 % (Kynaston *et al.*, 1993).

6.3.4 Results

6.3.4.1 The effect of prolonged lactulose administration on breath hydrogen excretion

Day 1: A significant increase in breath hydrogen excretion (i.e. a sustained increase greater than 10 ppm (*Appendix 19*)) was observed in one pony only (Pony No. 114). In all other animals, hydrogen excretion was negligible after lactulose administration.

Pretest, the mean plasma ammonia concentration was 64.7 μmol/L (range, 61-73 μmol/L). No marked decrease in plasma NH_3^- concentration was detected at any time during the 8 hour sampling period following oral lactulose dosing (**Fig. 6.2**).

Day 14: Breath hydrogen excretion was negligible in all 6 animals after lactulose administration. Similarly, plasma NH_3^- concentrations recorded in samples from all subjects (both 'treated' and 'control' animals) during the eight hour test period were not markedly different from the data recorded on Day 1 (**Fig. 6.2**).

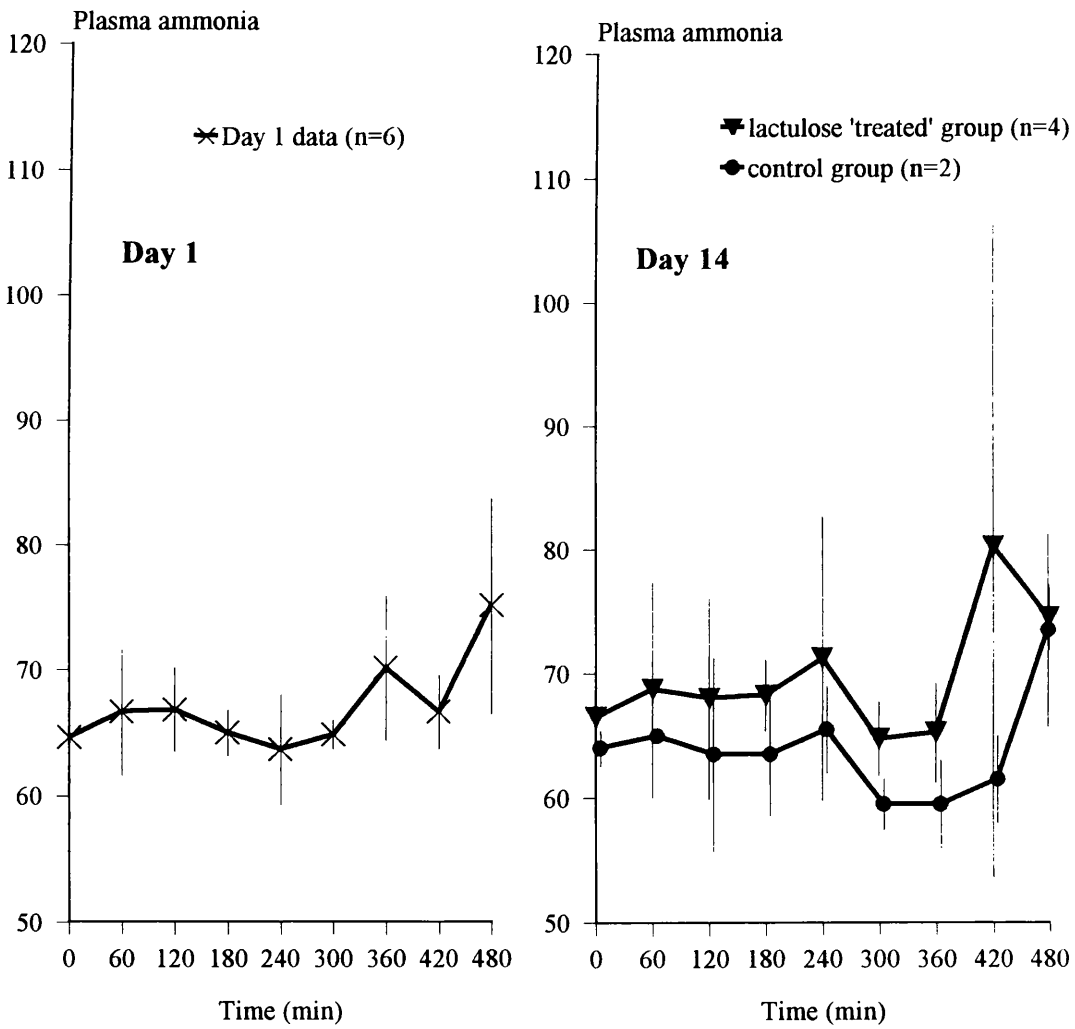


Fig. 6.2: Day 1 - Mean (±SD) plasma ammonia concentrations (μmol/L) measured during a lactulose breath hydrogen test in each of six ponies. Day 14 - Mean (±SD) plasma ammonia concentrations (μmol/L) measured during a lactulose breath hydrogen test in i) four ponies which had been exposed to lactulose for the previous 12 days and ii) two ponies which had not received lactulose during that time.

6.3.4.2 Urinary excretion of lactulose in ponies

Mean urinary recovery of lactulose (i.e. percentage of the administered dose) during the 10 hour urine collection period was 0.075 % (range, 0.027-0.142 %) (**Table 6.3**).

A significant increase in breath hydrogen excretion was detected in one pony (Pony No. 123) at 30 min after oral lactulose loading rising to a 'peak' of 90 ppm at 90 min. In all other animals, hydrogen excretion was negligible (i.e. did not exceed 10 ppm at any time during the eight hour sampling period).

	0-5 hours			5-10 hours		
	urine output (ml)	lactulose (mg)	% of oral dose	urine output (ml)	lactulose (mg)	% of oral dose
Pony 123	570	42.5	0.017	350	25.0	0.01
Pony 124	755	75.0	0.03	530	45.0	0.018
Pony 125	720	75.0	0.03	560	25.0	0.01
Pony 127	405	221.5	0.089	1000	70.6	0.028
Pony 128	400	309	0.124	560	44.7	0.018
Pony 129	900	135.0	0.054	540	60.0	0.024

Table 6.3: Total urine output in each of six ponies and lactulose recovery in urine following oral lactulose dosing.

6.3.5 Discussion

The results of this study confirm the observation made in **STUDY 6**, that oral lactulose loading infrequently results in breath hydrogen excretion in ponies.

In man, lactulose is neither hydrolysed by intestinal disaccharidases nor metabolised by other tissues, and thus transfer across the intestinal mucosa, which is thought to occur via intercellular pathways (Kynaston *et al.*, 1993), can be accurately and quantitatively reflected by excretion in urine. In this study, a normal range for urinary recovery of lactulose in healthy ponies was established: The percentage of the administered lactulose dose excreted in urine over the 10 hour sampling period is less than that reported in man, which in one study using a similar analytical technique was 0.15±0.09 % (mean±SD) for a five hour urine collection period (Fleming, Duncan, Russell & Laker, 1996). The range of values for the

urinary recovery of lactulose seen in the present study, reflects the natural individual variation in the absorption of this sugar.

Although this study confirms that lactulose is absorbed only in small amounts from the healthy equine small intestine, there is no evidence from this study that the unabsorbed component is consistently fermented by bacteria in the large intestine. When this sugar was administered to lactulose-naive yearlings, a significant increase in hydrogen excretion was observed in only one of six ponies. In addition, repeated administration of lactulose, and thus exposure of large intestinal bacteria to this novel carbohydrate did not apparently result in adaptation of sugar metabolism of intestinal bacteria and did not result in induction of detectable increases in hydrogen excretion in a subsequent lactulose-hydrogen breath test. Indeed, the one pony which had a 'positive' initial test failed to excrete hydrogen in breath on the second occasion. These results indicate that failure of breath hydrogen excretion in ponies following lactulose administration is not due to inadequate exposure of large intestinal bacteria to this sugar.

Similarly, there was no apparent effect on the plasma ammonia concentrations recorded during both the initial and second lactulose-hydrogen breath tests. These results may reflect the failure of large intestinal bacteria to ferment lactulose and consequently insignificant alteration (i.e. acidification) of the intra-luminal environment. Interestingly, no studies have been reported which confirm the proposed therapeutic benefit of lactulose for the treatment of equine hepatic encephalopathy. However, it must be remembered that the present study was conducted on healthy individuals and that, in all animals, the resting (i.e. pretest) plasma ammonia concentrations were within normal limits. Portal venous blood characteristically contains relatively high concentrations of ammonia and this is effectively removed by the healthy liver, with the result that blood in the systemic circulation contains only low concentrations of ammonia (Centre, 1995). Therefore, it could be argued that administration of lactulose to individuals with a normal functioning liver is unlikely to result in an observable effect on plasma ammonia concentration.

In conclusion, from this study, it is clear that the inability of an individual to excrete hydrogen in breath following oral lactulose loading is not due to either inadequate exposure of the intestinal flora to what is essentially a novel carbohydrate or absorption of the sugar from the small intestine. The fate of lactulose, administered orally, in the horse remains to be elucidated.

STUDY 8

6.4 Dietary Influences on Equine Gastrointestinal Tract Function: Assessment by Breath Hydrogen Measurement

6.4.1 Background

The rapid ingestion of large quantities of energy-dense feeds has been implicated in the development of clinical abnormalities such as hyper-excitability, wood-chewing, diarrhoea, flatulent colic and large intestinal volvulus (Clarke, Roberts & Argenzio, 1990). However, accurate assessment of the physiological effects of diet type and feeding frequency on gastrointestinal tract (GIT) function in the horse has been limited by the requirement for expensive, complicated and invasive techniques. Many of the conventional methods which are used to provide precise data on motility function require some surgical preparation, for example myoelectrical activity recording (Sarna, 1986), extra-mural strain gauge transducers (Davies & Gerring, 1983; Clarke, Thompson, Becht & Moore, 1988) or intraluminal pressure recording (Sellars, Lowe & Brondum, 1979). Similarly, *in vivo* evaluation of fermentation in the large intestine requires the use of animals with caecal/colonic fistulae (Willard *et al.*, 1977). In human gastroenterology, breath hydrogen measurement is commonly employed as a simple, non-invasive technique for the investigation of GIT function. This is based on the principle that, in healthy individuals, the detection of hydrogen in breath, following a test meal, indicates incomplete small intestinal absorption and bacterial fermentation of carbohydrate in the large intestine (Levitt, 1969). Consequently, in man, breath hydrogen analysis has been successfully applied to the investigation of carbohydrate malabsorption (Stephen, Haddad & Phillips, 1983; Casellas, Chicharro & Malagelada, 1993) and assessment of MCTT (Ladas, Latoufis, Giannopoulou, Hatziioannou & Raptis, 1989).

6.4.2 Experimental objective

The objective of the study was to investigate, indirectly, the effect of maintenance diet on intestinal function in healthy ponies by evaluating breath hydrogen excretion patterns following the administration of a standard test meal.

6.4.3 Materials and methods

6.4.3.1 Animals and breath collection

Seven adult British native-breed ponies were used in this study (**Table 2.1**). All were considered to be healthy based on history and clinical examinations. The ponies were

maintained on either a hay only diet and bedded on barley straw (Diet 1) or a complete pelleted ration and bedded on shredded paper (Diet 2). Either the hay or the pelleted ration was fed twice daily, at 08:00 and 15:30 hours. Breath samples were collected from ponies, stored and analysed as described above (*Section 2.2.4*).

6.4.3.2 Experimental design

Following an overnight fast (14-16 hours), breath samples were obtained, in duplicate, at times zero min (i.e. pre-test) and every 30 min thereafter for eight hours following either no test meal (i.e. fasted) or the administration of 1.6g wheat flour (Allinson Original 100% Wholemeal Flour, Westmills Foods Ltd) per kg bwt, as a 40% w/v solution, by stomach tube. All samples were analysed for hydrogen concentration within 30 min of collection. Animals under test had access to water at all times and access to food was denied until the end of the sampling period. Breath hydrogen tests were performed on all animals while maintained on Diet 1 and Diet 2, each procedure (i.e. fasted and test meal) repeated on three occasions while on each diet. A period of 4 weeks was allowed for acclimatisation to each diet before tests were carried out. At least 72 hours were allowed between tests on a single individual. Each pony was weighed on a weekly basis and these weights were used to determine the required amount of test substrate.

6.4.3.3 Data analyses

All analyses utilized the mean of duplicate measurements at each time point (*Appendix 18*).

As the data were units and followed a Poisson distribution, a $\log_e (x+1)$ transformation was performed before statistical analyses. Statistical analyses for significance were performed by use of two-way ANOVA, with repeated measures, for each of the following combinations, fasted (Diet 1) versus test meal (Diet 1), fasted (Diet 2) versus test meal (Diet 2), test meal (Diet 1) versus test meal (Diet 2), with factors being time, animal and treatment (e.g. fasted (Diet 1) or test meal (Diet 1)). Test replicates were analysed separately and significance was set at the 5% level.

For all tests, following the administration of the test meal, the total area under the hydrogen excretion curve (AUC_{8hours}) was calculated using the equation

$$AUC_{8hours} \text{ (ppm x hour)} = (0.5H_1+H_2+\ldots+H_{n-1}+0.5H_n) \text{ x 1 hour}$$

where H_n is the breath hydrogen concentration (ppm) at time point n. The AUC at hourly intervals (hourly AUC data), for each test was calculated and expressed as a percentage of the AUC_{8hours}.

6.4.4 Results

Data which contributed to the results presented in this section are detailed in Appendices 19, 21 and 22.

When the ponies were fasted, hydrogen concentrations excreted in breath for the eight hour sampling period were consistently low and the mean breath hydrogen concentrations during this period ranged from 1.29 (±1.37) ppm to 3.71 (±3.26) ppm when maintained on Diet 1 and from 0.67 (±1.15) ppm to 1.48 (±1.45) ppm when maintained on Diet 2.

Following administration of the test meal, breath hydrogen concentrations were significantly greater, over time, than the respective 'fasting data', when maintained on Diet 1 ($p<0.001$) and Diet 2 ($p<0.001$) (i.e. there was a significant time x treatment interaction). In addition, analysis of the AUC_{8hours} data indicated that, although considerable within- and between-animal variability existed (**Table 6.4**), cumulative excretion of hydrogen, following administration of the test meal, was greater when ponies are maintained on a hay diet (range 23-875 ppm x hour) compared with when on a complete pelleted ration (range 8-261 ppm x hour) (**Table 6.4**).

		Pony						
Procedure (Replicate)	**Variable**	**123**	**124**	**125**	**126**	**127**	**128**	**129**
Diet 1								
(1)	**AUC_{8hours} (ppm x hour)**	875	83	38	57	350	107	343
(2)	**AUC_{8hours} (ppm x hour)**	421	28	32	125	341	43.5	106
(3)	**AUC_{8hours} (ppm x hour)**	376	30	24	91.5	23	131.5	812
Diet 2								
(1)	**AUC_{8hours} (ppm x hour)**	192.5	13	17	184	65	11	26
(2)	**AUC_{8hours} (ppm x hour)**	98	20.5	27.5	54.5	261	37	29
(3)	**AUC_{8hours} (ppm x hour)**	141.5	8	10	218	87	31	26.5

Table 6.4: Area under the hydrogen excretion curve (AUC_{8hours}, ppm x hour) for each of three breath hydrogen tests performed on each of seven ponies, following the administration of wheat flour, while maintained on Diet 1 (hay only) and Diet 2 (pelleted ration only).

When the effect of diet on hydrogen excretion over the eight-hour sampling period following administration of the test meal was assessed (i.e. test meal (Diet 1) versus test meal (Diet 2)), a significant time x diet interaction was detected ($p<0.001$) (i.e. there was a significant difference between treatments in the response over time). Comparison of mean hourly hydrogen excretion (expressed as a percentage of AUC_{8hours}) indicates that a greater proportion of the total hydrogen excretion occurs earlier during the sampling period when on the complete pelleted ration compared with when maintained on the hay diet (**Fig. 6.3**).

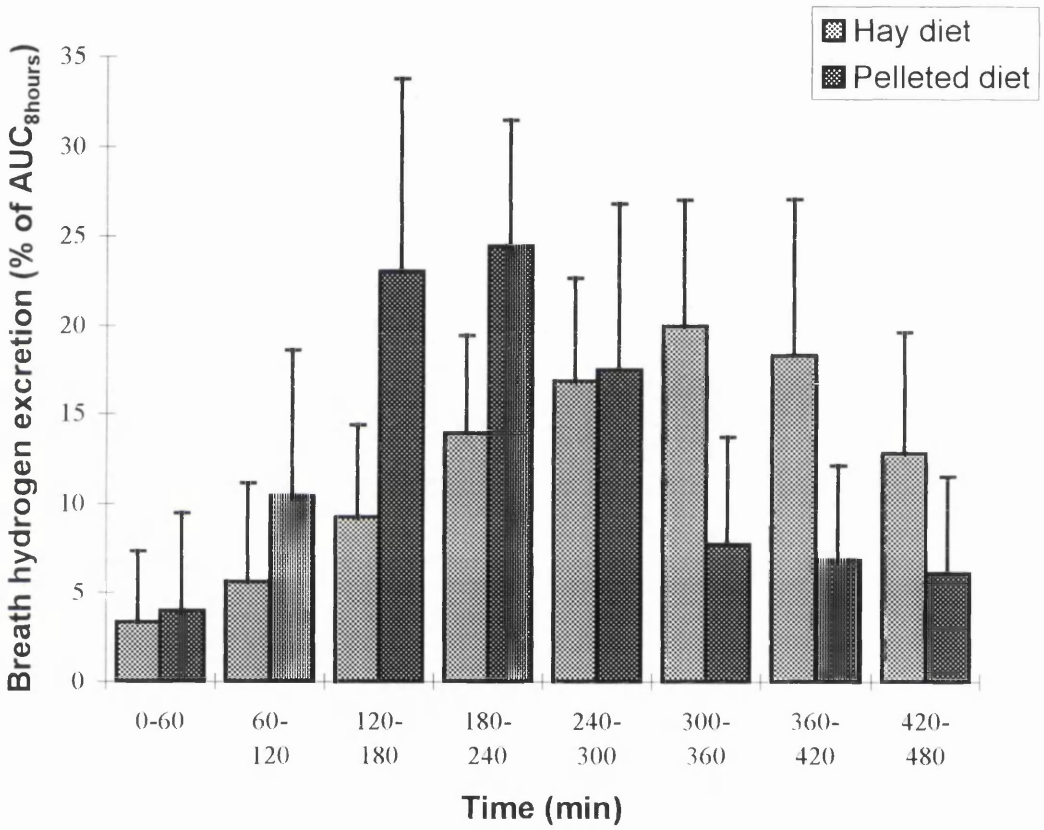


Fig. 6.3: Mean (±SD) hourly hydrogen excretion (expressed as a percentage of the total AUC), following administration of the test meal, when maintained on Diet 1 (hay only) compared with when maintained on Diet 2 (pelleted ration).

6.4.5 Discussion

The results of this study indicate that, in ponies, maintenance diet has a marked effect on breath hydrogen excretion patterns and, by inference, gastrointestinal function.

The use and interpretation of breath hydrogen measurement for the investigation of GIT function requires that 1) intestinal flora are capable of liberating appreciable quantities of hydrogen during carbohydrate fermentation, 2) bacterial fermentation in the gut is the sole source of hydrogen production, 3) hydrogen production, in healthy individuals,

originates almost entirely from bacterial fermentation in the large intestine and 4) pulmonary hydrogen excretion reflects intestinal hydrogen production (Bond & Levitt, 1975). Based on the results of **STUDY 6**, it has been shown that hydrogen excretion in fasted ponies is negligible and increases only after the ingestion/administration of certain test meals. In addition, although Mackie & Wilkins (1988) demonstrated that substantial populations of bacteria are present in the stomach and small intestine of healthy horses and that they contribute to the digestion of carbohydrates, the microbial counts were 100-fold higher in the caecum and colon confirming that these are more favourable sites for microbial colonisation and fermentation. Similarly, studies detailing VFA production in the equine intestine concluded that the caecum and colons were the primary sites for microbial digestion (Argenzio, Southworth & Stevens, 1974). Therefore, in the present study, it was assumed that hydrogen detected in breath originates from bacterial fermentation in the large intestine.

Because hydrogen excretion in breath is an indirect measure of bacterial fermentation of unabsorbed carbohydrate in the intestine, the differences observed in cumulative hydrogen excretion indicate that when the ponies in the present study were maintained on hay, either a greater proportion of the test substrate was available for fermentation because of less efficient digestion/absorption from the small intestine or conditions in the intestinal lumen facilitated increased production and/or absorption of hydrogen. Studies in the rat have demonstrated that dietary composition can modify the mucosal architecture and the intestinal mucus composition and therefore alter the functional characteristics of the small intestine (Sharma, Schumacher, Ronassen & Coates, 1995). On the other hand, Kern *et al.*, (1973) demonstrated that, in the horse, diet has a marked effect on bacteria numbers in the caecum and other authors indicated that the microbial population and micro-environment of the equine large intestine are substantially and rapidly altered following dietary change (Garner, Moore, Johnson, Clark, Amend, Tritschler, Coffman, Sprouse, Hutcheson & Salem, 1978). Changes in the microbial populations of the gut may influence the amount of hydrogen available for absorption by variation in the numbers of either hydrogen-producing or hydrogen-utilising bacteria.

In human studies, Florent, Flourie, Leblond, Rautureau, Bernier & Rambaud (1985) demonstrated that when individuals ingested a non-absorbable carbohydrate (lactulose) over a period of eight days, hydrogen excretion in breath decreased, whereas the molar ratio of acetic acid to total VFA and lactic acid of caecal content increased. It was suggested that the associated decrease in caecal pH favours the growth of certain species of bacteria which

catabolise carbohydrates to acetic and lactic acid and, consequently, is responsible for a marked decrease in hydrogen production. Studies in the horse have indicated that twice daily concentrate feeding is responsible for a significant reduction in caecal pH compared with when maintained on hay (Willard *et al.*, 1977). In addition, those authors demonstrated a significant dietary influence on VFA profiles in caecal content, indicative of differences in the metabolic pathways of bacterial carbohydrate fermentation. When maintained on the concentrate ration, the molar percentage of proprionate in caecal content was increased, indicating the passage of soluble carbohydrate into the large intestine and subsequent fermentation (Willard *et al.*, 1977). Therefore, the reduction in hydrogen excretion observed in the present study, when ponies were maintained on the pelleted diet, possibly represents an adaptation of intestinal bacteria to the repeated caecal/colonic load of fermentable carbohydrate and an associated alteration in the luminal environment of the large intestine.

Hydrogen is consumed by a number of bacterial reactions including methanogenesis, reduction of sulphate and acetate production and it has been demonstrated that hydrogen consumption, as indicated by methane-producing status, influences the results of breath hydrogen tests (Corazza, Strocchi, Sorge, Benati & Gasbarrini, 1993). Therefore, breath hydrogen measurements reflect net rather than absolute hydrogen production. The factors which influence hydrogen consumption within the intestinal lumen are not fully understood. However, it is recognised that poor mixing of ingesta produces a high faecal hydrogen tension which facilitates rapid hydrogen consumption (Strocchi & Levitt, 1992). Based on this information, it is interesting to speculate that one of the possible reasons for lower hydrogen excretion when these ponies were maintained on the pelleted ration is that consumption of hydrogen is greater due to altered composition of ingesta resulting in less effective caecal/colonic mixing.

In the present study, the amount of hydrogen excreted in breath, following administration of the test meal, varied considerably within and between individuals, on occasion resulting in low/negligible breath hydrogen excretion. In human gastroenterology, failure of individuals to excrete appreciable quantities of hydrogen in breath after the administration of non-absorbable carbohydrates is well recognised. It has been commonly accepted that an inability to excrete detectable/significant quantities of hydrogen primarily reflects a lack of hydrogen-producing large intestinal flora and that animal variation in hydrogen excretion is the result of individual/daily variability in bacterial populations in the gut (LaBrooy, Male, Beavis & Misiewicz, 1983; Hammer, 1993). Numerous other

variables which may influence breath hydrogen excretion in healthy individuals, following a test meal, include small intestinal digestive/absorptive capacity, methane-producing status, colonic mucosal function and ventilation rate/sampling technique (Bjorneklett & Jenssen, 1980; Cloarec, Bornet, Gouilloud, Barry, Salim & Galmiche, 1990; Strocchi, Ellis & Levitt, 1991). In addition, in the present study, the amount of test meal for individual animals was calculated based on bodyweight, therefore, it is likely that different quantities of test substrate were available for bacterial fermentation in each pony and may account for some of the between-animal variability observed. The variability in test results which have been detected in this and human studies (Corazza *et al.*, 1993) emphasises the need to regard breath hydrogen tests as semi-quantitative assessments of intestinal hydrogen production.

Despite the observed individual differences and dietary influences on the quantities of hydrogen excreted, assessment of the patterns of hydrogen excretion over the eight hour sampling period (hourly AUC data) indicate more rapid presentation of unabsorbed components of the test meal to intestinal bacteria when on the pelleted ration compared with when maintained on hay. Based on the assumption that exhaled hydrogen originates almost entirely in the large intestine, these results may be explained by a dietary influence on the intrinsic motor activity of the gut resulting in an altered rate of gastric emptying/small intestinal transit (i.e. MCTT).

The influence of feeding and dietary composition on equine intestinal motility is not fully understood. However, it has been suggested that horses on different feeding regimens (steady-state feeding versus 'bolus' feeding) will experience markedly different small intestinal motor activity in response to feeding (Clarke, Roberts & Argenzio, 1990). Although the regulatory peptides and the precise mechanisms involved are poorly understood, it is recognised that intestinal motor activity is mediated to some extent by neurohormonal responses to feeding and the presence of digestive byproducts in the alimentary tract. In addition, it has been documented that secretion of certain regulatory peptides is influenced by ration type: Smyth, Young & Hammond (1989) reported increased and prolonged (>12 hours) postprandial gastrin secretion after refined (pelleted) diets when compared to hay, even when *in vitro* analysis indicated that both diets had a similar nutrient composition. Moreover, gastrin secretion was influenced by the duration of feeding of a particular diet in that all mean gastrin concentrations measured after seven days on the pelleted diet were significantly higher than during the first feed of pellets (Smyth, Young & Hammond, 1989). Therefore, in the present study, it is plausible that neurohormonal

influences were, in part, responsible for the apparent increased rate of small intestinal transit of the test meal while on the pelleted ration.

In conclusion, the results of the present study indicate that maintenance diet has an apparent effect on gastric/small intestinal motility and add weight to the theory that the episodic feeding of large quantities of 'concentrate' ration results in increased transit of ingesta. Although, logically, this could be expected to decrease the efficiency of small intestine digestion/absorption and result in greater amounts of soluble carbohydrate reaching the large intestine, in turn fueling intense caecal/colonic fermentation (Clarke, Roberts & Argenzio, 1990), there is no direct evidence from this study to suggest increased bacterial fermentation when the ponies were maintained on the pelleted diet. Bearing in mind the economic and welfare implications of conventional methods used for the evaluation of gastrointestinal function in the horse (e.g. scintigraphy, radiography, myoelectrography, intestinal fistulation), the results of this study indicate that breath hydrogen measurement may prove to be a simple, cheap and non-invasive alternative for the assessment of MCTT and alterations in caecal/colonic function. On this basis, further studies investigating the potential of this technique are justified.

STUDY 9

6.5 Effect of Cisapride and Codeine Phosphate on Intestinal Transit in Healthy Ponies: Assessment by Breath Hydrogen Measurement.

6.5.1 Background

Drugs which alter gastrointestinal motility are frequently used for the treatment of numerous medical conditions in the horse. However, due to a lack of simple, non-invasive techniques, little objective information is available with regard to their efficacy at influencing gastrointestinal transit in this species. Roberts & Argenzio (1986) evaluated the effect of a variety of motility-modifying drugs in healthy horses by monitoring both their clinical effects and alterations in whole gut transit, using polyethylene glycol as a marker substrate. Although this paper provided useful clinical/physiological information, the techniques used were subjective and lack detail about transit through specific sections of the gut. Similarly, Davies & Gerring (1983) investigated the effect of spasmolytic drugs on motility patterns of the equine small intestine by recording electrical and smooth muscle activity at pre-selected sites. While these were valid techniques for the investigation of gastrointestinal physiology, they are invasive and are not a direct measure of the rate of transit of ingesta.

The ideal technique to investigate the efficacy of motility-modifying drugs should assess ingesta transit through specific parts of the intestine without directly influencing the physiological control of gastrointestinal motility. In man the 'gold standard' technique for this purpose is the imaging of meals labeled with radio-isotopes, using scintigraphy (Sciarretta, Furno, Mazzoni, Garagnani & Malaguti, 1994). Although this method has been used in the horse, its application has been limited to the assessment of gastric emptying (Ringger, Lester, Neuwirth, Merritt, Vetro & Harrison, 1996). In addition, the technique requires expensive, complex equipment.

At present, breath hydrogen measurement is the most commonly used simple, non-invasive, non-radiological method of assessing intestinal transit in man. Determination of MCTT, by measuring the time taken to detect a significant and sustained increase in breath hydrogen measurement, following the ingestion of a test meal, was first introduced by Bond & Levitt (1975).

6.5.2 Experimental objective

The objective of the study was to assess, in healthy ponies, the effects of cisapride and codeine phosphate on MCTT, determined by breath hydrogen measurement.

6.5.3 Materials and methods

6.5.3.1 Animals

Three adult British native-breed ponies (mean age, five years) were used in this study (**Table 2.1**). All were considered to be healthy based on history and clinical examinations. The ponies were stabled, bedded on wood shavings and maintained on a high-fibre complete pelleted ration, fed twice daily.

6.5.3.2 Experimental design

Following an overnight fast (14-16 hours), one of three procedures ([A]-[C]) was assessed by monitoring breath hydrogen excretion. Breath hydrogen concentrations were determined, in duplicate, at 30 min intervals for eight hours following the ingestion of one kg of the high-fibre pelleted meal (i.e. the test meal) without premedication [A] or ingestion of the test meal one hour after premedication with either cisapride (Prepulsid, Janssen-Cilag; 0.5 mg/kg bwt, *per os* (PO)) [B] or codeine phosphate (Codeine Phosphate 30 mg, Norton; 2 mg/kg bwt, PO) [C]. Procedures were selected randomly for each pony on a particular test day. Each procedure was repeated on three occasions on each pony and at least three days were allowed between tests on any individual. Breath samples were collected, stored and analysed as outlined previously (*Section 2.2.4*).

6.5.3.3 Data analyses

All analyses utilised the mean of duplicate measurements at each time point (*Appendix 18*). For all breath hydrogen tests, increases in breath hydrogen concentration greater than 5 ppm were regarded as being a 'positive' test, if sustained for two consecutive readings (i.e. 30 min) (*Appendix 19*). For all tests, the time at which the significant increase in breath hydrogen concentration occurred (T_{sig}) and the time of peak hydrogen concentration ($T_{\Delta H2}$) were noted. In the present study, T_{sig} was considered to indicate the time at which the head of the test meal entered the caecum (i.e. MCTT).

As the data were units and followed a Poisson distribution a $\log_e (x+1)$ transformation was performed before statistical analyses. The effect of treatment on hydrogen excretion following the administration of the test meal was statistically analysed by two-way ANOVA, with repeated measures, with factors being time, animal and treatment (i.e. [A], [B] or [C]). Significance was set at the 5 % level.

For all tests, the total area under the hydrogen excretion curve (AUC_{8hours}) was calculated using the equation,

$$AUC_{8hours} \text{ (ppm x hour)} = (0.5H_1+H_2+....+H_{n-1}+0.5H_n) \text{ x 1 hour,}$$

where H_n is the breath hydrogen concentration (ppm) at time point n. The AUC at hourly intervals (hourly AUC data), for each test was calculated and expressed as a percentage of the AUC_{8hours}.

6.5.4 Results

Data which contributed to the results presented in this section are detailed in Appendix 23.

Significant concentrations of hydrogen (i.e. >5 ppm, sustained for two consecutive readings) were excreted in the breath of all ponies, on all occasions, following ingestion of the test meal (i.e. on all occasions for procedures [A], [B] and [C]) (**Table 6.5**).

When the effect of treatment on hydrogen excretion over the eight-hour sampling period, following administration of the test meal, was assessed, a significant time x treatment interaction was detected ($p<0.001$) (i.e. there was a significant difference between treatments in the response over time). Comparison of mean hourly hydrogen excretion (expressed as a percentage of AUC_{8hours}) (**Fig. 6.4**) indicates that, compared to the control data, a greater proportion of the total hydrogen excretion occurs earlier during the sampling period following premedication with cisapride [B]. By contrast, following premedication with codeine [C], a greater proportion of the total hydrogen excretion occurred at a later stage during the eight hour sampling period.

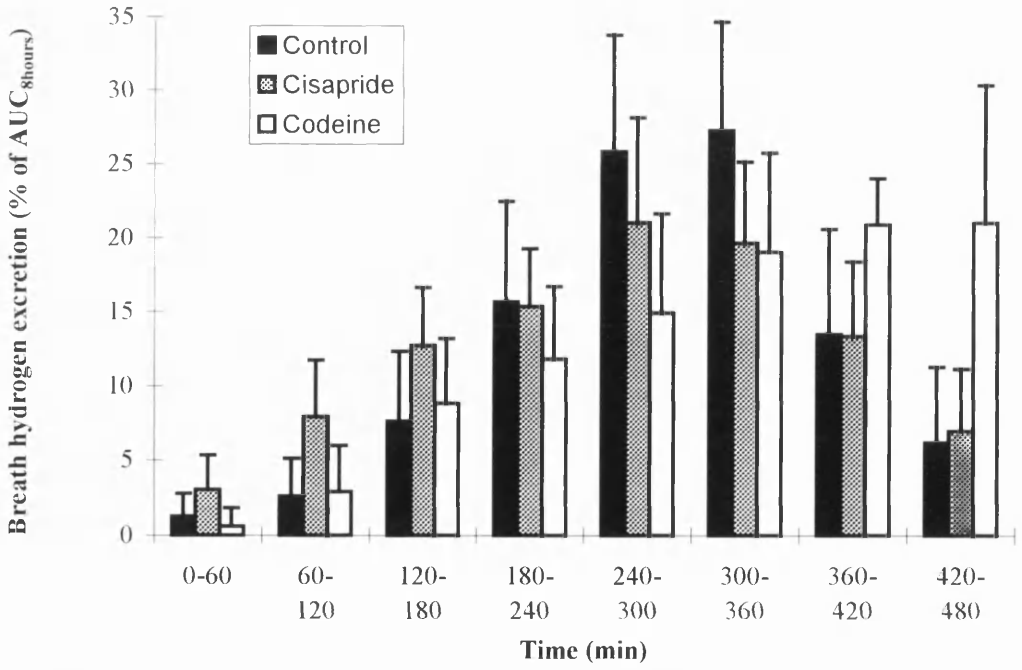


Fig. 6.4: Mean (±SD) hourly hydrogen excretion (expressed as a percentage of the total AUC), following the ingestion of the test meal without premedication [A] or ingestion of the test meal one hour after premedication with either cisapride [B] or codeine phosphate [C].

Pony (Procedure)	Variable	[A] Control	[B] Cisapride + [A]	[C] Codeine + [A]
PONY 129				
1	T_{sig} (min)	210	120	150
	$T_{\Delta H2}$ (min)	300	300	420
	AUC_{8hours} (ppm x hour)	58.5	43	67
2	T_{sig} (min)	180	90	180
	$T_{\Delta H2}$ (min)	330	240	450
	AUC_{8hours} (ppm x hour)	53.5	96	66
3	T_{sig} (min)	150	210	240
	$T_{\Delta H2}$ (min)	270	270	390
	AUC_{8hours} (ppm x hour)	26	36	27.5
PONY 126				
1	T_{sig} (min)	240	120	180
	$T_{\Delta H2}$ (min)	240	390	300
	AUC_{8hours} (ppm x hour)	19	88	101
2	T_{sig} (min)	300	90	120
	$T_{\Delta H2}$ (min)	360	300	390
	AUC_{8hours} (ppm x hour)	34	103.5	61.5
3	T_{sig} (min)	150	90	180
	$T_{\Delta H2}$ (min)	360	420	210
	AUC_{8hours} (ppm x hour)	43	32	57
PONY 122				
1	T_{sig} (min)	90	120	60
	$T_{\Delta H2}$ (min)	300	270	420
	AUC_{8hours} (ppm x hour)	102	141	210
2	T_{sig} (min)	150	60	120
	$T_{\Delta H2}$ (min)	330	180	450
	AUC_{8hours} (ppm x hour)	273	233.5	117
3	T_{sig} (min)	180	120	120
	$T_{\Delta H2}$ (min)	330	390	450
	AUC_{8hours} (ppm x hour)	45.5	147.5	97
median (range)	T_{sig} (min)	180 (90-300)	120 (60-210)	150 (60-240)
	$T_{\Delta H2}$ (min)	330 (240-360)	300 (180-420)	420 (210-450)
	AUC_{8hours} (ppm x hour)	45.5 (19-273)	96 (32-233.5)	67 (27.5-210)

Table 6.5: Breath hydrogen excretion in individual ponies following the administration of a test meal after either no premedication [A] or premedication with cisapride [B] or codeine phosphate [C]. Each procedure was repeated on three occasions. AUC_{8hours} = total area under the hydrogen excretion curve for the eight hour sampling period, $T_{\Delta H2}$ = time of peak hydrogen excretion, T_{sig} = time at which significant increases in hydrogen excretion were first noted.

These observations were supported by assessment of T_{sig} and $T_{\Delta H2}$ recorded for each procedure. The median T_{sig} and $T_{\Delta H2}$ for the control data were 180 min (range, 90-300 min) and 330 min (range, 240-360 min), respectively. For procedure [B], T_{sig} (median, 120 min; range, 60-210 min) and $T_{\Delta H2}$ (median, 300 min; range, 180-420 min) were notably shorter than the control data. Although administration of codeine phosphate did not result in an appreciable delay in T_{sig} (median, 150 min; range, 60-240 min), the time of peak hydrogen excretion (median, 420 min; range, 210-450 min) was markedly delayed relative to the control data. In addition, hydrogen excretion at the end of the eight hour sampling period was greater following the administration of codeine than for either of the other procedures (**Fig. 6.4**). For all procedures ([A]-[C]), considerable within and between animal variability in T_{sig}, $T_{\Delta H2}$ and AUC_{8hours} was observed (**Table 6.5**).

6.5.5 Discussion

The results of this study indicate that both cisapride and codeine phosphate modify the pattern of breath hydrogen excretion in healthy ponies following ingestion of a test meal.

In this study, the use of breath hydrogen measurement to assess small bowel transit time assumes that hydrogen production occurs entirely in the large intestine, where the unabsorbed component of the test meal is presented to and fermented by intestinal bacteria. Clearly, overgrowth of bacteria within the small intestine or a caecal/colonic intraluminal environment which does not favour the production of hydrogen may compromise the validity of this technique. However, for all animals in this study, a marked increase in breath hydrogen excretion was detected on all occasions for each procedure and the pattern of hydrogen excretion was suggestive of a gradual presentation of substrate to intestinal bacteria rather than a rapid and transient hydrogen 'peak' which would be expected in the case of bacterial colonisation of the small intestine. Moreover, in man, it is considered that, in the absence of massive bacterial overgrowth, hydrogen production in the small bowel is unimportant (Bond & Levitt, 1972) and, as previously discussed, a number of studies have been conducted which indicate that the caecum and colons are the primary sites for microbial digestion in the equine intestinal tract (Argenzio, Southworth & Stevens, 1974; Mackie & Wilkins, 1988). In addition, studies undertaken to validate breath hydrogen measurement for the assessment of MCTT in man, have demonstrated that, in healthy individuals, there is a highly significant linear correlation between the rise in breath hydrogen excretion and the onset of colonic filling as determined by scintigraphy (i.e. the

'gold' standard) (Read, Miles, Fisher, Holgate, Kime, Mitchell, Reeve, Roche & Walker, 1980; Read, Al-Janabi, Edwards & Barber, 1984; Sciarretta *et al.*, 1994)

It has been suggested that, as a strict measure of transit time, it is advantageous for the test substrate to be totally non-absorbable (e.g. lactulose), since individual differences in the rate of absorption of other test materials would affect the size of the intestinal bolus and hence influence transit time (Bond & Levitt, 1975). However, more recent studies have indicated that, when lactulose alone is used, it induces abnormally rapid MCTT and yields poorly reproducible results (Staniforth & Rose, 1989), while the use of a standardised test meal (with or without lactulose) produced more consistent results (LaBrooy *et al.*, 1983; Ladas *et al.*, 1989). Moreover, the results of **STUDY 6** and **STUDY 7** have indicated that lactulose is totally unsuitable for this purpose in the horse since hydrogen is infrequently excreted in breath following its administration. For these reasons, voluntary ingestion of a high-fibre meal was considered appropriate as a test meal, in that it would provide more physiologically relevant information. In addition, the pelleted feed was chosen as the maintenance diet because previous observations (*unreported observations*) indicated that T_{sig}, as defined above, is less variable between individuals following the administration of a test meal when on this diet compared to when maintained on hay only. However, despite the test protocol adopted in an attempt to reduce variability, variation in T_{sig} (i.e. MCTT) was observed, for all procedures, both between subjects and in the same subject at different times. While it is likely that individual differences in the rate of meal ingestion contributed to the variation observed, perhaps the most important factor was between and within animal variation in intestinal function itself.

Cisapride is a relatively new gastrointestinal prokinetic agent which acts selectively on the gut by facilitating the release of acetylcholine at the myenteric plexus (Schuurkes, Van Nueten, Van Daele, Reyntjens & Janssen, 1985). King & Gerring (1988) demonstrated that it enhances motor activity in the stomach, small intestine and colon of healthy ponies and based on this knowledge it is commonly employed for the prophylaxis/treatment of post operative ileus (Gerring & King, 1989). In the present study, T_{sig} and the time of peak hydrogen excretion were reduced by cisapride pretreatment when compared to the control data. These results indicate an acceleration of gastric emptying and/or small intestinal transit and verify the proposed effect of this drug on gastrointestinal tract function in healthy animals.

From our results, the effect of codeine on gastrointestinal motility is less clear. Codeine phosphate is a morphine derivative which is used for the symptomatic relief of

chronic diarrhoea in man (Palmer, Corbett & Holdsworth, 1980) and the horse (Love, Mair & Hillyer, 1992). The proposed mechanism of action of opiates on gastrointestinal transit is that they block acetylcholine release in the myenteric plexus, by presynaptic inhibition, which in turn diminishes propulsive motility (Schiller, Davis, Santa Ana, Morawski & Fordtran, 1982). Although codeine has been shown to prolong small intestinal transit in man (Pressman, Hofmann, Witztum, Gertler, Steinbach, Stokes, Kelts, Stone, Jones & Dharmsathaphorn, 1987), no such effect on T_{sig} was observed in the present study. However, the time to peak hydrogen excretion was delayed when compared to the control data and hydrogen excretion at the end of the eight hour sampling period was greater. Therefore, it is possible that the premedication time of one hour did not allow sufficient time for the drug to have an effect prior to the test and that it only began to influence ingesta transit after the time of presentation of the head of the test meal to large intestinal flora.

In conclusion, despite the limitations associated with this indirect measure of MCTT and the between and within-individual variability recorded, the results of the present study indicate that breath hydrogen measurement appears to be sufficiently sensitive to detect the contrasting effects of both cisapride and codeine phosphate on small intestinal transit. Therefore, further studies to investigate the clinicopharmacological potential of this technique are justified.

CHAPTER 7

GENERAL CONCLUSIONS AND FURTHER RESEARCH AREAS

7.1 Review of Clinical Cases

Although there are numerous known and potential causes of chronic gastrointestinal disease in the adult horse, the results presented in Chapter 3 indicated that the diagnoses most commonly encountered at the University of Glasgow Veterinary School were CIBD, cyathostomosis and alimentary lymphosarcoma. Despite employing numerous diagnostic techniques for the investigation of these cases, there was considerable overlap in the clinical features of different disease entities such that a definitive ante mortem diagnosis was often difficult to achieve: few investigative techniques provided specific diagnostic information and, not infrequently, test results were open to misinterpretation.

In this case series, the most useful diagnostic test was, simply, the identification of large numbers of cyathostome larvae in faeces. In the absence of such a feature, the definitive diagnosis of cyathostomosis is difficult but may be aided by historical and clinical features such as age, season of presentation, presence of diarrhoea, pyrexia, leucocytosis and hypoalbuminaemia. Interestingly, this study also serves to emphasise that cyathostome-associated disease is not limited to the classical presentation of acute larval cyathostomosis and that, as reported in other studies (Mair, 1993, 1994; Lyons *et al.*, 1994; Mair & Pearson, 1995; Matthews & Morris, 1995), clinical manifestations may range from chronic weight loss to acute, life-threatening gastrointestinal disease.

The fact that the majority of the remaining cases had either a severe inflammatory or neoplastic infiltrate of the intestine suggests a potentially valuable role for intestinal biopsy in the investigation of such cases. Although obtaining intestinal biopsies via laparotomy may be of limited practical value because of financial considerations and the increased risks associated with abdominal surgery in debilitated and/or hypoalbuminaemic animals, the results presented in this study suggested that histopathological examination of biopsied rectal tissue is a safe, simple and relatively non-invasive means of effecting a definitive diagnosis in a small proportion of cases. These results are in broad agreement with the results of a larger and more comprehensive study on the usefulness of the rectal biopsy technique reported by Lindberg, Nygren & Persson (1996).

It is recognised that the accurate description of the pattern of a disease within a population is essential to the understanding of that disease and that, in certain instances, such a description can help identify factors which may be associated with predisposition to

or initiation of the disease under study. Knowledge of predisposing factors for a given disease also allows for the identification of high-risk groups. With this in mind, a limited epidemiological study was performed in an attempt to highlight any factors which may be associated with an increased risk of developing a particular chronic enteropathy. A control population was established using cases presented to the referral clinic for a period of one year: This time period was chosen for the principal reason that the computing system and the method of data entry used facilitated rapid, easy data retrieval. Therefore it could be suggested that this population is not an ideal control because it is not time matched and is based on a referral population. However, it could be argued that if conclusions based on a control population are to be compared with those generated at other referral centres, then the use of a referral population, rather than a local general population as the control, would be appropriate.

When historical data from our clinical group was compared to that of a control population, it appeared that, ponies are at a greater risk of developing either cyathostomosis or alimentary lymphosarcoma than horses and that female animals are at a greater risk of developing either CIBD or alimentary lymphosarcoma than males. Although it is accepted that there is a relationship between the age of an individual and the risk of developing clinical disease due to cyathostome infection (Reid *et al.*, 1995), the associations between animal type and/or gender and the development of either CIBD, cyathostomosis and/or alimentary lymphosarcoma have not been previously reported. This study does serve to highlight that specific animal factors may be associated with a greater risk of developing certain chronic equine enteropathies and warrants further investigation.

Chronic diseases of the gastrointestinal tract are relatively uncommon, but they have important welfare implications, in that they can be severely debilitating and often have a guarded to poor prognosis. While this study fulfills the intended objective by highlighting factors which may help differentiate between disease entities and in doing so expanding the information base on chronic gastrointestinal tract diseases in the adult horse, it also serves to emphasise the difficulties associated with achieving a definitive diagnosis in these cases and the limited means of objectively assessing gastrointestinal function in the horse. Further studies into the casual factors, epidemiological associations and pathogenesis of chronic gastrointestinal diseases are justified and because of the relatively low prevalence of these diseases this could possibly be best approached by seeking input from colleagues in general practice and other referral institutions (i.e. a multicentre study). Such studies may lead to the development of improved diagnostic tests and/or help define approaches to therapy.

7.2 Fructosamine Analysis

In Chapter 4, a validation study for measurement of fructosamine in equine plasma was outlined and the use of this parameter for monitoring alterations in protein metabolism in ponies using, as a model, experimental cyathostome infection was evaluated.

The results of the validation study indicated that the assay is rapid and precise and accurate for measuring low concentrations of fructosamine in equine plasma, which is in broad agreement with validation studies performed in other species (Jensen, 1992; Reusch *et al.*, 1993; Graham, 1995). Using this assay, a reference range for plasma fructosamine in healthy, stabled British native-breed ponies was calculated to be 203-310 μmol/L. While the reference range for fructosamine in equine plasma, established in the present study, is appropriate for stabled British native-breed ponies, it should not be considered appropriate for the general equine population and, if fructosamine is to gain acceptance as a marker of protein metabolism in equine medicine, it would be necessary to establish a reference range based on a greater number and more diverse population of animals, and to investigate the possible effects of age, breed or environment on plasma fructosamine concentrations.

It was possible to monitor the effects of experimental cyathostome infections in groups of ponies by sequential clinical measurements. All infected animals failed to gain weight at the same rate as the uninfected controls and one pony developed overt clinical disease. As expected, fructosamine concentrations decreased in all infected animals post-infection and this was considered to be due to increased rate of protein turnover and/or enteric protein loss and/or altered composition of serum proteins. Although fructosamine is used as a marker of the rate of protein turnover in human medicine (Lloyd & Marples, 1986; Waterson & Mills, 1988), it was not possible, from the results of the present study, to state categorically that fructosamine decreased in parasitised animals due to an increased rate of protein turnover. However, there is evidence to suggest that this parameter may be a more effective means, than albumin measurement, for monitoring alterations in protein metabolism. With this in mind, further research should be directed towards evaluating the use of this parameter for monitoring the relatively subtle effects of low-level (i.e. subclinical) cyathostome infection and for the assessment and/or clinical monitoring of overt clinical disease due to equine chronic enteropathies.

It is acknowledged that the statistical analysis of data generated from the experimental cyathostome infection study is compromised by small group sizes. Therefore, the emphasis in interpretation was weighted towards reporting the results in a descriptive manner, using the statistical analysis to support observations. A useful and practical implication of the

statistical analysis performed in this study is that, having quantified the variability in the system, this information can be used to calculate the number of animals which should be included in future studies to produce statistically useful results.

7.3 The Oral Glucose Tolerance Test

The results of Chapter 5 provide further confirmation that extraintestinal factors can influence the OGTT: Both age and diet had a significant effect on plasma glucose concentrations in ponies, following oral glucose loading. These results suggest that both variables should be considered when interpreting tests in the clinical situation. Although not investigated in the present study, there are numerous mechanisms by which both age and diet may hypothetically influence OGTT results. Perhaps one of the most interesting theories for the diet-associated effect is the suggestion that there was evidence of an improvement in insulin sensitivity when ponies were maintained on the pelleted versus the hay diet. This theory had been advocated previously by Smyth, Young & Hammond (1989) and warrants further investigation, because it may have important implications for the management of conditions associated with insulin insensitivity, in particular laminitis in obese ponies.

Retrospective analysis of OGTTs performed on clinical cases, from a variety of backgrounds, indicated that the conventional OGTT protocol as described by Roberts & Hill (1973) offers no advantage over a modified test protocol, based on a single sample taken at 120 min following oral glucose administration for the detection/exclusion of small intestinal pathology. Use of the modified protocol is both cheaper and more suited to the practice situation. Because this study was based on test results from a relatively small number of animals (n=21), it would seem appropriate to confirm this observation by repeating these analyses on a different, larger group of clinical cases. Again, as observed by Mair *et al.* (1991), detection of total malabsorption suggested a diagnosis of either CIBD or alimentary lymphosarcoma and was, therefore, considered to be an indicator of poor prognosis.

7.4 Breath Hydrogen Analysis

The studies on breath hydrogen measurement began by assessing the patterns of breath hydrogen excretion in healthy adult ponies following the ingestion/administration of a variety of test meals, and interpretation of the results was based on the assumption that, in the horse, hydrogen excreted in breath originated from bacterial fermentation of unabsorbed

carbohydrate in the caecum/colon. When fasted, hydrogen concentration in breath was negligible, but significant concentrations of hydrogen were detected in breath of all ponies one to five hours after the ingestion of oats or the administration of wheat flour by stomach tube suggesting that hydrogen is excreted in response to feeding and that a proportion of both meals was unabsorbed. However, there were two unexpected, yet interesting, findings: Some ponies excreted hydrogen in breath following the administration of simple sugars (glucose and xylose) and the majority of ponies failed to excrete hydrogen in breath following the administration of unabsorbable sugars (lactulose and lactose). The fact that dramatic increases in hydrogen excretion were noted in healthy ponies following either glucose or xylose administration indicates that a proportion of the oral sugar load escaped absorption from the small intestine, which is in agreement with the findings of Breukink (1974), and is likely to preclude the use of this test for the investigation of small intestinal malabsorption in individual clinical cases. The reasons for the majority of these adult ponies 'failing' to excrete hydrogen in breath following the administration of either lactulose or lactose were unclear, but suggested that these sugars were either removed from the small intestine or were not fermented by large intestinal bacteria. However, the results of a study designed to investigate the fate of lactulose following oral administration to ponies demonstrated that, as in human (Fleming *et al.*, 1996) and small animal species (Papasouliotis *et al.*, 1993), only a small fraction of an oral lactulose load is absorbed from the equine small intestine. In addition, there was no evidence to suggest that lactulose is fermented by large intestinal bacteria, even after repeated exposure of intestinal bacteria to this sugar. Therefore the fate of lactulose is still unclear. Regardless of the reasons for lactulose 'failure', this finding would have implications with regard to the use of lactulose in the medical management of hepatic encephalopathy in the horse. One positive aspect of this study was the technical success of the urine collection method and the analysis for urine lactulose concentration. These observations suggest that determination of the percentage of urinary recovery of orally administered sugars may be a safe, simple and minimally-invasive diagnostic and/or research tool for the assessment of small intestinal permeability in the horse and is, therefore, worthy of further investigation.

In further studies, maintenance diet was found to have a significant effect on breath hydrogen excretion patterns and, by inference, gastrointestinal tract function. Following the administration of wheat flour, cumulative hydrogen excretion was greater when the ponies were maintained on hay compared with when maintained on the pelleted ration. Although the precise mechanisms involved are unclear, these findings suggest diet-related changes in

either the functional characteristics of the small intestine (i.e. a greater proportion of the administered test meal is available for bacterial fermentation when maintained on the hay diet) or the large intestinal intra-luminal environment (i.e. conditions facilitated greater net production of hydrogen when maintained on the hay diet). In addition, despite the within and between animal variation in hydrogen excretion, the patterns of hydrogen excretion recorded when maintained on the pelleted ration indicated more rapid presentation of the unabsorbed component of the test meal to intestinal bacteria compared with when maintained on the hay diet. This was interpreted as a diet-related change in gastric/small intestinal motility and, consequently, a reduced MCTT when maintained on the pelleted diet.

The potential use of breath hydrogen measurement for the assessment of MCTT (defined as the time at which a significant, sustained increase in hydrogen excretion is detected) was further investigated using the motility modifying drugs, cisapride and codeine phosphate. In man, it has been shown that there is a highly significant correlation between MCTT's as determined by breath hydrogen measurement and that calculated using the gold-standard technique, scintigraphy (Sciarretta *et al.*, 1994). In the present study, cisapride did appear to reduce MCTT. In addition, although MCTT was not prolonged following codeine pretreatment, the patterns of breath hydrogen excretion recorded did indicate a delay in presentation of the test meal to intestinal bacteria. Therefore, based on the results of this preliminary study, it was concluded that breath hydrogen measurement was sufficiently sensitive to detect the contrasting effects of both cisapride and codeine phosphate on gastrointestinal motility in the horse.

These findings suggest that breath hydrogen measurement may be a useful, non-invasive means of assessing certain aspects of gastrointestinal tract function in groups of animals. However, more accurate interpretation of test results would necessitate further validation studies in which breath hydrogen measurement was compared with more conventional, direct methods of assessing gastrointestinal tract function in the horse such as myoelectrography. In addition, interpretation of test results in the present series of studies assumes that, in healthy ponies, hydrogen excreted in breath originates from the large intestine and although this has been confirmed in man (Levitt, 1969), this aspect remains to be investigated in the horse.

LIST OF APPENDICES

APPENDIX 1

SUPPLIERS OF REAGENT AND EQUIPMENT

ABX,

ABX Hematologie, Parc Euro Medicine, Rue du Caducee, BT 7290 34184, Mont Pellier, ZX CEDEX 4, France.

Bard Limited,

Crawley, West Sussex, England.

Bayer Diagnostics,

Bayer plc, Evans House, Hamilton Close, Houndsmills, Basingstoke, England.

BDH Chemicals,

Merck Ltd, Hunter Boulevard, Magna Park, Lutterworth, Leics., England.

Beckman Instruments Inc.,

Oakley Court, Kingsmead Business Park, London Road, High Wycombe, Bucks., England.

Becton Dickinson,

Becton Dickinson UK Limited, Between Towns Road, Cowley, Oxford, England.

Cox Pharmaceuticals,

A.H. Cox and Co. Ltd., Whiddon Valley, Barnstaple, England.

GMI Medical Ltd,

Gas Measurement Instruments Limited, Inchinnan Estate, Renfrew, Scotland.

IDEXX Laboratories Ltd,

Milton Court, Churchfield Road, Chalfont St Peter, Bucks., England.

Janssen-Cilag Ltd,

Saunderton, High Wycombe, Bucks., England.

Microsoft Corporation,

One Microsoft Way, Redmond, WA 98052-6399, USA.

Minitab Incorporated,

3081 Enterprise Drive, State College, PA 16801, USA.

Norton,

H.N. Norton & Co Ltd, Harlow, Essex, England.

Nycomed,

P.O. Box 4284, Torshov, N-0401 Oslo 4, Norway.

Randox Laboratories Ltd.,

Ardmore, Diamond Road, Crumlin, Co. Antrim, Northern Ireland.

Roche,

Roche Products Limited, PO Box 8, Welwyn Garden City, Herts., England.

Rocket of London,

Watford, England.

Scottish Quality Assessment Scheme,

Biochemistry Department, Victoria Infirmary, Glasgow, Scotland.

Sigma-Aldrich Company Ltd,

Fancy Road, Poole, Dorset, England.

Software Publishing Corporation,

P.O. Box 7210, 1901 Landings Drive, Mountain View, CA 94039-7210, USA.

Spillers Horse Feeds,

Old Wolverton Road, Old Wolverton, Milton Keynes, England.

Technicon Instruments Corporation,

Bayer plc, Evans House, Hamilton Close, Houndsmills, Basingstoke, England.

Westmills Foods Ltd.,

Vanwall Road, Maidenhead, Berks., England.

UKEQAS,

United Kingdom External Quality Assurance Scheme, Department of Clinical Chemistry, Queen Elizabeth Medical Centre, Edgbaston, Birmingham, England.

APPENDIX 2

Hospital case numbers of the 29 cases of chronic equine gastro-/enteropathies included in Chapter 3.

Identification No.	Case No.
1	115705
2	119519
3	121786
4	123918
5	123455
6	107652
7	128325
8	128368
9	128234
10	122259
11	123810
12	124258
13	104810
14	105806
15	104206
16	128620
17	116133
18	122783
19	127091
20	109739
21	116328
22	119669
23	125597
24	105402
25	114660
26	124992
27	128581
28	105698
29	120147

APPENDIX 3

Case numbers, age, breed/type, sex and 'reason for referral' of all animals included in the control population generated for use in Chapter 3. NR=not recorded, TB=Thoroughbred, STB= Standardbred, ID=Irish Draught, F=female, M=male, MN=gelding.

Case No.	Age	Breed	Sex	Referral Group
128324	13	Warmblood	F	Surgery
128325	4	Dales	F	Medicine
128326	8	TB	MN	Surgery
128327	1	TB	M	Surgery
128328	15	TB x	F	Surgery
128329	10	Riding pony	F	Surgery
128330	8	Hackney x	MN	Surgery
128331	8	TB x	F	Surgery
128332	9	Shire	MN	Medicine
128333	15	Riding pony	F	Surgery
128334	5	Hunter	F	Medicine
128335	6	Hanovarian	MN	Surgery
128336	15	Riding pony	MN	Medicine
128337	16	Riding pony	F	Medicine
128338	4	TB	MN	Medicine
128339	3	STB	MN	Surgery
128340	8	Riding pony	MN	Surgery
128341	7	ID	MN	Surgery
128342	3	TB	F	Surgery
128343	5	TB x	MN	Medicine
128344	9	ID x	F	Surgery
128345	15	Welsh cob	F	Surgery
128346	5	Warmblood	M	Surgery
128347	7	Hunter	MN	Medicine
128348	11	Appaloosa	MN	Surgery
128349	15	Arab x	F	Medicine
128350	14	TB x	MN	Medicine
128351	6	TB x	MN	Medicine
128352	16	TB	F	Surgery
128353	11	Hunter	MN	Surgery
128354	8	TB x	F	Medicine
128355	5	Shetland	M	Surgery

Case No.	Age	Breed	Sex	Referral Group
128356	2	Shetland	F	Surgery
128357	2	Shetland	F	Surgery
128358	16	TB x	MN	Surgery
128359	21	Clydesdale	MN	Medicine
128360	17	Shetland	F	Medicine
128361	7	Shetland	F	Medicine
128362	8	Welsh cob	MN	Surgery
128363	3	Shetland	MN	Surgery
128364	16	Shetland x	MN	Medicine
128365	6	Warmblood	M	Surgery
128366	16	TB x	F	Medicine
128367	5	TB x	MN	Surgery
128368	4	Welsh cob	F	Medicine
128369	4	Shetland	F	Surgery
128370	3	Shetland	F	Surgery
128371	20	Connemara	MN	Medicine
128372	11	Riding pony	MN	Surgery
128373	11	TB x	F	Surgery
128374	11	TB	MN	Surgery
128375	8	TB	MN	Surgery
128376	6	TB	F	Medicine
128377	7	Hunter	MN	Surgery
128378	11	TB	MN	Surgery
128379	13	TB x	MN	Surgery
128380	16	TB x	MN	Surgery
128381	3	Fell	MN	Surgery
128382	5	TB x	MN	Surgery
128383	12	TB x	F	Surgery
128384	12	TB	F	Medicine
128385	3	Shetland	M	Surgery
128386	2	Shetland	F	Surgery
128387	9	Shetland	F	Surgery
128388	NR	TB x	F	Surgery
128389	10	TB x	F	Surgery
128390	NR	TB x	MN	Surgery
128391	8	TB	F	Surgery
128392	20	Riding horse	MN	Medicine

Case No.	Age	Breed	Sex	Referral Group
128393	21	TB	MN	Surgery
128394	15	TB	MN	Surgery
128395	15	TB	MN	Surgery
128396	20	TB	MN	Surgery
128397	30	Donkey	F	Medicine
128398	16	Connemara x	MN	Medicine
128399	5	TB x	F	Medicine
128400	8	Warmblood	F	Medicine
128401	8	Riding pony	F	Medicine
128402	7	TB x	F	Surgery
128403	15	TB x	F	Surgery
128404	17	Highland	MN	Medicine
128405	11	TB x	MN	Surgery
128406	13	TB x	MN	Surgery
128407	20	Donkey	F	Medicine
128408	9	Clydesdale	F	Medicine
128410	7	TB	MN	Surgery
128411	10	Riding pony	F	Surgery
128412	11	Highland	F	Medicine
128413	6	TB x	MN	Surgery
128414	9	Arab	MN	Surgery
128415	18	Connemara x	MN	Surgery
128416	19	TB	F	Surgery
128417	5	Hackney x	MN	Surgery
128418	14	Riding pony	MN	Surgery
128419	33	Riding horse	MN	Medicine
128420	6	TB x	MN	Surgery
128421	2	Riding pony	M	Surgery
128422	4	Riding pony	M	Medicine
128423	0.1	Warmblood	F	Surgery
128424	3	Warmblood	M	Surgery
128425	7	STB	MN	Surgery
128426	6	Arab x	F	Medicine
128427	9	TB	MN	Surgery
128428	NR	Riding horse	F	Surgery
128429	8	TB x	MN	Surgery

Case No.	Age	Breed	Sex	Referral Group
128430	8	Clydesdale x	MN	Medicine
128431	6	TB	F	Medicine
128432	20	Clydesdale	F	Medicine
128433	3	Welsh cob	M	Surgery
128434	11	TB	MN	Surgery
128435	20	Riding pony	MN	Surgery
128436	16	Riding pony	MN	Surgery
128437	9	TB	F	Surgery
128438	7	TB	MN	Medicine
128439	4	Warmblood	F	Medicine
128440	16	Donkey	F	Medicine
128441	3	Welsh mt	F	Medicine
128442	12	TB	F	Surgery
128443	16	Hunter	MN	Surgery
128444	6	Welsh mt	MN	Surgery
128445	28	Highland	MN	Medicine
128446	28	Shetland	MN	Medicine
128447	2	TB	F	Surgery
128448	5	TB x	F	Medicine
128449	11	Hunter	MN	Surgery
128450	13	Shire x	MN	Medicine
128451	7	Hunter	MN	Medicine
128452	13	TB	F	Medicine
128453	2	Hanovarian	F	Surgery
128454	6	TB	MN	Medicine
128456	9	Clydesdale x	MN	Medicine
128457	4	Clydesdale x	MN	Medicine
128458	8	Shetland	F	Medicine
128459	11	Riding pony	F	Medicine
128460	5	NR	MN	Medicine
128461	6	NR	MN	Medicine
128462	6	Riding pony	F	Medicine
128463	16	Arab x	MN	Surgery
128464	2	Hanovarian x	M	Surgery
128465	1	TB x	F	Surgery
128466	11	Connemara	F	Surgery
128467	8	Riding pony	F	Medicine

Case No.	Age	Breed	Sex	Referral Group
128468	15	Highland	MN	Surgery
128469	4	STB	MN	Surgery
128470	0.4	Clydesdale	F	Medicine
128471	9	Hunter	MN	Surgery
128472	14	NR	MN	Surgery
128473	18	NR	F	Surgery
128474	10	TB	MN	Surgery
128475	2	Warmblood x	F	Medicine
128476	10	Riding pony	F	Surgery
128477	10	TB x	F	Surgery
128478	10	Riding pony	MN	Surgery
128479	4	Riding horse	NR	Medicine
128481	4	TB	MN	Medicine
128482	14	Connemara x	F	Surgery
128483	4	Arab x	MN	Surgery
128484	11	TB	MN	Surgery
128485	6	Welsh cob	F	Medicine
128486	20	Riding pony	MN	Surgery
128487	9	Riding pony	MN	Surgery
128488	5	TB x	F	Surgery
128489	6	Riding horse	MN	Surgery
128490	4	STB	MN	Surgery
128491	7	Riding horse	F	Medicine
128493	1	Welsh mt	M	Surgery
128494	8	Welsh cob	MN	Medicine
128495	4	Riding horse	F	Surgery
128496	10	TB x	F	Medicine
128497	6	TB x	F	Surgery
128498	5	Warmblood x	F	Medicine
128499	8	Hunter	MN	Medicine
128500	12	Riding horse	MN	Medicine
128501	13	Arab x	F	Medicine
128502	4	Riding horse	F	Medicine
128503	6	TB	MN	Medicine
128504	14	Riding horse	MN	Surgery
128505	2	ID x	M	Surgery
128506	22	TB x	MN	Medicine

Case No.	Age	Breed	Sex	Referral Group
128507	0.9	Hanovarian	F	Surgery
128508	21	TB x	MN	Medicine
128509	20	Connemara x	MN	Surgery
128510	2	TB x	F	Surgery
128511	18	TB x	F	Medicine
128512	15	Highland x	MN	Medicine
128513	14	Connemara x	MN	Surgery
128514	6	Clydesdale x	MN	Surgery
128515	5	TB x	F	Surgery
128516	5	TB x	MN	Medicine
128517	22	Welsh mt	F	Medicine
128518	12	TB x	MN	Medicine
128519	7	TB x	MN	Medicine
128520	16	Riding pony	F	Surgery
128521	5	Arab x	MN	Surgery
128522	6	Riding pony	F	Surgery
128523	7	Highland	F	Surgery
128524	8	Riding pony	MN	Medicine
128525	14	Riding pony	F	Surgery
128526	21	Riding pony	MN	Medicine
128527	6	Warmblood	MN	Surgery
128528	8	Riding horse	MN	Surgery
128529	4	Heavy cob	MN	Surgery
128530	8	TB x	MN	Surgery
128531	12	Riding pony	MN	Medicine
128532	9	Riding horse	MN	Surgery
128533	8	Clydesdale	MN	Medicine
128534	7	TB	MN	Surgery
128535	7	TB	MN	Surgery
128536	5	Riding pony	MN	Medicine
128537	2	Riding pony	F	Surgery
128538	6	Riding pony	F	Surgery
128539	10	Riding pony	MN	Surgery
128540	7	TB	F	Medicine
128541	15	TB x	MN	Medicine
128542	1	Riding pony	M	Medicine
128543	24	TB x	MN	Medicine

Case No.	Age	Breed	Sex	Referral Group
128544	19	Welsh cob	F	Medicine
128545	NR	Warmblood	MN	Surgery
128546	12	Riding horse	MN	Surgery
128547	6	TB x	F	Surgery
128548	0.8	Riding pony	F	Medicine
128549	3	STB	MN	Surgery
128550	13	Appaloosa	MN	Surgery
128551	1	Arab x	M	Surgery
128552	17	Highland x	MN	Medicine
128553	9	Welsh mt	MN	Medicine
128554	5	Riding pony	F	Surgery
128555	15	TB x	MN	Surgery
128556	9	TB	MN	Surgery
128557	7	TB	F	Surgery
128558	7	Riding pony	MN	Surgery
128559	9	STB	F	Surgery
128563	4	TB x	MN	Surgery
128564	5	Riding horse	F	Surgery
128565	4	ID x	F	Surgery
128566	7	TB x	F	Surgery
128567	9	Hanovarian	MN	Surgery
128568	20	Riding horse	MN	Medicine
128569	6	Welsh cob	MN	Surgery
128570	12	Riding horse	MN	Surgery
128571	19	TB x	F	Medicine
128572	4	TB	MN	Surgery
128573	2	TB x	M	Surgery
128574	30	Riding pony	MN	Medicine
128575	17	Hunter	MN	Medicine
128576	3	Riding pony	MN	Medicine
128577	7	Welsh mt	F	Medicine
128578	9	Riding pony	MN	Surgery
128579	5	TB x	F	Medicine
128580	0.5	Highland	F	Medicine
128581	20	TB x	F	Medicine
128582	9	Warmblood	F	Surgery
128583	6	Connemara	F	Surgery

Case No.	Age	Breed	Sex	Referral Group
128584	16	TB x	F	Surgery
128585	17	Riding pony	F	Surgery
128586	5	Riding horse	F	Surgery
128589	5	NR	MN	Surgery
128591	2	Warmblood x	MN	Surgery
128592	6	TB	F	Surgery
128593	6	TB x	MN	Surgery
128594	20	TB x	MN	Medicine
128595	3	Arab x	F	Surgery
128596	NR	TB x	MN	Surgery
128597	17	Riding pony	F	Medicine
128598	17	Connemara x	MN	Medicine
128599	6	Highland	MN	Medicine
128600	9	TB x	F	Medicine
128601	12	Hunter	F	Surgery
128602	1	Clydesdale	M	Medicine
128604	15	Riding pony	F	Surgery
128605	12	Welsh cob	MN	Medicine
128606	7	TB x	MN	Medicine
128607	3	ID x	F	Medicine
128608	6	TB x	MN	Surgery
128609	NR	Riding horse	MN	Medicine
128610	3	Riding pony	MN	Medicine
128611	6	Riding pony	F	Surgery
128612	8	Hunter	MN	Surgery
128613	6	Hanovarian x	MN	Surgery
128614	8	Riding pony	MN	Surgery
128615	7	TB x	F	Surgery
128616	8	Riding pony	F	Surgery
128617	10	Riding horse	F	Surgery
128618	7	TB	MN	Surgery
128619	6	TB x	MN	Surgery
128620	20	Fell	F	Medicine
128621	12	TB x	F	Medicine
128622	7	Shetland	F	Medicine
128623	7	TB	F	Surgery
128624	14	TB x	F	Medicine

APPENDIX 4

Serum and plasma fructosamine concentrations (μmol/L) measured in adult (Group A) and yearling (Group B) ponies for five consecutive weeks.

Animal Identification		Week 1	Week 2	Week 3	Week 4	Week 5	mean±SD plasma fructosamine concentration
Group A							
123	serum	250	272	268	282	249	
	plasma	250	255	243	256	246	250.0±5.61
124	serum	260	315	285	298	258	
	plasma	246	280	279	279	252	267.2±16.75
125	serum	279	304	317	298	282	
	plasma	247	291	302	285	255	276.0±23.79
126	serum	301	331	316	344	287	
	plasma	285	315	300	329	279	301.6±20.73
127	serum	261	293	288	297	248	
	plasma	252	271	275	279	253	266.0±12.65
128	serum	265	308	281	308	267	
	plasma	264	290	273	291	257	275.0±15.25
129	serum	234	274	264	279	239	
	plasma	226	261	251	269	233	248.0±18.22
Group B							
114	serum	304	306	312	334	289	
	plasma	289	310	317	329	272	303.4±22.79
115	serum	201	223	241	246	217	
	plasma	192	211	221	232	209	213.0±14.88
116	serum	166	297	306	322	268	
	plasma	267	278	290	296	257	277.6±16.04
117	serum	284	287	306	324	287	
	plasma	277	290	291	304	278	288.0±11.07
118	serum	227	223	258	255	227	
	plasma	222	233	243	243	233	234.8±8.73
119	serum	250	246	206	138	240	
	plasma	233	236	304	266	229	253.6±31.75
120	serum	232	255	254	244	235	
	plasma	227	238	246	245	231	237.4±8.38
121	serum	282	286	275	275	247	
	plasma	272	263	249	265	240	262.4±18.28
Mean (±SD) Serum Fructosamine Concentration*							270.4±37.7
Mean (±SD) Plasma Fructosamine Concentration*							263.3±29.1

*calculated based on the mean of 75 observations.

APPENDIX 5

Plasma fructosamine concentrations (μmol/L) measured in yearling (Group C) ponies for five consecutive weeks.

Animal Identification	Week 1	Week 2	Week 3	Week 4	Week 5	mean±SD
Group C						
101	274	269	263	267	238	262.2±14.1
102	261	258	249	271	245	256.8±10.26
103	199	168	183	201	181	186.4±13.7
104	233	232	227	245	224	232.2±8.04
105	251	230	223	229	213	229.2±13.94
106	279	257	238	238	223	247.0±21.58
111	271	276	242	285	283	271.4±17.36
112	258	254	242	295	286	267.0±22.47
113	259	265	239	277	287	265.4±18.3

APPENDIX 6

Fructosamine concentrations (μmol/L) of two plasma pools assayed repeatedly for calculation of intra-assay precision.

Number	Adult (Group A) Plasma Pool	Foal (Group B) Plasma Pool
1	261	251
2	258	251
3	265	247
4	260	246
5	258	248
6	252	253
7	253	251
8	255	246
9	253	250
10	258	240
Mean±SD	457.3±4.11	248.3±3.77
CV	1.6 %	1.52 %

APPENDIX 7

Fructosamine concentrations (μmol/L) of two plasma pools assayed repeatedly for calculation of inter-assay precision.

Assay Number	Adult (Group A) Plasma Pool	Foal (Group B) Plasma Pool
1	261	251
2	256	240
3	245	233
4	264	255
5	235	236
Mean±SD	252.2+12.03	243+9.57
CV	4.77 %	3.94 %

APPENDIX 8

Fructosamine concentrations (μmol/L) of a plasma sample measured at 4 dilutions for assessment of assay linearity.

Dilution	Predicted Fructosamine Concentration (μmol/L)	Measured Fructosamine Concentration (μmol/L)
0	255.6	255.6
1:1.5	192.0	197.0
1:2	128.0	137.3
1:4	64.0	74.0
1:8	32.0	36.6

APPENDIX 9

Fructosamine concentrations (μmol/L) measured in plasma samples at different times after sample collection for assessment of the effects of sample storage on plasma fructosamine measurement.

Pony	Immediate[1]	72 hours[2]	7 days[3]	30 days[4]
114	288	246	272	271
115	305	260	310	297
116	304	259	304	293
117	333	280	319	317
118	333	285	307	309
119	343	296	321	322
123	313	268	304	300
124	308	261	292	286
125	293	236	294	273
126	327	277	305	321
127	308	267	299	294
128	320	268	338	301
129	279	234	258	268
Mean±SD	311.85±18.94	264.0±18.23	301.0±20.54	296.3±18.26

[1]analysed within 4 hours of sample collection.

[2]analysed following storage of whole blood for 72 hours at room temperature.

[3]analysed following storage of plasma for 7 days at 4^0C.

[4]analysed following storage of plasma for 28 days at -20^0C.

APPENDIX 10

Individual pony weights (kg) measured following experimental cyathostome infections.

	Infected Group 1			Infected Group 2			Control Group 3		
	101	**104**	**105**	**111**	**112**	**113**	**102**	**103**	**106**
Week									
-8	130	79	103	109	76	83	95	80	102
-6	129	79	103	112	80	88	90	80	103
-4	133	84	105	114.5	82	89	93	84	106
-2	131	88	106	114.5	87	94	96	87	106
0	138	90	111	113	91	98	104	91	113
1	143	93	116	-	-	-	110	94	119
2	141	89	113	108	86	91	106	92	119
3	142	93	111	-	-	-	110	95	120
4	142	92	111	114.5	92	100	111	93	121
5	147	92	114	-	-	-	118	101	131
6	143	90	112	112	91	100	118	101	128
7	143	92	112	103	88	98	121	102	132
8	142	90	112	99	84.5	90.5	118	102	130
9	142	95.5	116	96	84.5	91	127	111	137
10	145	98	117	-	86.8	91	130	112	138
11	147	99	119	-	88	93	132	117	141
12	148	99	118	-	-	-	135	121	139
13	146	100	118	-	90	93	137	122	145
14	153	98	126	-	92	93	142	125	147
15	150	95.5	119	-	94	98	134	122	144
16	154	94	121	-	99	107	136	127	145
17	154	91	122	-	101	106	136	124	140
18	154	96	120	-	98	107	136	126	143
19	-	-	-	-	-	-	-	-	-
20	159	99.5	128	-	99.5	107	144	136	152

APPENDIX 11

Individual plasma fructosamine concentrations (μmol/L) measured following experimental cyathostome infections.

	Infected Group 1			Infected Group 2			Control Group 3		
	101	**104**	**105**	**111**	**112**	**113**	**102**	**103**	**106**
Week									
-4	274	233	251	271	258	259	261	199	279
-3	269	232	230	276	254	265	258	168	257
-2	263	227	223	242	242	239	249	183	238
-1	267	245	229	285	295	277	271	201	238
0	238	224	213	283	286	287	245	181	223
1	257	232	230	331	283	296	255	204	241
2	240	226	219	257	242	252	246	196	251
3	241	222	224	281	274	279	255	197	244
4	216	208	210	282	314	314	242	196	247
5	247	238	240	289	276	275	275	215	265
6	233	227	238	256	281	268	280	214	280
7	210	212	228	252	263	269	258	228	275
8	232	225	231	219	250	257	286	241	294
9	209	224	222	186	218	250	281	210	278
10	215	228	226	-	222	222	287	248	290
11	207	216	219	-	226	193	277	235	288
12	205	204	230	-	227	186	291	247	288
13	201	207	196	-	208	175	285	254	260
14	211	202	208	-	217	188	285	261	294
15	237	213	225	-	232	203	293	285	296
16	216	194	209	-	184	185	296	258	279
17	223	177	203	-	190	189	285	258	274
18	249	193	218	-	196	193	289	275	288
19	261	200	234	-	203	199	312	294	316
20	257	194	248	-	250	258	314	294	328

APPENDIX 12

Individual plasma albumin concentrations (g/L) measured following experimental cyathostome infections.

	Infected Group 1			Infected Group 2			Control Group 3		
	101	**104**	**105**	**111**	**112**	**113**	**102**	**103**	**106**
Week									
-4	28	21	27	31	27	30	26	16	27
-3	28	21	23	32	28	30	24	15	24
-2	26	21	23	30	27	30	23	15	23
-1	26	23	25	35	31	33	26	17	25
0	24	22	22	35	33	33	25	16	22
1	25	24	25	37	31	28	25	18	25
2	25	24	25	35	32	33	25	18	24
3	26	24	26	35	34	37	27	18	26
4	24	23	25	34	34	34	26	18	26
5	24	24	25	36	35	34	27	21	25
6	25	25	26	35	34	32	29	22	30
7	22	23	23	37	33	32	26	21	31
8	24	24	23	32	32	30	28	24	31
9	22	24	22	31	26	24	29	22	29
10	23	26	23	-	25	22	30	27	31
11	21	24	23	-	29	26	29	26	31
12	21	25	25	-	30	28	26	25	29
13	22	23	19	-	33	28	30	29	29
14	23	21	20	-	29	26	29	28	29
15	23	22	22	-	31	29	30	28	30
16	24	22	23	-	30	30	30	29	31
17	25	20	22	-	30	30	30	31	29
18	27	21	23	-	28	27	29	29	28
19	28	22	26	-	30	30	33	32	33
20	29	24	28	-	31	31	33	33	33

APPENDIX 13

Individual serum globulin concentrations (α2, β1, β2, γ) (g/L) measured following experimental cyathostome infections.

α2-globulin concentrations (g/L)

	Infected Group 1			Infected Group 2			Control Group 3		
	101	**104**	**105**	**111**	**112**	**113**	**102**	**103**	**106**
Week									
-4	7.18	9.97	8.86	7.85	9.01	6.83	9.29	7.54	10.42
-3	7.72	8.96	9.43	8.44	8.47	7.56	8.97	5.84	9.74
-2	7.04	9.97	8.16	7.08	8.49	7.98	8.27	6.79	9.92
-1	7.56	9.80	9.07	7.54	8.53	7.74	8.03	7.27	9.88
0	6.85	9.83	7.15	8.00	8.56	7.50	7.74	6.84	8.14
1	6.78	9.35	7.79	7.90	9.00	7.75	7.84	8.84	9.06
2	6.75	10.32	8.06	7.81	9.41	8.10	7.69	8.14	9.46
3	6.75	8.53	8.57	9.11	9.42	7.88	7.61	6.76	9.50
4	7.10	7.73	8.80	10.60	10.21	8.49	7.34	6.02	9.06
5	7.70	7.78	9.13	11.36	11.21	10.27	7.99	6.21	7.69
6	8.32	8.06	9.92	10.02	10.03	9.48	7.84	5.83	9.58
7	8.22	8.93	15.94	9.24	9.86	10.14	8.99	6.66	10.53
8	8.37	10.21	12.52	7.79	12.42	11.04	8.82	7.00	8.68
9	8.58	9.07	10.80	7.76	10.20	9.41	9.46	7.84	8.93
10	8.96	8.45	12.29	-	9.11	9.26	8.64	7.07	8.97
11	7.70	7.00	10.44	-	11.27	10.60	7.45	6.60	7.83
12	8.69	7.80	10.69	-	11.47	11.02	7.52	5.99	8.18
13	8.18	8.50	9.64	-	10.46	9.42	7.80	6.21	8.46
14	7.56	7.55	9.39	-	8.68	9.15	8.13	6.61	9.73
15	8.32	8.83	10.30	-	9.68	8.78	7.13	6.44	9.47
16	6.63	7.31	9.30	-	9.75	9.77	6.61	5.99	8.38
17	7.17	7.67	8.80	-	11.78	9.95	7.02	6.50	9.11
18	6.20	7.28	9.09	-	10.89	10.95	6.27	6.10	8.80
19	8.90	9.92	11.52	-	10.04	9.64	10.94	8.51	8.82
20	9.30	10.52	11.25	-	10.79	9.57	8.70	8.00	11.08

β1-globulin concentrations (g/L)

	Infected Group 1			Infected Group 2			Control Group 3		
	101	104	105	111	112	113	102	103	106
Week									
-4	3.13	5.10	4.07	6.37	5.13	4.37	7.41	6.03	6.45
-3	2.97	5.4	4.08	6.05	5.01	4.88	7.54	6.22	5.92
-2	3.06	5.54	3.74	6.55	5.36	4.86	6.97	6.02	5.64
-1	3.78	5.42	4.43	7.09	4.80	4.87	7.54	6.21	7.69
0	3.25	4.89	4.31	7.69	4.37	4.88	6.94	5.98	6.66
1	3.37	5.64	4.40	7.35	4.29	5.00	6.78	5.69	6.84
2	2.90	5.70	4.51	6.98	4.22	5.16	7.42	5.84	6.56
3	3.25	5.39	4.48	7.30	5.27	5.54	7.24	6.35	5.90
4	2.83	4.82	4.07	7.48	5.79	5.70	6.91	6.20	6.24
5	3.20	5.10	4.27	8.23	5.61	5.61	6.80	5.75	5.62
6	3.33	5.57	5.05	6.99	4.85	4.80	7.06	5.88	6.04
7	3.80	6.32	7.17	6.39	5.07	5.54	7.83	6.03	6.31
8	4.27	6.31	6.01	5.62	5.10	4.86	7.25	5.55	6.26
9	4.06	6.93	6.14	6.38	5.10	4.80	7.93	4.95	4.99
10	5.13	7.09	6.66	6.44	4.85	4.78	7.71	6.05	5.55
11	5.20	5.88	6.90	-	6.08	6.14	6.78	5.25	5.92
12	5.41	8.45	9.04	-	6.51	7.26	6.95	5.67	6.01
13	6.22	9.18	8.63	-	6.30	6.08	7.38	5.87	5.70
14	6.66	8.73	8.51	-	5.54	5.96	7.25	5.92	5.63
15	5.59	9.86	9.11	-	5.43	5.55	6.61	5.67	5.76
16	5.14	8.76	8.37	-	6.04	6.99	6.56	5.30	5.37
17	5.82	8.90	8.56	-	6.62	6.83	7.02	5.51	5.37
18	6.43	8.62	6.22	-	6.66	7.99	6.27	5.40	5.83
19	5.50	8.99	9.36	-	6.70	6.73	7.20	6.08	5.67
20	4.68	8.69	10.26	-	6.88	6.73	6.91	6.08	5.85

β2-globulin concentrations (g/L)

	Infected Group 1			Infected Group 2			Control Group 3		
	101	104	105	111	112	113	102	103	106
Week									
-4	5.94	7.06	5.45	3.60	5.47	4.87	3.36	5.34	6.57
-3	5.99	5.99	5.72	3.53	5.45	5.25	2.75	4.45	6.44
-2	5.41	6.55	5.04	2.83	5.87	5.46	2.55	4.73	6.78
-1	6.10	6.04	5.67	3.00	5.20	5.26	2.53	4.92	6.65
0	5.50	5.30	4.95	3.22	4.60	5.06	2.70	4.69	6.60
1	5.61	6.04	5.57	3.28	4.30	5.14	2.86	5.17	6.84
2	5.60	6.60	5.57	3.33	4.05	5.22	2.33	4.78	6.27
3	5.30	6.44	5.77	3.35	5.15	5.73	2.75	4.97	6.61
4	6.38	4.98	5.56	3.32	5.66	5.70	2.57	4.89	6.72
5	6.05	4.87	5.51	3.40	5.80	6.62	2.48	4.51	5.04
6	6.55	5.45	6.32	2.96	5.13	6.12	2.46	4.65	5.61
7	6.92	6.45	8.26	3.16	5.42	6.49	2.32	4.66	5.59
8	7.24	7.15	8.49	3.23	6.42	7.02	2.84	4.45	5.46
9	6.92	6.99	8.08	3.36	5.81	5.98	3.11	4.90	5.34
10	7.18	7.93	9.22	-	5.05	5.77	2.20	4.81	5.12
11	6.78	7.11	8.94	-	5.84	7.07	2.30	4.52	5.05
12	7.47	7.87	10.23	-	6.63	8.05	2.17	4.37	5.33
13	7.75	8.16	10.27	-	6.24	7.69	2.28	4.62	4.92
14	7.68	7.38	11.21	-	5.60	7.61	2.02	4.70	5.50
15	6.67	7.49	10.56	-	6.55	7.87	1.74	3.41	5.25
16	6.41	7.48	9.80	-	6.56	7.94	1.71	3.87	4.78
17	6.66	6.44	8.93	-	6.87	7.93	2.22	4.12	5.21
18	7.20	6.89	10.98	-	6.27	7.40	1.68	3.73	6.01
19	7.04	7.19	11.45	-	7.50	8.91	2.95	4.80	5.73
20	7.41	6.87	11.70	-	7.38	9.04	2.82	4.65	5.98

γ-globulin concentrations (g/L)

	Infected Group 1			Infected Group 2			Control Group 3		
	101	**104**	**105**	**111**	**112**	**113**	**102**	**103**	**106**
Week									
-4	4.32	6.05	4.24	7.49	6.56	6.33	6.38	4.05	4.90
-3	3.89	6.53	4.35	7.80	7.54	6.71	6.77	3.28	4.70
-2	3.62	6.55	4.27	7.20	6.56	6.48	6.19	4.13	4.96
-1	3.94	6.56	4.88	7.57	6.26	6.50	6.33	4.09	5.37
0	3.70	6.08	4.41	7.94	5.96	6.53	6.15	3.74	4.90
1	3.83	6.50	4.88	8.50	5.69	7.00	6.04	4.04	5.16
2	4.05	7.38	5.04	9.09	5.42	7.92	6.04	3.91	4.79
3	4.55	7.42	5.54	9.98	6.88	8.38	5.99	4.05	5.02
4	4.66	6.05	5.56	9.30	7.15	8.18	5.78	3.43	4.92
5	5.30	6.50	5.62	9.93	7.31	9.39	5.67	3.31	4.08
6	5.72	7.31	6.03	8.82	6.90	10.20	5.77	3.48	4.64
7	5.56	8.31	6.21	8.06	7.01	10.65	10.44	3.87	5.01
8	5.78	8.00	6.45	5.88	6.24	7.68	6.03	3.45	5.15
9	5.62	7.88	6.14	6.11	5.20	5.59	6.47	3.87	4.58
10	5.83	8.13	6.66	-	4.61	9.57	6.32	4.32	4.82
11	5.46	7.11	6.48	-	6.20	11.72	6.16	4.11	4.87
12	6.04	8.32	7.39	-	7.75	11.55	6.90	4.27	5.64
13	6.66	8.70	7.18	-	6.74	9.67	7.38	4.39	4.62
14	6.24	8.14	7.56	-	5.82	9.20	6.87	4.29	5.31
15	5.59	8.58	7.72	-	6.14	8.72	6.21	4.73	5.18
16	5.35	8.12	7.38	-	7.01	9.89	6.10	4.13	4.90
17	5.71	7.56	7.75	-	7.81	9.88	6.48	4.47	5.15
18	6.55	7.67	7.26	-	8.10	11.62	6.10	4.16	5.70
19	5.70	7.32	9.22	-	8.43	9.77	6.41	4.93	6.11
20	5.72	7.06	9.65	-	7.63	9.90	6.02	4.53	6.46

APPENDIX 14

The results of individual OGTTs performed on three occasions on each of seven adult ponies when maintained on a hay only diet.

Replicate 1

Pony	**123**	**124**	**125**	**126**	**127**	**128**	**129**
Time (min)	Plasma Glucose Concentrations (mmol/L)						
0	4.2	4.4	4.4	4.2	4.1	4.9	4.2
30	6.1	6.0	7.4	6.5	5.8	7.2	7.1
60	6.8	8.6	9.3	8.3	8.6	8.5	8.6
90	7.4	9.6	10.6	10.1	10.9	10.1	9.3
120	7.6	10.6	11.7	10.1	11.5	10.7	8.5
150	6.4	11.0	11.7	10.0	10.2	10.9	7.1
180	6.9	10.4	10.7	9.4	9.8	9.0	5.4
240	6.0	8.4	9.7	6.6	7.1	8.7	3.3
300	4.9	5.9	6.9	3.5	5.0	8.3	3.2
360	5.9	5.6	6.1	3.1	3.3	6.3	4.3

Replicate 2

Pony	**123**	**124**	**125**	**126**	**127**	**128**	**129**
Time (min)	Plasma Glucose Concentrations (mmol/L)						
0	4.5	4.8	4.5	4.7	4.5	4.9	4.5
30	5.5	7.7	6.3	5.6	6.8	6.8	7.1
60	6.0	10.0	9.0	6.9	9.7	7.9	8.1
90	6.8	11.3	11.3	7.0	11.3	9.6	8.7
120	7.6	12.8	11.3	6.6	12.3	9.7	8.9
150	6.9	13.5	13.0	7.2	12.2	10.4	8.9
180	7.3	13.7	11.7	5.8	11.7	8.6	7.7
240	6.6	9.2	9.7	4.1	8.2	8.2	5.8
300	6.6	5.1	8.2	4.0	5.0	6.2	3.2
360	5.6	2.8	6.8	4.1	3.2	3.8	3.3

Replicate 3

Pony	123	124	125	126	127	128	129
Time (min)	Plasma Glucose Concentrations (mmol/L)						
0	4.6	4.4	5.1	6.0	4.1	4.9	4.3
30	5.7	6.6	7.3	7.4	6.0	5.6	6.4
60	6.3	9.2	8.9	8.5	7.3	6.9	6.9
90	7.1	11.0	8.6	10.9	8.8	8.3	7.3
120	7.5	10.4	8.2	10.7	9.5	8.7	6.9
150	7.1	10.4	7.7	10.5	9.5	9.3	7.3
180	6.9	10.3	7.9	8.3	8.4	9.6	5.5
240	6.4	7.3	8.2	5.3	5.5	8.5	2.8
300	6.0	7.0	6.6	3.0	3.9	6.9	3.7
360	5.3	5.6	7.2	4.3	2.9	4.8	4.8

APPENDIX 15

The results of individual OGTTs performed on three occasions on each of seven adult ponies when maintained on a pelleted ration only.

Replicate 1

Pony	123	124	125	126	127	128	129
Time (min)	Plasma Glucose Concentrations (mmol/L)						
0	4.1	4.0	4.1	4.0	4.0	4.2	4.7
30	5.3	5.5	5.4	5.8	4.5	4.7	5.5
60	5.3	6.8	5.2	6.3	6.2	5.2	6.3
90	5.2	6.7	5.9	6.5	7.0	6.1	5.4
120	4.7	5.6	5.6	6.0	7.3	6.6	5.1
150	4.6	5.1	4.9	6.0	5.5	6.0	3.8
180	4.6	4.6	4.7	4.5	3.1	5.8	3.9
240	4.7	3.4	4.7	3.6	1.9	4.9	4.6
300	4.3	4	4.6	4.1	3.9	3.2	3.9
360	4.2	4.1	5.0	3.9	3.9	4.2	4.3

Replicate 2

Pony	123	124	125	126	127	128	129
Time (min)	Plasma Glucose Concentrations (mmol/L)						
0	4.2	4.0	4.3	4.1	4.3	4.2	4.5
30	5.2	6.3	5.8	6.4	5.6	4.6	6.8
60	6.0	6.9	6.5	6.8	6.3	5.5	9.1
90	6.1	5.0	5.7	7.0	7.7	6.2	9.9
120	5.5	4.5	4.6	6.0	8.2	6.7	10.0
150	5.0	3.4	4.7	5.4	8.3	6.6	9.8
180	4.4	4.9	4.4	5.0	5.4	6.2	9.6
240	3.5	4.2	4.3	3.3	2.5	3.3	6.0
300	4.0	4.7	4.4	3.8	1.5	3.5	2.5
360	4.1	4.4	5.5	4.0	3.5	4.5	2.0

Replicate 3

Pony	**123**	**124**	**125**	**126**	**127**	**128**	**129**
Time (min)	Plasma Glucose Concentrations (mmol/L)						
0	4.5	3.9	4.6	4.2	4.3	4.5	4.7
30	5.5	5.1	5.8	6.6	6.7	5.9	6.1
60	5.7	6.9	6.7	7.9	8.0	7.1	7.7
90	5.7	7.5	5.7	7.9	9.0	8.7	8.1
120	6.2	7.0	5.2	6.7	9.3	8.8	7.3
150	5.9	6.5	5.3	6.1	8.0	7.9	5.8
180	5.1	4.5	4.4	5.8	6.8	8.3	4.7
240	4.8	4.7	5.3	4.7	6.0	5.4	5.0
300	4.8	5.1	5.1	3.6	3.5	4.9	5.4
360	5.6	4.9	5.1	3.7	1.7	3.8	5.8

APPENDIX 16

The results of individual OGTTs performed on three occasions on each of seven pony foals when maintained on a pelleted ration only.

Replicate 1

Pony	101	102	103	105	106	112	113
Time (min)	Plasma Glucose Concentrations (mmol/L)						
0	4.7	3.8	4.0	4.7	4.9	4.8	4.6
30	8.2	8.1	7.0	8.8	7.3	8.0	7.7
60	9.8	11.0	8.7	11.0	10.2	11.2	9.9
90	11.8	12.0	11.5	12.2	12.3	12.5	11.1
120	10.4	11.6	11.4	13.4	12.0	12.2	10.1
150	10.1	10.5	10.0	13.3	10.9	10.5	7.6
180	9.4	8.7	10.5	12.6	8.1	8.2	4.9
240	7.8	5.5	8.6	12.0	4.7	3.5	3.1
300	3.8	3.3	6.2	6.2	2.4	2.1	4.2
360	2.1	3.1	3.6	3.0	3.7	4.0	4.1

Replicate 2

Pony	101	102	103	105	106	112	113
Time (min)	Plasma Glucose Concentrations (mmol/L)						
0	4.7	4.2	4.3	4.5	4.4	4.1	4.5
30	7.1	7.5	8.3	8.3	7.8	6.3	7.4
60	8.6	10.8	10.6	11.5	10.7	7.5	10.2
90	9.1	12.9	12.6	13.1	13.6	10.4	10.8
120	10.1	12.9	13.0	14.1	15.1	9.8	7.3
150	9.3	12.3	13.9	14.2	16.1	8.0	5.6
180	8.6	10.6	13.2	14.1	13.9	6.1	3.0
240	9.2	7.7	9.7	10.6	10.1	4.7	2.2
300	6.7	4.7	6.2	5.8	5.9	4.1	3.6
360	5.6	2.3	3.7	3.0	2.7	2.4	3.8

Replicate 3

Pony	101	102	103	105	106	112	113
Time (min)	Plasma Glucose Concentrations (mmol/L)						
0	3.9	4.1	3.6	3.9	3.1	4.9	4.5
30	6.1	6.6	5.8	6.9	5.8	7.6	6.8
60	8.2	8.6	8.0	9.9	9.4	9.8	8.0
90	10.0	9.1	10.0	12.3	10.6	11.8	8.9
120	11.9	8.2	12.2	12.2	9.4	11.4	9.7
150	10.6	7.1	12.6	10.0	8.0	6.1	6.7
180	8.4	5.9	11.4	7.4	6.2	5.5	5.3
240	5.0	3.1	8.2	4.0	2.9	2.7	2.7
300	2.4	3.1	5.2	2.2	2.9	2.6	2.8
360	3.6	3.6	2.9	3.8	3.4	4.2	3.8

APPENDIX 17

Characteristics of the individual OGTTs which were included in the study to assess the sensitivity and specificity of the OGTT for detecting/excluding a diagnosis of small intestinal pathology in the horse (**STUDY 5**).

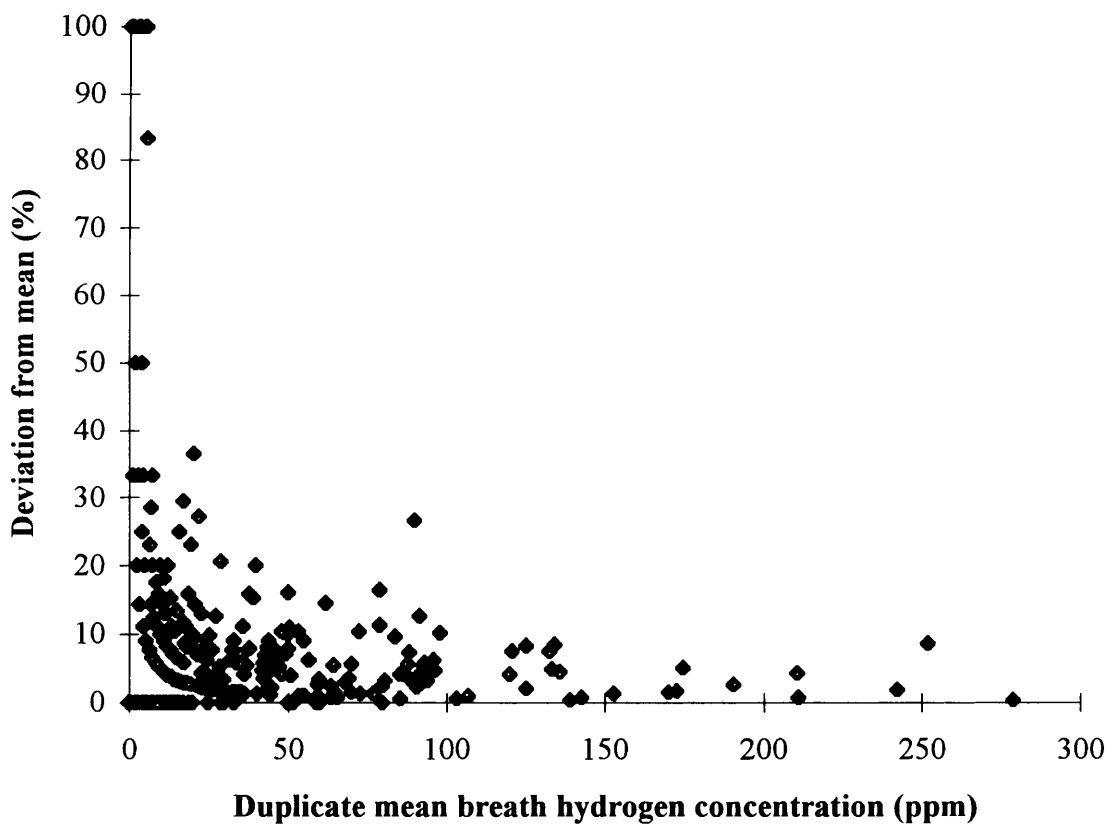
Case Number	Peak glucose concentration*	Glucose concentration at 120 min*
Small intestinal pathology present		
104810	157 %	145 %
124258	119 %	109 %
123810	111 %	107 %
105698	134 %	106 %
122783	126 %	126 %
127091	121 %	117 %
119669	125 %	104 %
125597	196 %	196 %
114660	168 %	168 %
124992	128 %	118 %
118875	131 %	0 %
Small intestinal pathology absent		
105806	236 %	236 %
119519	187 %	187 %
116328	200 %	200 %
103815	135 %	122%
110578	184 %	184 %
120147	124 %	124 %
113360	107 %	0 %
120387	141 %	139 %
121616	300 %	300 %
125466	141 %	120 %

*data presented as a percentage of basal glucose concentration.

APPENDIX 18

Repeatability of the breath collection/storage technique.

Repeatability of the breath collection/storage technique was determined by analysing the duplicate breath hydrogen concentrations detected for all tests performed, in all ponies, following the ingestion/administration of each different test meal/carbohydrate substrate as described in **STUDY 6** (i.e. 42 separate tests). For all duplicate measurements, the mean of each pair was taken as 100 % and the percentage deviation from the mean value was determined. The average deviation from the mean value for all pairs analysed (n = 714), over a range of concentrations from 0 ppm to 280 ppm was calculated to be 15.00 %, with 308 identical paired readings. When the analysis was repeated on duplicate measurements, with an initial reading of 5 ppm or greater (n = 342) the average deviation decreased to 6.26 % and included 79 identical paired readings.



Analysis of duplicate breath hydrogen measurements (n=714). The mean of each duplicate was taken as 100 % and each point represents the deviation of each measurement from the mean value.

These results compare favourably with those of Metz, Gassull, Leeds, Blendis & Jenkins (1976b) who, when using the same data analysis, reported a mean variation of 11.6 % in hydrogen concentrations in duplicate, end-expiratory breath samples.

The collection technique was similar to a 'nasal prong' method described by Perman, Barr & Watkins (1978) for breath collection from children. Although these authors acknowledged a potential for error due to dilution of the sample if collection begins too early in expiration, they found that the method was highly correlated with other reliable collection procedures. In addition, they observed that hydrogen concentrations in breath samples stored in plastic syringes were not significantly altered over an eight hour period (Perman, Barr & Watkins, 1978).

Based on the analysis described above, the reproducibility of the collection/storage method was regarded as being satisfactory and all data analyses performed in Chapter 6 utilized the mean of duplicate measurements at each time point.

APPENDIX 19

Assessment of the pattern of breath hydrogen excretion following a test meal in relation to fasting data.

When the adult ponies were maintained on either the hay only diet (Diet 1) or the pelleted ration (Diet 2) the group mean (±SD) breath hydrogen concentrations at each time point during the 'fasting' tests (i.e. 21 separate tests when maintained on each diet) were determined. Breath hydrogen excretion curves following the various test meals/carbohydrate substrates were analysed individually and were regarded as being biologically significantly different from fasting if there was a detected increase in breath hydrogen concentration greater than the greatest mean+2SD value for the respective 'fasting' data (i.e. 10 ppm* and 5 ppm* when the maintenance diets were Diet 1 and Diet 2, respectively), sustained for at least 2 consecutive time points (i.e. 30 min).

	Diet 1				**Diet 2**			
Time	mean	SD	mean+2SD		mean	SD	mean+2SD	
0	2.90	2.37	7.64		1.48	1.45	4.38	
30	3.71	3.27	10.25	**10***	1.38	1.58	4.54	**5***
60	3.33	2.77	8.88		1.48	1.32	4.11	
90	3.31	2.47	8.25		1.33	1.30	3.93	
120	3.05	2.13	7.31		1.19	1.26	3.71	
150	2.17	1.70	5.56		1.10	1.29	3.68	
180	2.17	2.03	6.22		0.98	1.25	3.48	
210	2.21	1.78	5.77		0.81	1.15	3.10	
240	1.93	1.58	5.10		0.88	1.01	2.90	
270	2.17	1.74	5.65		0.98	1.26	3.50	
300	2.02	1.96	5.94		0.79	0.99	2.77	
330	2.10	1.42	4.93		0.90	1.40	3.71	
360	1.67	1.29	4.24		0.86	1.06	2.98	
390	1.33	1.42	4.17		0.83	1.42	3.67	
420	1.50	1.57	4.65		0.67	1.15	2.98	
450	1.71	1.58	4.87		0.69	1.26	3.21	
480	1.29	1.38	4.05		0.83	1.36	3.56	

*greatest mean±2SD rounded to the nearest whole number.

APPENDIX 20

Concentrations of hydrogen (mean of duplicate measurements) detected in excreted breath of adult ponies following the administration/ingestion of a variety of 'test meals'.

T_{sig} (min) = the time at which significant increases in hydrogen (if any) were first noted.
$\Delta H2$ (ppm) = the peak hydrogen concentration measured during the test period.
$T_{\Delta H2}$ (min) = the time of peak hydrogen concentration.
AUC_{8hours} (ppm x hours) = the total area under the hydrogen excretion curve.
NA = not applicable.

Glucose

Pony	**123**	**124**	**125**	**126**	**127**	**128**	**129**
Time (min)	Breath Hydrogen Concentrations (ppm)						
0	4	2	3.5	3	1	1.5	6
30	4	2	3.5	2.5	0	1	10.5
60	8	0.5	4	6	1	2	28.5
90	26.5	1	2.5	5.5	2	2	48
120	55.5	2	2	7	0	4	70
150	65.5	1	2	10	1.5	2.5	64.5
180	91	2	3	8.5	3	2.5	84
210	89	4	2	11	4	2.5	93.5
240	85.5	3.5	3	10.5	4	2	93
270	125.5	2	1.5	15	1	3.5	96.5
300	134.5	0	0.5	23	1	2	62
330	94	1.5	0	36	3	3	42.5
360	32.5	4	1.5	13	2	6.5	14.5
390	27	2.5	0	6.5	4	2.5	11.5
420	15	3	3	4	2	2	11.5
450	13	1.5	1.5	2	2	1.5	16
480	11	2	1	1	0	1	11.5
T_{sig} (min)	90	NA	NA	210	NA	NA	30
$\Delta H2$ (ppm)	134.5	NA	NA	36	NA	NA	96.5
$T_{\Delta H2}$ (min)	300	NA	NA	330	NA	NA	270
AUC_{8hours} (ppm x hours)	437	16.25	16.125	81.25	15.5	20.375	377.625

Xylose

Pony	123	124	125	126	127	128	129
Time (min)	Breath Hydrogen Concentrations (ppm)						
0	2.5	8.5	7	4	0	2	3.5
30	2.5	4	7.5	4.5	2	0.5	8
60	5.5	5.5	6	5	2	0	17.5
90	16.5	5.5	5.5	5.5	4	2	17
120	27	4	11	13	3	0	23.5
150	50	0.5	3	11	2.5	1	25
180	70	2	1.5	19.5	2	0	25.5
210	107	0.5	1.5	29	1.5	0	35.5
240	125.5	2.5	4	38	2.5	0.5	29
270	136	2	3	42.5	2	0	16
300	153	2.5	1	32.5	0	0.5	10.5
330	133.5	0	2	39	1	1	10.5
360	139.5	2	2	10	3	2	14
390	143	1	3	4.5	2	3	9.5
420	211	2	1	3	1	3	12
450	175	1	2	5.5	2	2	9.5
480	173	0	0	1	2	0	12
T_{sig} (min)	90	NA	NA	120	NA	NA	60
$\Delta H2$ (ppm)	211	NA	NA	42.5	NA	NA	35.5
$T_{\Delta H2}$ (min)	420	NA	NA	270	NA	NA	210
AUC_{8hours} (ppm x hours)	791.375	19.625	28.75	132.5	15.75	8.25	135.375

Oats

Pony	123	124	125	126	127	128	129
Time (min)	Breath Hydrogen Concentrations (ppm)						
0	5	3	1	3.5	1	2.5	9
30	3.5	6	0.5	4	0.5	1.5	7
60	2	6	0	4	2	2	17
90	5	6	2	6	2	1	20.5
120	3.5	5	3	3	2	6	45
150	5.5	6	4	4.5	2	15.5	43
180	7	8	2	6	3	28.5	50
210	6.5	8.5	3	10	5	53.5	51
240	8.5	10	4	13.5	5.5	50	56.5
270	9.5	18.5	3	21	12.5	80	59.5
300	16	12.5	12	38	13.5	53.5	59.5
330	16.5	25	19	29	18.5	30	69.5
360	17.5	55	19	27.5	18.5	23.5	59
390	19.5	60	21.5	28.5	16.5	24.5	63.5
420	33.5	73	30	29	23	42	44.5
450	30.5	49.5	26	18	22.5	49	27.5
480	36.5	17	40	9	25.5	34.5	18
T_{sig} (min)	300	240	300	210	270	150	60
$\Delta H2$ (ppm)	36.5	73	40	38	25.5	80	69.5
$T_{\Delta H2}$ (min)	480	420	480	300	480	270	330
AUC_{8hours} (ppm x hours)	102.625	179.5	84.75	124.125	80.125	239.5	343.25

Wheat Flour

Pony	123	124	125	126	127	128	129
Time (min)	Breath Hydrogen Concentrations (ppm)						
0	3	16	0.5	1	0.5	0.5	2
30	1	8.5	0	3	0	1	0.5
60	3	7	0	3.5	0	0	2
90	4	4	2.5	6.5	2	0	5
120	5.5	4	2.5	5.5	8	1.5	9
150	23.5	11	4	7	22	5	10.5
180	52	23	6	4	33	17	14.5
210	77	12.5	6.5	5.5	79	11	18.5
240	120	14.5	10	4.5	80.5	11	32.5
270	88.5	15.5	5	10	92	10	60
300	170.5	10	11.5	11.5	90	15	79
330	133	12	14.5	14	91.5	17.5	103.5
360	242.5	13	3.5	16	88	47	121
390	279	9.5	3	10	68	36	98
420	191	8	4.5	5	26.5	23	80
450	252	5	1.5	6	15	13	33
480	211.5	2	2	4	8	10.5	35.5
T_{sig} (min)	150	150	240	270	150	180	150
$\Delta H2$ (ppm)	279	23	14.5	16	92	47	121
$T_{\Delta H2}$ (min)	390	180	330	360	270	360	360
AUC_{8hours} (ppm x hours)	874.875	83.25	38.125	57.25	349.875	106.75	342.875

Lactulose

Pony	123	124	125	126	127	128	129
Time (min)	Breath Hydrogen Concentrations (ppm)						
0	6	3.5	1	5	3	0	1.5
30	6	1	7.5	5	3	0.5	2
60	3	2	0	27.5	3	0	4
90	4	2	1	28.5	1	0	4
120	2.5	1	1	34	1.5	1	4.5
150	5.5	1	0	43.5	1	0	4
180	8.5	1	0	43.5	2	0	2.5
210	10	0.5	0	48	3.5	1	3
240	14.5	2	0.5	54.5	1	0	2.5
270	19	1	0	50	1	2	1
300	22.5	1	0	50.5	1	0	0
330	17	1	0	44	3.5	1	0.5
360	35.5	2	3	36.5	3	0	1
390	45	2.5	0.5	45	2	0.5	1
420	40.5	2	2.5	46.5	0.5	0	2
450	65	1.5	2	37	2	1	0
480	44	0	2	20	2	0	0.5
T_{sig} (min)	210	NA	NA	60	NA	NA	NA
$\Delta H2$ (ppm)	65	NA	NA	54.5	NA	NA	NA
$T_{\Delta H2}$ (min)	450	NA	NA	240	NA	NA	NA
AUC_{8hours} (ppm x hours)	161.75	11.625	9.75	303.25	15.75	3.5	16.5

Lactose

Pony	123	124	125	126	127	128	129
Time (min)	Breath Hydrogen Concentrations (ppm)						
0	2	4.5	2	0	0	0	1.5
30	3.5	6	0.5	0.5	0.5	1	1
60	6	5.5	0	4	2	0.5	2
90	7.5	8	1.5	4.5	0.5	0	2
120	11	7	0	1	2	0.5	4.5
150	21.5	9	0.5	2	2	0.5	7.5
180	32	7.5	1	1.5	2	1	4
210	44	9.5	0	0.5	2	1.5	7
240	27	6.5	0.5	0	2.5	1.5	7.5
270	36	11	0	0	2	0	9.5
300	63.5	9	0	0	1.5	0	14
330	72.5	5	0.5	1	2	0.5	19
360	47.5	4	1	0.5	2	0	20
390	65.5	3.5	0	6	0	0	17
420	96	3	0	5.5	0	0	14
450	85.5	0.5	0	6.5	0	0	10.5
480	93	1.5	0	6	1	0	9
T_{sig} (min)	120	NA	NA	NA	NA	NA	300
$\Delta H2$ (ppm)	96	NA	NA	NA	NA	NA	20
$T_{\Delta H2}$ (min)	420	NA	NA	NA	NA	NA	360
AUC_{8hours} (ppm x hours)	333.25	49	3.25	18.25	10.75	3.5	72.375

APPENDIX 21

Concentrations of hydrogen (mean of duplicate measurements) detected in excreted breath of adult ponies following the administration of wheat flour by stomach tube when maintained on a hay only diet. Tests were repeated on each pony on three occasions.

T_{sig} (min) = the time at which significant increases in hydrogen (if any) were first noted.
$\Delta H2$ (ppm) = the peak hydrogen concentration measured during the test period.
$T_{\Delta H2}$ (min) = the time of peak hydrogen concentration.
AUC_{8hours} (ppm x hours) = the total area under the hydrogen excretion curve.
NA = not applicable.

Replicate 1

Pony	**123**	**124**	**125**	**126**	**127**	**128**	**129**
Time (min)	Breath Hydrogen Concentrations (ppm)						
0	3	16	0.5	1	0.5	0.5	2
30	1	8.5	0	3	0	1	0.5
60	3	7	0	3.5	0	0	2
90	4	4	2.5	6.5	2	0	5
120	5.5	4	2.5	5.5	8	1.5	9
150	23.5	11	4	7	22	5	10.5
180	52	23	6	4	33	17	14.5
210	77	12.5	6.5	5.5	79	11	18.5
240	120	14.5	10	4.5	80.5	11	32.5
270	88.5	15.5	5	10	92	10	60
300	170.5	10	11.5	11.5	90	15	79
330	133	12	14.5	14	91.5	17.5	103.5
360	242.5	13	3.5	16	88	47	121
390	279	9.5	3	10	68	36	98
420	191	8	4.5	5	26.5	23	80
450	252	5	1.5	6	15	13	33
480	211.5	2	2	4	8	10.5	35.5
T_{sig} (min)	150	150	240	270	150	180	150
$\Delta H2$ (ppm)	279	23	14.5	16	92	47	121
$T_{\Delta H2}$ (min)	390	180	330	360	270	360	360
AUC_{8hours} (ppm x hours)	874.875	83.25	38.125	57.25	349.875	106.75	342.875

Replicate 2

Pony	123	124	125	126	127	128	129
Time (min)	Breath Hydrogen Concentrations (ppm)						
0	3	4	3	0	1.5	0.5	0
30	2.5	2.5	2	0.5	0	0	1
60	2.5	3	3	0	0	0	1
90	6	8	6	5	2	0	5.5
120	7	4.5	5.5	12.5	2	0	5
150	15	5	4.5	10	5	0	6
180	23.5	2	3.5	6.5	17	0	14
210	48.5	3.5	3.5	16	67	0	17.5
240	66	0	2	15	90.5	4	16.5
270	106.5	2.5	3.5	12	61.5	6	21
300	108.5	4.5	3.5	28.5	77	6	30.5
330	135	3.5	3.5	30.5	72.5	8	17
360	121.5	3.5	5	41	88	9	12.5
390	84	3	4.5	30.5	123	19.5	13.5
420	64	3.5	6	18.5	39	18	18.5
450	35.5	4.5	5	20	28.5	13.5	21
480	30	2.5	3	8	15	5.5	22
T_{sig} (min)	150	NA	NA	120	180	390	180
$\Delta H2$ (ppm)	135	NA	NA	41	123	19.5	30.5
$T_{\Delta H2}$ (min)	330	NA	NA	360	390	390	300
AUC_{8hours} (ppm x hours)	421.25	28.375	32	125.25	340.625	43.5	105.75

Replicate 3

Pony	123	124	125	126	127	128	129
Time (min)	Breath Hydrogen Concentrations (ppm)						
0	2.5	5.5	0.5	2.5	0	2	27.5
30	2	3	1	2	1	3	58
60	3	2.5	1	4.5	2	2.5	51.5
90	1	3	2.5	7	2	4	56.5
120	7	1.5	5	8.5	1	8	108
150	29.5	1.5	3	17	4.5	17	133
180	63	4.5	5.5	15.5	1.5	29.5	107
210	94	5.5	3.5	18	4	24.5	77.5
240	86	4.5	3.5	12.5	5.5	40.5	135.5
270	76	6	2	8	3.5	39	145
300	126	6	2	7.5	2.5	35	118
330	165.5	5	2	8.5	3	22	123.5
360	38	5	3.5	13.5	2	12	117
390	16	4.5	3.5	12.5	5	7	98
420	16.5	2	4	14	4	8.5	118
450	19	2	3.5	27	3	7	132
480	17.5	3	4	11.5	2	5	62.5
T_{sig} (min)	150	NA	NA	150	NA	150	30
$\Delta H2$ (ppm)	165.5	NA	NA	27	NA	40.5	145
$T_{\Delta H2}$ (min)	330	NA	NA	450	NA	240	270
AUC_{8hours} (ppm x hours)	376.25	30.375	23.875	91.5	22.75	131.5	811.75

APPENDIX 22

Concentrations of hydrogen (mean of duplicate measurements) detected in excreted breath of adult ponies following the administration of wheat flour by stomach tube when maintained on the pelleted ration. Tests were repeated on each pony on three occasions.

T_{sig} (min) = the time at which significant increases in hydrogen (if any) were first noted.
$\Delta H2$ (ppm) = the peak hydrogen concentration measured during the test period.
$T_{\Delta H2}$ (min) = the time of peak hydrogen concentration.
AUC_{8hours} (ppm x hours) = the total area under the hydrogen excretion curve.
NA = not applicable.

Replicate 1

Pony	123	124	125	126	127	128	129
Time (min)	Breath Hydrogen Concentrations (ppm)						
0	3	1.5	2.5	0.5	0	0.5	0.5
30	2	0.5	1	0	0.5	0	1.5
60	3.5	2	0	2	0.5	0	1
90	2.5	2	2	15	2	0	4
120	13	2.5	0	37.5	2.5	0	5.5
150	25	5.5	4.5	38.5	24	0	6.5
180	41	4	8.5	46	25.5	0.5	6.5
210	51	3	5	52.5	23	2	4.5
240	70	3	1	70.5	16.5	3.5	5.5
270	69.5	1	1.5	54	11	3	3.5
300	31	0.5	0.5	21	8.5	2	2
330	15	0.5	0	7.5	4	1	2
360	14.5	0	0	8	4	3	2.5
390	13	0	1	6.5	2.5	3	3
420	13.5	0	3	4	2.5	1.5	0.5
450	13	0	3	2.5	2	2	2
480	12	0	3	3	1.5	2	2
T_{sig} (min)	120	NA	180	90	150	NA	120
$\Delta H2$ (ppm)	70	NA	8.5	70.5	25.5	NA	6.5
$T_{\Delta H2}$ (min)	240	NA	180	240	180	NA	150
AUC_{8hours} (ppm x hours)	192.5	12.625	16.875	183.625	64.875	11.375	25.875

Replicate 2

Pony	123	124	125	126	127	128	129
Time (min)	Breath Hydrogen Concentrations (ppm)						
0	3	0	0.5	0	1	0.5	4.5
30	3	0	0	0	1	0.5	5.5
60	3	1.5	0	1	2	0	7
90	3	2	2.5	4	3.5	0	4
120	8	4	3	5.5	2	4	4
150	15.5	3.5	7.5	11	15.5	5	4.5
180	24.5	4.5	7	13	23.5	7	2
210	21.5	3	10	22	50.5	11	5
240	30.5	4	13	28.5	93.5	8.5	4
270	17.5	3.5	2	13	111.5	9.5	2.5
300	13	3.5	0	3.5	74.5	6	2.5
330	13.5	2	0.5	0.5	69	5	0.5
360	10.5	1.5	2	0	40	4	0.5
390	11	2.5	2	2	16	4.5	2.5
420	10.5	3	2.5	2	8	5	3.5
450	7.5	2	2	2	7.5	3	5
480	5.5	1	1.5	2	5.5	1.5	4
T_{sig} (min)	120	NA	150	120	150	150	NA
$\Delta H2$ (ppm)	30.5	NA	13	28.5	111.5	11	NA
$T_{\Delta H2}$ (min)	240	NA	240	240	270	210	NA
AUC_{8hours} (ppm x hours)	98.375	20.5	27.5	54.5	260.625	37	28.625

Replicate 3

Pony	123	124	125	126	127	128	129
Time (min)	Breath Hydrogen Concentrations (ppm)						
0	9	0	5	1.5	2	0	1
30	4.5	0	0.5	2.5	1	0	1
60	4	0.5	1.5	2	7	0.5	1.5
90	29	3	1.5	13	18	2.5	2
120	39	3	3	29	48.5	7	3
150	40	3	5.5	48	22.5	12	5.5
180	39	2.5	2.5	91	56	7	7.5
210	37.5	1	2	54	5	7	4
240	31.5	0	1	67.5	5	7.5	3.5
270	9.5	1.5	0	69.5	0.5	5.5	4
300	14	0.5	0	23.5	1	3	2.5
330	6.5	0	0	11	3.5	3.5	4
360	10	0	0	9	4	3	3.5
390	8.5	0.5	0	5	1	1	3
420	3	0	0	4	0	1	3
450	2	0	0	4	0.5	1	3
480	1	0.5	0.5	4	0.5	0.5	3
T_{sig} (min)	90	NA	NA	90	60	120	150
$\Delta H2$ (ppm)	40	NA	NA	91	56	12	7.5
$T_{\Delta H2}$ (min)	150	NA	NA	180	180	150	180
AUC_{8hours} (ppm x hours)	141.5	7.875	10.125	217.875	87.375	30.875	26.5

Appendix 23

Concentrations of hydrogen (mean of duplicate measurements) detected in excreted breath of adult ponies following the ingestion of a test meal after either no premedication or premedication with cisapride or codeine phosphate. Tests were repeated on each pony on three occasions.

T_{sig} (min) = the time at which significant increases in hydrogen (if any) were first noted.
$\Delta H2$ (ppm) = the peak hydrogen concentration measured during the test period.
$T_{\Delta H2}$ (min) = the time of peak hydrogen concentration.
AUC_{8hours} (ppm x hours) = the total area under the hydrogen excretion curve.

Control (i.e no premedication)

Pony	122			126			129		
Replicate	1	2	3	1	2	3	1	2	3
Time (min)									
0	5.5	2	1.5	0	0	0	1	2	0
30	4.5	1	0	0	2	0	0	0	0
60	2	0	2.5	0	0	0.5	0.5	0	0
90	8.5	2.5	3	0	1.5	0	0	2.5	0.5
120	11.5	4.5	1	0.5	1.5	0.5	0	3	0.5
150	11	8	3	2	0	5	1	2.5	5
180	21.5	18.5	6	3	1	6	2.5	5.5	5
210	10	33	7	4.5	0.5	7.5	8.5	5.5	7.5
240	16	76	10	6	2	8.5	15	6.5	7.5
270	17	107	10.5	5	4.5	7.5	22	15.5	10.5
300	38.5	104	9.5	5.5	6	9.5	24.5	16	4.5
330	35.5	120	15	3	11	11.5	21.5	18.5	2
360	16	35	10.5	4	12	13.5	12	12.5	3
390	6	21.5	6	2	10	7	6	8.5	1.5
420	2.5	7.5	3	0.5	7.5	4.5	2.5	3	1
450	1	5	2.5	2	6.5	3.5	0.5	4.5	3
480	0	3	1	0	3.5	2	0	3.5	1
T_{sig} (min)	90	150	180	240	300	150	210	180	150
$\Delta H2$ (ppm)	38.5	120	15	6	12	13.5	24.5	18.5	10.5
$T_{\Delta H2}$ (min)	300	330	330	240	360	360	300	330	270
AUC_{8hours} (ppm x hours)	102	273	45.5	19	34	43	58.5	53.5	26

Premedication with cisapride

Pony	122			126			129		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Time (min)									
0	1	13	2.5	0.5	0	3	1.5	6.5	1
30	1	6	1.5	2	0.5	2	1	2.5	0
60	2	17	1.5	4.5	3.5	3	2.5	4.5	1
90	3.5	20.5	3.5	4.5	9.5	6	2.5	8	3.5
120	8.5	35.5	15	6.5	11	5.5	6	13.5	3
150	15.5	36.5	25.5	7	17	4.5	6.5	11	1.5
180	14.5	50.5	28	7	18	3.5	5.5	20.5	2.5
210	19	48	22	11	14	3.5	6	21	5
240	34	41	18	12	24	2	5	22.5	7.5
270	54	36	26.5	16	25	3.5	9	20.5	11
300	51	32.5	26	23	30	3.5	12.5	19	9
330	32	33	24	25	19	3.5	11	12.5	9
360	27.5	32.5	22.5	17.5	15.5	5	5	10.5	10
390	11.5	26	29.5	29	9	4.5	4	7	4
420	1	21.5	27.5	6	5	6	6	6.5	2.5
450	4	17	16.5	3.5	4	5	2	6	1.5
480	4.5	13.5	13	3	3.5	3	2	5.5	0
T_{sig} (min)	120	60	120	120	90	90	120	90	210
$\Delta H2$ (ppm)	54	50.5	29.5	29	30	6	12.5	22.5	11
$T_{\Delta H2}$ (min)	270	180	390	390	300	420	300	240	270
AUC_{8hours} (ppm x hours)	141	233.5	147.5	88	103.5	32	43.	96	36

Premedication with codeine phosphate

Pony	122			126			129		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Time (min)									
0	6.5	0.5	0	0	2	0	0	0.5	0
30	5	0	0	0	0	0	0	0.5	0
60	14.5	1	0	0	0	0	0	0.5	0
90	26	3.5	1	2	3	0	0	0	0
120	18	11.5	7.5	1.5	5	2.5	2.5	2	0.5
150	7	22.5	11	2.5	7.5	4.5	6	3	1.5
180	8.5	21	23.5	6	8	11.5	7	7	3
210	8.5	8.5	3.5	6	7	15.5	8.5	7	4
240	14.5	11.5	5	18.5	6	8.5	7	9	6.5
270	18	12.5	8.5	27.5	7	12.5	8.5	9	5
300	37	13.5	7	40	8	15	5	12	5
330	49	23	9	37.5	12.5	15	9.5	9.5	4
360	55.5	20.5	15	20	12	8.5	16	11.5	3.5
390	35	22	24.5	21	15	9.5	10	15	7
420	73	21.5	30.5	12	11.5	6	24.5	18	6.5
450	34	33	35	4	12.5	2.5	21	19	5.5
480	26	16	25.5	7.5	14	4.5	17.5	17.5	6
T_{sig} (min)	60	120	120	180	120	180	150	180	240
$\Delta H2$ (ppm)	73	33	35	40	15	15.5	24.5	19	7
$T_{\Delta H2}$ (min)	420	450	450	300	390	210	420	450	390
AUC_{8hours} (ppm x hours)	210	117	97	101	61.5	57	67	66	27.5

GLOSSARY

ANOVA	analysis of variance
AP	alkaline phosphatase
AUC	area under curve
AUC_{8hours}	area under curve for the eight hour test period
BGC	basal glucose concentration
bwt	body weight
^{51}Cr	^{51}Chromium
CIBD	chronic inflammatory bowel disease
CV	coefficient of variation
EDTA	ethylenediaminetetra-acetate
EGE	eosinophilic gastroenteritis
GE	granulomatous enteritis
GGT	gamma-glutamyl transferase
GIT	gastrointestinal tract
GLDH	glutamate dehydrogenase
Glu_{MAX}	peak glucose concentration
H_2	hydrogen
hr	hour
L_3	cyathostome third stage larvae
MCTT	mouth-to-caecum transit time
min	minutes
n	number of observations
NBT	nitroblue tetrazolium
NH_3	ammonia
NA	not applicable
NAD	no abnormality detected
NSAID	non-steroidal anti-inflammatory drug
OGTT	oral glucose tolerance test
p	probability
PI	post infection
ppm	parts per million
SD	standard deviation

SI	small intestine
SPE	serum protein electrophoresis
spp.	species
VFA	volatile fatty acids
w/v	weight over volume
$\Delta H2$	peak hydrogen excretion
$T_{\Delta H2}$	time of peak hydrogen excretion
T_{sig}	time at which significant and sustained increases in hydrogen excretion are detected.
^{111}In	^{111}Indium

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